3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of PBDEs. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

PBDEs are classes of brominated hydrocarbons that were previously used as flame retardant additives in plastics, textiles, and other materials. Production of PBDEs began in the 1970s and has continued until recently. PentaBDE and octaBDE mixtures were voluntarily withdrawn from the U.S. marketplace by their manufacturers at the end of 2004; however, the manufacture and use of decaBDE continued past that date (EPA 2010). In December of 2009, the two remaining U.S. producers of decaBDE and the largest U.S. importer of this product announced commitments to phase out manufacture and importation of decaBDE for most uses in the United States by December 31, 2012, and to end manufacture and import for all uses by the end of 2013 (EPA 2013j). Although PBDEs are no longer produced or used, concern continues to exist for health effects of PBDEs due to evidence that PBDE congeners have become ubiquitously distributed in the environment and are present in tissues and breast milk of the general population (EPA 2010; Meijer et al. 2008; Park et al. 2011; Rawn et al. 2014; Schecter et al. 2010). PBDEs comprise compounds in which 1–10 bromine atoms are attached to the biphenyl structure in up to 209 different combinations. Based on the number of bromine substituents, there are 10 homologous groups of PBDEs (monobrominated through decabrominated), each containing one or more isomers. PBDEs are structurally similar when viewed in one dimension, differing only in the ether linkage between the two phenyl rings in PBDEs, but the oxygen bridge confers three-dimensional conformational differences that can influence toxicological properties. Consequently, on the basis of chemical structure, it cannot be assumed that the health effects of PBDE congeners are necessarily similar. Reviews on the health effects and other aspects of PBDEs include those by Bellinger (2013), Darnerud et al. (2001), de Boer et al. (2000a), de Wit (2002), Dingemans et al. (2011), EPA (2008a, 2008b, 2008c, 2008d), Gill et al. (2004), Hardy (1999, 2002a, 2002b), Hardy et al. (2009), Markowski (2007), McDonald (2002), Rahman et al. (2001), Silberhorn et al. (1990), and WHO (2006). Discussions of health effects are divided into lower-brominated congeners and decaBDE due to important differences in pharmacokinetics and toxicity of decaBDE compared to lower-brominated PBDEs. Toxicity data for previously used

PBDE mixtures as well as individual PBDE congeners are included in this profile, with mixtures being categorized by their most prominent congener (see Table 4-3, Physical and Chemical Properties of Technical PBDE Mixtures). Using current health effects evaluation procedures, toxicity data for individual congeners may over- or underestimate the actual health risk of PBDE mixtures because congeners vary in toxic potency and may be influenced by other congeners in an additive, less-than-additive, or more-than-additive way. It is also important to recognize that the PBDEs to which people may be exposed may be different from the original PBDE source because of possible changes in congener composition resulting from differential partitioning and transformation in the environment and/or differential biological metabolism and retention.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

A few studies have examined groups of chemical workers involved in the manufacture and distribution of PBDEs (Bahn et al. 1980; Brown et al. 1981; Chanda et al. 1982; Landrigan et al. 1979; Rosenman et al. 1979; Stross et al. 1981). These people are believed to have been exposed predominantly by dermal contact and inhalation, although the oral route cannot be ruled out. Results from these studies, therefore, are discussed in this section as well as in Section 3.2.3. The highest NOAEL and all LOAEL values from each reliable inhalation study of health effects end points in each species and duration category for PBDEs are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.1 Death

No studies were located regarding death in humans after inhalation exposure to PBDEs.

No deaths occurred in groups of five male and five female rats that were chamber-exposed to pentaBDE aerosol (compound dissolved in corn oil), octaBDE dust, or decaBDE dust in concentrations as high as 200,000, 60,000, or 48,200 mg/m^3, respectively, for 1 hour and observed for the following 14 days (IRDC 1974, 1975a, 1975b). Confidence in these studies is limited by a lack of control data. There was no mortality in rats that were exposed to dusts of commercial octaBDE products at concentrations of 174 mg/m^3 for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978) or $\leq$202 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2000).

Table 3-1 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Systemic								
1	Rat (CD)	14 d 8 hr/d	Resp	3.7	24 (reversible rapid breathing)		Great Lakes Chemical Corporation 1978 OctaBDE (technical)	
			Cardio	165				
			Gastro	165				
			Hemato	165				
			Hepatic	0.6	3.7 (hepatocytomegaly and focal hepatocellular degeneration)			
			Renal	165				
			Endocr	165				
			Ocular	165				
			Bd Wt	165				

Table 3-1 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE								
Systemic								
2	Rat (CD)	13 wk 5 d/wk 6 hr/d	Resp	16	202 (alveolar histiocytosis, chronic active lung inflammation)		Great Lakes Chemical Corporation 2000 OctaBDE (technical)	
			Cardio	202				
			Gastro	202				
			Hemato	202				
			Musc/skel	202				
			Hepatic	1.1	16 (centrilobular hepatocellular hypertrophy)			
			Renal	202				
			Endocr	1.1[b]	16 (decreased serum T4, increased serum TSH)			
			Dermal	202				
			Ocular	202				
			Bd Wt	202				
Immuno/ Lymphoret								
3	Rat (CD)	13 wk 5 d/wk 6 hr/d		16	202 (grossly discolored and enlarged bronchial and mediastinal lymph nodes associated with chronic active lung inflammation and alveolar histiocytosis)		Great Lakes Chemical Corporation 2000 OctaBDE (technical)	

Table 3-1 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
Reproductive								
4	Rat (CD)	13 wk 5 d/wk 6 hr/d		16 F	202 F (absence of corpora lutea in ovaries)		Great Lakes Chemical Corporation 2000 OctaBDE (technical)	

a The number corresponds to entries in Figure 3-1.

b Used to derive an intermediate-duration (15-364 days) inhalation minimal risk level (MRL) of 0.006 mg/m3 for lower brominated diphenyl ethers. The MRL was derived by converting the animal NOAEL of 1.1 mg/m3 to a duration-adjusted human equivalent concentration (NOAELHEC) of 0.53 mg/m3, and dividing by an uncertainty factor of 30 (3 for species to species extrapolation with dosimetric adjustments and 10 for human variability) and a modifying factor of 3 (for an incomplete data base).

Note on chemical form: The chemical form in all studies was a technical octaBDE mixture (exact composition was not reported).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest observed adverse effect level; Musc/skel = muscular/skeletal; NOAEL = no observed adverse effect level; Resp = respiratory; T4 = thyroxine; TSH = thyroid stimulating hormone; wk = week(s)

Figure 3-1. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Inhalation

Acute (≤14 days)

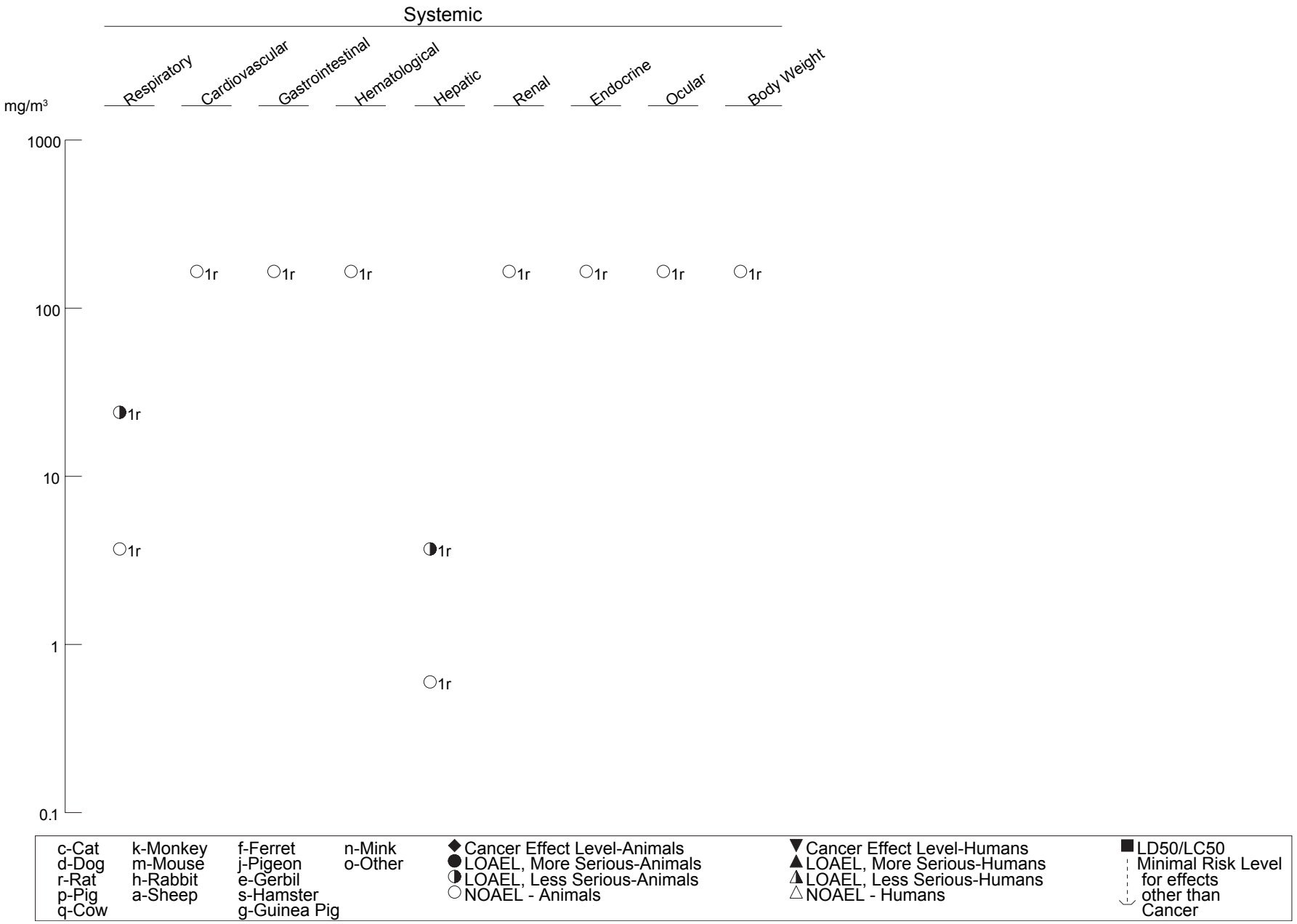
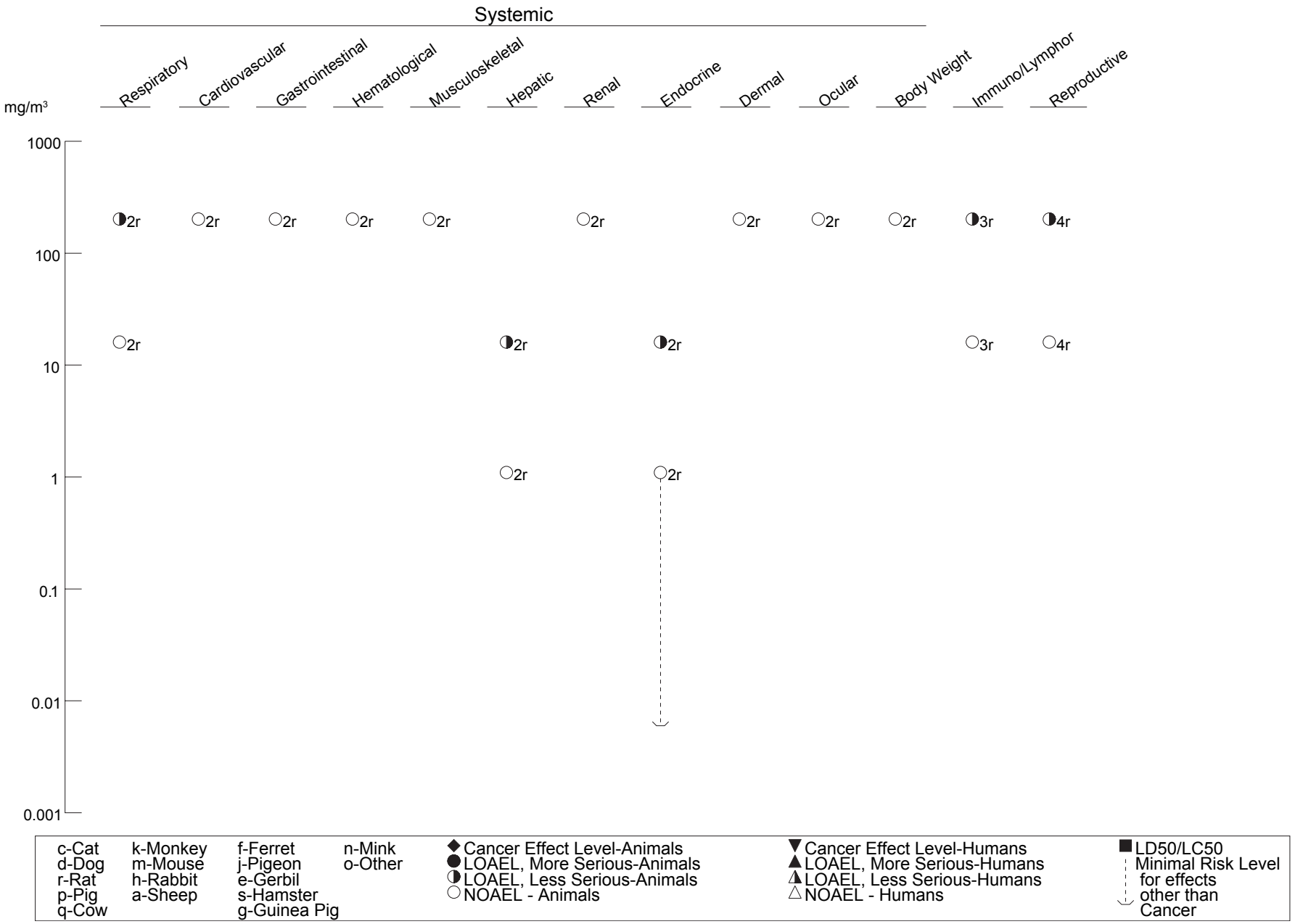


Figure 3-1. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Inhalation *(Continued)*

Intermediate (15-364 days)



3.2.1.2 Systemic Effects

Systemic effects that have been observed in humans and animals following inhalation exposure to PBDEs are described below.

Respiratory Effects. No studies were located regarding respiratory effects in humans after inhalation exposure to PBDEs.

Transient signs of respiratory distress that included tachypnea or dyspnea developed in rats that were chamber-exposed to pentaBDE aerosol (compound dissolved in corn oil), octaBDE dust, or decaBDE dust in very high concentrations of 200,000, 60,000, and 48,200 mg/m^3, respectively, for 1 hour (IRDC 1974, 1975a, 1975b). Confidence in these effect levels is low due to a small number of tested animals and lack of control data.

One 14-day inhalation study of commercial octaBDE has been conducted. In this study, rats were chamber-exposed to concentrations of 0, 0.6, 3.7, 23.9, or 165.2 mg/m^3 as powdered dust for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978). Increased respiration rate occurred at ≥23.9 mg/m^3. The rapid breathing pattern developed by the end of each exposure period, always disappeared by the following morning, and was not observed at lower exposure concentrations. Histological examinations of the control and 165.2 mg/m^3 rats (other groups not examined) showed no changes in tissues that included nasal turbinates, trachea, lungs, and mediastinal lymph nodes).

Histological changes in the lungs, but no clearly observed changes in the nasal cavity, were found in a study of rats that were nose-only exposed to 0, 1.1, 16, or 202 mg/m^3 as dust aerosol for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2000). The pulmonary effects included alveolar histiocytosis and chronic active inflammation, which occurred in both sexes, and were only clearly induced at 202 mg/m^3. Total incidences of alveolar histiocytosis in the 0, 1.1, 16, and 202 mg/m^3 exposure groups were 3/10, 5/10, 5/10, and 10/10 in males, respectively, and 0/10, 5/10, 2/10, and 10/10 in females, respectively. Respective total incidences of chronic active lung inflammation were 0/10, 0/10, 2/10, and 10/10 in males, and 0/10, 1/10, 1/10, and 10/10 in females. Both lesions were predominantly minimal or mild in severity, with moderate severity occurring in a few high-dose animals. Additional effects included gross pulmonary changes in both sexes at 202 mg/m^3; these included lung firmness and white discoloration and/or enlargement in the bronchial and/or mediastinal lymph nodes. The gross lymph node changes correlated with the histological granulomatous inflammation. Effects in

nasal tissues were equivocal. Incidences of nasal goblet cell hypertrophy were slightly increased in nasal level II of both sexes at ≥1.1 mg/m^3, but changes in incidence were not clearly dose-related and there was essentially no increase in severity from minimal levels with increasing dose. Total incidences of goblet cell hypertrophy in nasal level II in the 0, 1.1, 16, and 202 mg/m^3 exposure groups were 4/10, 9/10, 6/10, and 10/10 respectively, in males, and 2/10, 6/10, 4/10, and 8/10, respectively, in females. Minimal severity goblet cell hypertrophy was also slightly increased in nasal level IV in males at 202 mg/m^3 (4/10, 0/10, 1/10, and 8/10), but not in females.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation exposure to PBDEs.

No histopathological changes were observed in the heart of rats that were exposed to dusts of commercial octaBDE products at concentrations of 174 mg/m^3 for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978), or ≤202 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2000).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after inhalation exposure to PBDEs.

No histopathological changes were observed in the stomach and lower gastrointestinal tract of rats that were exposed to dusts of commercial octaBDE products at concentrations of 174 mg/m^3 for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978), or ≤202 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2000).

Hematological Effects. No studies were located regarding hematological effects in humans after inhalation exposure to PBDEs.

No adverse hematological changes occurred in rats that were exposed to 24.4 or 174 mg/m^3 of commercial octaBDE dust aerosol for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978). Evaluation of a limited number of indices (hemoglobin, hematocrit, total erythrocyte count, and total and differential leukocyte counts) showed no unusual responses except for an elevation in leukocyte numbers. The observed increase in leukocyte counts was considered to be an unusual response by the investigators, although it was within the normal range for control rats in their laboratory. Comprehensive hematological assessments showed no unusual changes in rats exposed to commercial

octaBDE as dust aerosol at concentrations of ≤202 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2000).

Hepatic Effects. No studies were located regarding hepatic effects in humans after inhalation exposure to PBDEs.

Hepatic effects were observed in a 14-day inhalation study of dusts of commercial octaBDE mixtures. In this study, rats were chamber-exposed to concentrations of 0, 0.6, 3.7, 23.9, or 165.2 mg/m^3 as powdered dust for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978). Increased liver weight and hepatic histological changes occurred in rats exposed to concentrations ≥3.7 mg/m^3. At 3.7 mg/m^3, the liver lesions consisted of very slight to slight severity focal to multifocal cytoplasmic enlargement of the hepatocytes, accompanied by focal acidophilic degeneration of individual to small groups of cells. The liver lesions were similar in rats exposed to concentrations ≥24.4 mg/m^3, except that the hepatocyte enlargement was multifocal to diffuse in distribution and accompanied by focal, small to large areas of hepatocellular necrosis of very slight to marked degree.

Similar hepatic changes were found in a study of rats that were nose-only exposed to 0, 1.1, 16, or 202 mg/m^3 commercial octaBDE as dust aerosol for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2000). The liver was affected in both sexes as shown by dose-related increases in centrilobular hepatocellular hypertrophy at ≥16 mg/m^3 and increased liver weight (absolute and relative) at 202 mg/m^3. Respective total incidences of centrilobular hepatocellular hypertrophy (predominantly minimal to mild) in the 0, 1.1, 16, and 202 mg/m^3 groups were 1/10, 0/10, 3/10, and 10/10 in males, and 0/10, 0/10, 3/10, and 6/10 in females. Serum chemistry evaluations showed no clear effects of exposure. Serum cholesterol was significantly increased (66.2% more than controls, $p<0.01$) in 202 mg/m^3 females, but the magnitude of the elevation was not considered toxicologically significant. Some other statistically significant serum chemistry alterations (increased mean globulin and total protein, decreased albumin/globulin ratio) also occurred in females exposed to 202 mg/m^3, but these changes were not considered exposure-related due to small magnitudes of changes and lack of similar changes in the males.

Renal Effects. No studies were located regarding renal effects in humans after inhalation exposure to PBDEs.

No histopathological changes were observed in the kidneys or urinary bladder of rats that were exposed to dusts of commercial octaBDE products at concentrations of 174 mg/m^3 for 8 hours/day for 14 consecutive

days (Great Lakes Chemical Corporation 1978) or ≤202 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2000). Urinalyses were not performed in any of these studies.

Endocrine Effects. There is evidence suggestive of hypothyroidism in a small group of workers who were occupationally exposed to decaBDE (Bahn et al. 1980). In another study, plasma levels of thyroid hormones (T_3 and free T_4) and eight PBDE congeners (tetra- to heptaBDEs) were monitored for 198–221 days in three electronic dismantling workers (Pettersson et al. 2002). The hormones remained within normal ranges and there were no correlations between levels of hormones and the plasma concentrations of congeners.

An acute inhalation study of commercial octaBDE dust in rats showed no histopathological changes in the thyroids, parathyroids, adrenals, or pituitary following chamber exposure to 174 mg/m^3 as powdered dust for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978). Rats that were nose-only exposed to commercial octaBDE at concentrations of 1.1, 16, or 202 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks similarly showed no histological changes in the adrenals, pancreas, parathyroids, pituitary, or thyroids (Great Lakes Chemical Corporation 2000). Measurements of serum levels of thyroid hormones in the 13-week rat study, however, showed exposure-related decreases in mean thyroxine (total T_4) in both sexes exposed at ≥16 mg/m^3, and increases in TSH in males exposed at ≥16 mg/m^3 and in females exposed at 202 mg/m^3. The changes were usually statistically significant ($p<0.05$ or $p<0.01$) compared to controls and were considered by the investigators to be consistent with chemical-induced hypothyroidism. There were no serum T_3 changes, thyroid-attributable clinical signs or body weight effects, or gross or histopathological changes in the thyroid. The 1.1 mg/m^3 LOAEL for thyroid effects was used as the basis for the intermediate-duration MRL for inhalation exposure to octaBDE, as indicated in the footnote to Table 3-1 and discussed in Chapter 2 and Appendix A.

Dermal Effects. No studies were located regarding dermal effects in humans after inhalation exposure to PBDEs.

No gross or histological changes in the skin were observed in rats that were nose-only exposed to commercial octaBDE as dust aerosol at concentrations of ≤202 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2000).

Ocular Effects. No studies were located regarding ocular effects in humans after inhalation exposure to PBDEs.

Transient signs of ocular irritation that included eye squint, erythema, and/or ocular discharge were observed in rats that were chamber-exposed to pentaBDE aerosol (compound dissolved in corn oil), octaBDE dust, or decaBDE dust in concentrations of 2,000, 2,000, and 48,200 mg/m^3, respectively, for 1 hour (IRDC 1974, 1975a, 1975b). Confidence in these effect levels is low due to a small number of tested animals and lack of control data.

No histopathological changes were observed in eyes of rats that were chamber-exposed to ≤174 mg/m^3 of commercial octaBDE as powdered dust for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978). Opthalmoscopic and histological examinations showed no ocular effects in rats following nose-only exposure to ≤202 mg/m^3 of commercial octaBDE dust aerosol for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2000).

3.2.1.3 Immunological and Lymphoreticular Effects

A study conducted in China examined the association between serum levels of four BDEs (28, 47, 66, and 209), as well as PCBs and organochlorine pesticides, and the prevalence of asthma in children (3–6 years old) living in a heavily populated area (Meng et al. 2016). The study involved 620 cases and 218 controls. Serum analyses showed significantly higher levels of BDEs in cases than in controls. BDE 209 had the highest mean concentration in cases, 4.02 ng/g lipid, compared to 1.73 ng/g lipid in controls. In multivariable-adjusted analyses, the odds ratio (OR) for risk of asthma associated with 1 ng/g increase was statistically significant only for BDE 28 (OR 3.63; 95% confidence interval [CI] 1.23–10.70). Strafication of children based on asthma severity showed that BDE 209 was positively correlated with the severity of the condition (OR 1.40; 95% CI 1.14–1.72). PCBs and organochlorine pesticide also were associated with increased risk of asthma.

No histopathological changes were observed in the spleen, mesenteric or mediastinal lymph nodes, or bone marrow from rats that were exposed to 174 mg/m^3 of octaBDE dust for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978). Rats that were nose-only exposed to commercial octaBDE at concentrations of 1.1, 16, or 202 mg/m^3 as dust aerosol for 6 hours/day, 5 days/week for 13 weeks similarly showed no effects in bone marrow, spleen, or thymus, although gross changes in pulmonary lymph nodes were observed at 202 mg/m^3 (Great Lakes Chemical Corporation

2000). The effects included discolored and/or enlarged bronchial and mediastinal lymph nodes, and appeared to be associated with concurrent granulomatous inflammation of the lungs.

3.2.1.4 Neurological Effects

No studies were located regarding neurological effects in humans after inhalation exposure to PBDEs.

No clinical signs of neurotoxicity were observed in rats that were exposed to dusts of commercial octaBDE products at concentrations of 174 mg/m^3 for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978) or ≤202 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2000). Histological examinations of nervous system tissues, performed only in the 13-week study, showed no effects in the brain (forebrain, midbrain, hindbrain), optic nerve, or a peripheral nerve (sciatic).

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to PBDEs.

No histopathological changes were observed in testes or ovaries from rats that were exposed to commercial octaBDE at concentrations ≤174 mg/m^3 as powdered dust for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978). A histological effect in the ovaries was found in a study of rats that were nose-only exposed to 0, 1.1, 16, or 202 mg/m^3 as dust aerosol for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2000). Absence of corpora lutea, based on qualitative evaluation of step sections of the ovary, was found in 3/10 females at 202 mg/m^3, compared to 0/10 incidences in the control and both lower exposure groups. The investigators interpreted this 30% increase in incidence be treatment-related because an absence of corpora lutea was considered unusual in rats at 20 weeks of age. No gross or histopathological changes were observed in the oviduct, uterus, or vagina, or in male reproductive tissues (testes with epididymides and vas deferens).

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to PBDEs.

3.2.1.7 Cancer

No studies were located regarding cancer in humans or animals after inhalation exposure to PBDEs.

3.2.2 Oral Exposure

Human data presented below are primarily from studies that attempted to identify relationships between concentrations of PBDE in serum or other tissues and various health outcomes. Details of PBDE exposure in these study populations are typically unknown. However, exposure is presumed to have been primarily by the oral route for those studies presented below.

The highest NOAEL and all LOAEL values from each reliable study of health effects end points in each species and duration category for PBDEs are recorded in Tables 3-2 (lower PBDEs) or 3-3 (decaBDE) and plotted in Figures 3-2 (lower PBDEs) or 3-3 (decaBDE).

3.2.2.1 Death

Single-dose gavage LD_{50} values of 5,000 and 6,200 mg/kg were determined for pentaBDE (Saytex 115 and DE-71, respectively) in rats that were observed for 14 days (British Industrial Biological Research Association 1977; Pharmakon Research International Inc. 1984). Another study found that a single 5,000 mg/kg dose of pentaBDE caused deaths in four of five rats in the 14 days following treatment, whereas doses ≤500 mg/kg caused no mortality (IRDC 1975b). No deaths occurred in rats exposed to pentaBDE in estimated dietary doses of ≤90 mg/kg/day for 28 days (IRDC 1976) or ≤100 mg/kg/day for 90 days (WIL Research Laboratories 1984).

No deaths occurred in rats that were administered octaBDE by gavage in single doses ≤5,000 mg/kg and observed for the following 14 days (IRDC 1975a). Intermediate-duration dietary studies with octaBDE, resulted in no mortality in rats exposed to estimated dietary doses of ≤90 mg/kg/day for 28 days or ≤750 mg/kg/day for 13 weeks (IRDC 1976, 1977).

No deaths occurred in rats that were treated with a single gavage dose of ≤5,000 mg/kg of decaBDE or ≤2,000 mg/kg of 77.4% decaBDE (containing 21.8% nonaBDE and 0.8% octaBDE) and observed for the following 14 days (IRDC 1974; Norris et al. 1975a). No mortality was observed in rats and mice that were exposed to decaBDE via diet in estimated doses of ≤16,000 and ≤19,000 mg/kg/day, respectively, for 14 days (NTP 1986).

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Wistar)	once (GO)				6200 (44-day LD50)	British Industrial Biological Research Association 1977 PentaBDE (DE-71)	
2	Rat Spartan	once (GO)				5000 (4/5 died)	IRDC 1975b PentaBDE (technical)	
3	Rat (Sprague-Dawley)	once (GO)				5000 (14-day LD50)	Pharmakon Research International Inc. 1984 PentaBDE (Saytex 115)	
Systemic								
4	Rat (Sprague-Dawley)	once (GO)	Hepatic	1.2 M			Albina et al. 2010 PentaBDE (BDE99)	
			Renal		0.6 M (phagolysosomes in renal tubules)			
5	Rat (Sprague-Dawley)	once (GO)	Hepatic	1.2 M			Alonso et al. 2010 PentaBDE (BDE99)	No biologically relevant changes in hepatic serum chemistry.
			Renal	0.6 M	1.2 M (increased total protein in urine)			
			Endocr		0.6 M (reduced serum testosterone)			
6	Rat (CD)	10 d Gd 6-15 (GO)	Bd Wt	10	100 (20% reduction in maternal body weight gain)		Argus Research Laboratories 1985a PentaBDE (Saytex 115)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
7	Rat (Sprague-Dawley)	14 d Gd 6-19 (GO)	Bd Wt	2 F			Blanco et al. 2012 PentaBDE (BDE99)	
8	Rat (Wistar)	7 d 1 x/d (GO)	Hepatic	8 F	40 F (porphyria)		Bruchajzer 2011 PentaBDE (technical)	
			Bd Wt	200 F				
9	Rat (Wistar)	14 d 1 x/d (GO)	Hepatic		8 F (porphyria)		Bruchajzer 2011 PentaBDE (technical)	
			Bd Wt	200 F				
10	Rat (Wistar)	7 d 1 x/d	Hepatic	40 F	200 F (fatty degeneration)		Bruchajzer et al. 2010 PentaBDE (technical)	
			Bd Wt	40 F	200 F (7% decrease in body weight gain)			
11	Rat (Wistar)	14 d 1 x/d	Hepatic	40 F	200 F (fatty degeneration)		Bruchajzer et al. 2010 PentaBDE (technical)	
			Bd Wt	40 F	200 F (10% decrease in body weight gain)			

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form	Comments
12	Rat (Wistar)	once 5 d observation (GO)	Hepatic	200 F	2000 F (fatty degeneration)		Bruchajzer et al. 2011 PentaBDE (technical)	
			Bd Wt	200 F	2000 F (11% decrease in body weight gain)			
				200 F	2000 F (11% decrease in body weight gain)			
13	Rat (Wistar)	7 d 1 x/d (GO)	Hepatic	2 F	8 F (porphyria)		Bruchajzer et al. 2012 OctaBDE (technical)	
			Bd Wt	8 F	40 F (9% decrease in body weight gain)			
14	Rat (Wistar)	14 d 1 x/d (GO)	Hepatic	8 F	40 F (porphyria)		Bruchajzer et al. 2012 OctaBDE (technical)	
			Bd Wt	8 F	40 F (5% decrease in body weight gain)			
15	Rat (Sprague-Dawley)	14 d 1 x/d (GO)	Hepatic	76.6 M			Carlson, 1980b OctaBDE (technical)	
16	Rat (Sprague-Dawley)	14 d 1 x/d (GO)	Hepatic	56.4 M			Carlson, 1980b PentaBDE (technical)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
17	Rat (Sprague-Dawley)	14 d 1 x/d (GO)	Endocr		18 (reduced serum T4)		Darnerud and Sinjari 1996 PentaBDE (Bromkal 70)	
18	Rat (Sprague-Dawley)	6 d Gd 6.5-11.5 (GO)	Endocr	120 F			Ellis-Hutchings et al. 2009 PentaBDE (DE-71)	No exposure-related changes in serum thyroid hormone levels.
			Bd Wt	120 F				
19	Rat (Sprague-Dawley)	14 d 1 x/d (G)	Endocr	6 F	18 F (reduced serum T4, reduced T4 protein binding)		Hallgren and Darnerud 2002 TetraBDE (BDE47)	
			Bd Wt	18 F				
20	Rat (Sprague-Dawley)	14 d 1 x/d (GO)	Hepatic		18 F (reduced liver vitamin A)		Hallgren et al. 2001 PentaBDE (Bromkal 70-5DE)	
			Endocr		18 F (reduced serum T4)			
			Bd Wt	36 F				
21	Rat (Sprague-Dawley)	2 wk 1 x/d (GO)	Endocr		14 M (reduced serum T4)		Hoppe and Carey 2007 PentaBDE (technical)	No exposure-related changes in fat pad weight, adipocyte number, size, viability, lipolysis or glucose oxidation.
			Bd Wt	14 M				
			Metab	14 M				

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form	Comments
22	Rat Spartan	once (GO)	Bd Wt	5000			IRDC 1975a OctaBDE (technical)	
23	Rat Spartan	once (GO)	Bd Wt	500			IRDC 1975b PentaBDE (technical)	
24	Rat (Wistar)	once Gd 6 (GO)	Endocr		0.06[b] F (reduced serum T4)		Kuriyama et al. 2007 PentaBDE (BDE99)	
25	Rat (CD)	10 d Gd 6-15 (GO)	Bd Wt	25			Life Science Research Israel Ltd. (1987) OctaBDE (FR-1208)	
26	Rat (Wistar)	5 d Pnd 22-26 (GO)	Endocr	3 F	30 F (reduced serum T4)		Stoker et al. 2004 PentaBDE (DE-71)	
			Bd Wt	60 F				
27	Rat (Wistar)	5 d Pnd 23-27 (GO)	Endocr	3 M	30 M (reduced serum T4)		Stoker et al. 2004 PentaBDE (DE-71)	
			Bd Wt	60 M				
28	Rat (Wistar)	3 d 1 x/d (GO)	Endocr	30 M	60 M (increased serum LH)		Stoker et al. 2005 PentaBDE (DE-71)	
			Bd Wt	60 M				

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
29	Rat (Wistar)	9 d 1 x/d (GO)	Bd Wt	240 M			Stoker et al. 2005 PentaBDE (DE-71)	Hershberger Assay (castrated rats, supplemented with s.c. testosterone)
30	Rat (Wistar)	once Gd 6 (GO)	Bd Wt	0.7 F			Talsness et al. 2008 TetraBDE (BDE47)	
31	Rat (CD)	10 d Gd 6-15 (GO)	Bd Wt	25	50 (40% reduced maternal body weight gain)		WIL Research Laboratories 1986 OctaBDE (DE-79)	
32	Rat (Long-Evans)	4 d 1 x/d (GO)	Endocr	10 F	30 F (reduced serum T4)		Zhou et al. 2001 PentaBDE (DE-71)	
			Bd Wt	300 F				
33	Rat (Long-Evans)	4 d 1 x/d (GO)	Endocr	3 F	10 F (reduced serum T4)		Zhou et al. 2001 OctaBDE (DE-79)	
			Bd Wt	300 F				
34	Mouse (C57BL/6N)	14 d 1 x/d (GO)	Endocr		18 (reduced serum T4)		Darnerud and Sinjari 1996 PentaBDE (Bromkal 70)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form	Comments
35	Mouse C57BL/6J	once (GO)	Hepatic	500 F			Fowles et al. 1994 PentaBDE (DE-71)	
			Endocr	100 F	500 F (reduced serum T4)			
			Bd Wt	500 F				
36	Mouse (C57BL/6N)	14 d 1 x/d (GO)	Hepatic	72 F			Fowles et al. 1994 PentaBDE (DE-71)	
			Endocr		18 F (reduced serum T4)			
			Bd Wt	72 F				
37	Mouse (C57BL/6N)	14 d 1 x/d (GO)	Hepatic	18 F	36 F (reduced liver vitamin A)		Hallgren et al. 2001 PentaBDE (Bromkal 70-5DE)	
			Endocr		18 F (reduced serum T4)			
			Bd Wt	36 F				
38	Mouse (C57BL/6N)	14 d 1 x/d (GO)	Endocr		18 F (reduced serum T4)		Hallgren et al. 2001 TetraBDE (BDE47)	
			Bd Wt	18 F				

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
39	Mouse (C57BL/6)	4 d 1 x/d (GO)	Endocr	10 F	100 F (reduced serum T4)		Richardson et al. 2008 TetraBDE (BDE47)	
			Bd Wt	100 F				
Immuno/ Lymphoret								
40	Rat (Sprague-Dawley)	14 d 1 x/d (GO)		36			Darnerud and Thuvander 1998 PentaBDE (Bromkal 70-5DE)	No exposure-related changes in spleen or thymus weight, number or distribution of lymphocyte subpopulations, or in vitro IgG production.
41	Mouse (C57BL/6N)	14 d 1 x/d (GO)		18	36 (reduced in vitro production of IgG in mitogen- stimulated splenocytes)		Darnerud and Thuvander 1998 PentaBDE (DE-71)	
42	Mouse (C57BL/6N)	once (GO)		500 F			Fowles et al. 1994 PentaBDE (DE-71)	No exposure-related change in spleen or thymus weights or antibody respose to sheep red blood cells.
43	Mouse (C57BL/6N)	14 d 1 x/d (GO)		36 F	72 F (reduced antibody response to sheep red blood cells, decreased thymus weight)		Fowles et al. 1994 PentaBDE (DE-71)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
44	Mouse (C57BL/6N)	14 d 1 x/d (GO)		18 F			Hallgren et al. 2001 TetraBDE (BDE47)	No exposure-related changes in spleen or thymus weight
Neurological								
45	Rat (Sprague-Dawley)	once (GO)		1.2 M			Belles et al. 2010 PentaBDE (BDE99)	No changes in brain histology or in functional observation battery, open-field testing, passive avoidance test, or Morris water maze.
Reproductive								
46	Rat (Sprague-Dawley)	once (GO)			0.6 F (decreased serum testosterone)		Alonso et al. 2010 PentaBDE (BDE99)	
47	Rat (Sprague-Dawley)	14 d Gd 6-19 (GO)		2 F			Blanco et al. 2012 PentaBDE (BDE99)	No treatment-related changes in gravid uterine weight or number of implantation or resorptions.
48	Rat (Wistar)	3 d 1 x/d (GO)		30 M	60 M (increased serum LH)		Stoker et al. 2005 PentaBDE (DE-71)	
49	Rat (Wistar)	9 d 1 x/d (GO)			30 M (20% decrease in ventral prostate weight)		Stoker et al. 2005 PentaBDE (DE-71)	Hershberger Assay (castrated rats, supplemented with s.c. testosterone)

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form	Comments
50	Rat (Wistar)	once Gd 6 (GO)		0.7 F			Talsness et al. 2008 TetraBDE (BDE47)	No dose-related changes in ovary weight or histology.
51	Mouse (BALB/c)	3 d 1 x/d (GO)		300 F			Mercado-Feliciano and Bigsby 2008a PentaBDE (DE-71)	Mice were ovariectomized 3 weeks prior to exposure; no change in uterus weight or uterus/vaginal histology.
Developmental								
52	Rat (CD)	10 d Gd 6-15 (GO)		200			Argus Research Laboratories 1985a PentaBDE (Saytex 115)	No changes in number, sex, and weight of fetuses, fetal death, early and late resorptions, gross malformations, or skeletal or visceral abnormalities.
53	Rat (CD)	10 d Gd 6-15 (GO)		10	25 (increased resorptions and reduced fetal body weight)		Argus Research Laboratories 1985b OctaBDE (Saytex 115)	
54	Rat (Sprague-Dawley)	14 d Gd 6-19 (GO)		1	2 (delayed ossification, liver and heart hypertrophy)		Blanco et al. 2012 PentaBDE (BDE99)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
55	Rat (Long- Evans)	7 d Pnd 6-12 (GO)			30 M (impaired learning in visual discrimination task at Pnd 30-83)		Dufault et al. 2005 PentaBDE (DE-71)	
56	Rat (Sprague-Dawley)	6 d Gd 6.5-11.5 (GO)		120			Ellis-Hutchings et al. 2009 PentaBDE (DE-71)	No exposure-related changes in embryo viability, growth, or morphology.
57	Rat (Sprague-Dawley)	14 d Gd 6.5-19.5 (GO)		120			Ellis-Hutchings et al. 2009 PentaBDE (DE-71)	No exposure-related effects on fetal survival, growth, or malformations.
58	Rat (Sprague-Dawley)	once Pnd 10 (GO)			1 (impaired learning and memory at 2 months)		He et al. 2009 TetraBDE (BDE47)	
59	Rat (Sprague-Dawley)	once Pnd 10 (GO)			1 (impaired learning and memory and 23% decrease in relative uterine weight at 2 months)		He et al. 2011 TetraBDE (BDE47)	
60	Rat (Wistar)	once Gd 6 (GO)			0.06[b] (increased activity and impaired spermatogenesis in adult offspring)		Kuriyama et al. 2005 PentaBDE (BDE99)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form	Comments
61	Rat (Wistar)	once Gd 6 (GO)		0.06	0.3 (reduced serum T4 in Pnd 22 offspring)		Kuriyama et al. 2007 PentaBDE (BDE99)	
62	Rat (CD)	10 d Gd 6-15 (GO)		2.5	10 (minimal increased post-implantation loss)		Life Science Research Israel Ltd. (1987) OctaBDE (FR-1208)	
63	Rat (Wistar)	once Gd 6 (GO)			0.06[b] F (ultrastructural changes in ovaries in F1 females at Pnd 90, increased resorptions in F1 females mated to unexposed males)		Talsness et al. 2005 PentaBDE (BDE99)	
64	Rat (Wistar)	once Gd 6 (GO)			0.14 F (reduced number of secondary ovarian follicles at Pnd 38 and ultrastructural changes in the ovary at Pnd 100 in offspring)		Talsness et al. 2008 TetraBDE (BDE47)	
65	Rat (Sprague-Dawley)	once Pnd 10 (G)		0.8 M	8 M (decreased spontaneous activity, impaired habituation)		Viberg et al. 2005 PentaBDE (BDE99)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
66	Rat (CD)	10 d Gd 6-15 (GO)		25	50 (reduced fetal weight and increased skeletal variations associated with maternal tox)		WIL Research Laboratories 1986 OctaBDE (DE-79)	
67	Rat (Long-Evans)	14 d Gd 6-20 (GO)		1	10 (reduced serum T4 in fetuses)		Zhou et al. 2002 PentaBDE (DE-71)	
68	Mouse (C57BL/6)	once Pnd 10 (GO)		10 M			Costa et al. 2015 TetraBDE (BDE47)	No exposure-related changes in serum T3 or T4.
69	Mouse (C57BL/6N)	once Pnd 10 (G)			6.8 M (decreased post-tetanic and long term potential in hippocampal slices at Pnd 17-19)		Dingemans et al. 2007 TetraBDE (BDE47)	
70	Mouse (NMRI)	once Pnd 10 (G)			0.8 M (altered spontaneous activity and habituation at 2-4 months)		Eriksson et al. 2001 PentaBDE (BDE99)	
71	Mouse (NMRI)	once Pnd 10 (G)		0.7 M	10.5 M (altered spontaneous activity and habituation at 2-4 months)		Eriksson et al. 2001 TetraBDE (BDE47)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form	Comments
72	Mouse (NMRI)	once Pnd 3 (G)			8 M (altered spontaneous activity and habituation at 4 months)		Eriksson et al. 2002b PentaBDE (BDE99)	
73	Mouse (NMRI)	once Pnd 10 (G)			8 M (altered spontaneous activity and habituation at 4 months)		Eriksson et al. 2002b PentaBDE (BDE99)	
74	Mouse (NMRI)	once Pnd 19 (G)		8 M			Eriksson et al. 2002b PentaBDE (BDE99)	No exposure-related alterations in spontaneous activity at 4 months.
75	Mouse (NMRI)	once Pnd 10 (G)		0.8 M	12 M (altered spontaneous activity and habituation at 4-6 months)		Eriksson et al. 2006 PentaBDE (BDE99)	
76	Mouse (NMRI)	once Pnd 10 (G)			0.8 M (decreased activity and impaired learning and memory during at 2-6 months)		Fischer et al. 2008 PentaBDE (BDE99)	
77	Mouse (C57BL/6N)	once Pnd 10 (GO)			1 M (increased motor activity at 4 months)		Gee and Moser 2008 TetraBDE (BDE47)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
78	Mouse (C57BL/6N)	once Pnd 10 (GO)		30 M			Gee et al. 2008 TetraBDE (BDE47)	No exposure-related changes in offspring body weight or serum T3 or T4 levels.
79	Mouse (NMRI)	once Pnd 10 (GO)			12 M (decreased spontaneous activity and impaired habituation at 2 months)		Hallgren et al. 2015 PentaBDE (BDE99)	
80	Mouse (C57BL/6)	once Pnd 10 (G)		0.4	0.8 (decreased total activity at 2 months)		Sand et al. 2004 PentaBDE (BDE99)	
81	Mouse (NMRI)	once Pnd 10 (G)			8 M (decreased spontaneous activity, altered habituation, and altered response to cholinergic agent at 2 months)		Viberg et al. 2002 PentaBDE (BDE99)	
82	Mouse (NMRI)	once Pnd 10 (G)			0.45 M (decreased spontaneous activity at 6 months)		Viberg et al. 2003a HexaBDE (BDE153)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form	Comments
83	Mouse (C57/BL)	once Pnd 10 (G)		0.4	0.8 (decreased spontaneous activity and impaired habituation at 2-8 months)		Viberg et al. 2004a PentaBDE (BDE99)	
84	Mouse (NMRI)	once Pnd 10 (G)		0.4 M	12 M (decreased spontaneous activity, impaired habituation, and decreased density of cholinergic nicotinic receptors in hippocampus at 4 months)		Viberg et al. 2004b PentaBDE (BDE99)	
85	Mouse (NMRI)	once Pnd 3 (G)			16.8 M (decreased spontaneous activity and impaired habituation at 2 months)		Viberg et al. 2006 OctaBDE (BDE203)	
86	Mouse (NMRI)	once Pnd 10 (G)			16.8 M (decreased spontaneous activity, impaired habituation, and impaired learning and memory at 2-3 months)		Viberg et al. 2006 OctaBDE (BDE203)	
87	Mouse (NMRI)	once Pnd 3 (G)			15.2 M (decreased spontaneous activity at 2 months)		Viberg et al. 2006 HeptaBDE (BDE183)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form	Comments
88	Mouse (NMRI)	once Pnd 10 (G)		15.2 M			Viberg et al. 2006 HeptaBDE (BDE183)	No change in spontaneous activity or habituation at 2 months.
89	Mouse (NMRI)	once Pnd 3 (G)		18.5 M			Viberg et al. 2006 NonaBDE (BDE206)	No changes in spontaneous motor behavior or habituation at 2 months.
90	Mouse (NMRI)	once Pnd 10 (G)			18.5 M (decreased spontaneous activity and impaired habituation at 2 months)		Viberg et al. 2006 NonaBDE (BDE206)	
91	Rabbit (New Zealand)	13 d Gd 7-19 (GO)		5 F	15 F (delayed ossification of sternebrae with decreased maternal weight gain)		Breslin et al. 1989 OctaBDE (technical)	
INTERMEDIATE EXPOSURE								
Systemic								
92	Rat (Sprague-Dawley)	5 wk Gd 6 - Pnd 21 (IN)	Endocr	1 F	10 F (reduced serum T4 levels)		Bansal et al. 2014 PentaBDE (DE-71)	Dams were given DE-71-dosed vanilla wafers.

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
93	Rat (Sprague-Dawley)	15 d 1 x/d (GO)	Endocr	3 M	30 M (follicular cell hypertrophy and hyperplasia in thyroid, reduced serum T3 and T4, increased serum TSH)		Becker et al. 2012 PentaBDE (DE-71)	Study 1 (conducted at ILS)
			Bd Wt	60 M				
94	Rat (Sprague-Dawley)	15 d 1 x/d (GO)	Hepatic		3 M (centrilobular hepatocyte hypertrophy)		Becker et al. 2012 PentaBDE (DE-71)	Study 2 (conducted at RTI)
			Endocr	3 M	30 M (decreased serum T3 and T4, increased serum TSH)			
			Bd Wt	60 M				
95	Rat (Sprague-Dawley)	21 wk Pmd 70 - Pnd 42 (GO)	Hepatic	0.5 M	5 M (hepatocellular hypertrophy)		Bondy et al. 2011, 2013 PentaBDE (DE-71)	
			Renal	25				
			Endocr	0.5 M	5 M (reduced serum T4)			
			Bd Wt	25				
96	Rat (Sprague-Dawley)	6 wk Gd 1 - Pnd 21 (IN)	Endocr	3 F	30 F (reduced serum T3 and T4)		Bowers et al. 2015 PentaBDE (DE-71)	Dams were given DE-71-dosed cookies.
			Bd Wt	30 F				

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
97	Rat (Wistar)	21 d 1 x/d (GO)	Hepatic		8 F (porphyria)		Bruchajzer 2011 PentaBDE (technical)	
			Bd Wt		8 F (decreased body weight gain)			
98	Rat (Wistar)	28 d 1 x/d (GO)	Hepatic	2 F	8 F (porphyria)		Bruchajzer 2011 PentaBDE (technical)	
			Bd Wt	2 F	8 F (8% decrease in body weight gain)			
99	Rat (Wistar)	21 d 1 x/d	Bd Wt	40 F	200 F (12% decrease in body weight gain)		Bruchajzer et al. 2010 PentaBDE (technical)	
100	Rat (Wistar)	28 d 1 x/d	Bd Wt	40 F	200 F (14% decrease in body weight gain)		Bruchajzer et al. 2010 PentaBDE (technical)	
101	Rat (Wistar)	21 d 1 x/d (GO)	Hepatic	2 F	8 F (porphyria)		Bruchajzer et al. 2012 OctaBDE (technical)	
			Bd Wt	8 F	40 F (9% decrease in body weight gain)			

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
102	Rat (Wistar)	28 d 1 x/d (GO)	Bd Wt	8 F	40 F (8% decrease in body weight gain)		Bruchajzer et al. 2012 OctaBDE (technical)	
103	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	1.77 M			Carlson 1980a PentaBDE (technical)	
104	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	14.1 M			Carlson 1980a PentaBDE (technical)	
105	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	2.4 M			Carlson, 1980a OctaBDE (technical)	
106	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	19.2 M			Carlson, 1980a OctaBDE (technical)	
107	Rat (Sprague-Dawley)	90 d 1 x/d (GO)	Hepatic	0.015 M			Daubie et al. 2011 PentaBDE (BDE99)	No exposure-related changes in hepatic clinical chemistry
			Bd Wt	0.015 M				
108	Rat (Long- Evans)	125 d Pnd 1-125 (F)	Endocr		17.5 M (reduced serum T4)		Driscoll et al. 2009 PentaBDE (DE-71)	
			Bd Wt	26.2 M				

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
109	Rat (Wistar)	18 wk Gd 6 - Pnw 16 5-7 d/wk (GO)	Hepatic		50 (hepatocellular hypertrophy and vacuolization)		Dunnick et al. 2012 PentaBDE (DE-71)	
			Endocr		50 (thyroid gland follicular hypertrophy)			
			Bd Wt	50 M	50 F (14% decrease in body weight)			
110	Rat (Sprague-Dawley)	33 d Gd 6 - Pnd 18 (GO)	Hepatic		18 F (reduced liver vitamin A)		Ellis-Hutchings et al. 2006 PentaBDE (DE-71)	
			Endocr		18 F (reduced serum T4)			
			Bd Wt	18 F				
111	Rat (Sprague-Dawley)	70 d (F)	Hepatic	20 M			Ernest et al. 2012 52.1% penta-, 44.2% deca-, 0.4% octa-BDE	No exposure-related changes in hepatic clinical chemistry.
			Endocr	2 M	20 M (reduced serum T4; increased epithelial thickness of inner follicles and vacuolation of the luminal apices of epithelial cells in thyroid)			
			Bd Wt	20 M				
			Metab	2 M	20 M (reduced serum glucose level)			

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
112	Rat (Sprague-Dawley)	28 d (G)	Hepatic	2.5	25 (decreased hepatic vitamin A content)		Fattore et al. 2001 PentaBDE (Bromkal 70-5DE)	
113	Rat (Sprague-Dawley)	4 wk 1 x/d (GO)	Endocr		14 M (reduced serum T4)		Hoppe and Carey 2007 PentaBDE (technical)	
			Bd Wt	14 M				
			Metab		14 M (increased adipocyte lipolysis, decreased adipocyte glucose oxidation)			
114	Rat (CD)	28 d (F)	Hepatic		9 (increased liver weight and enlarged parenchymal cells)		IRDC 1976 PentaBDE (technical)	
			Renal	90				
			Endocr	90				
			Bd Wt	90				
115	Rat (CD)	28 d (F)	Hepatic		9 (increased liver weight and enlarged parenchymal cells)		IRDC 1976 OctaBDE (technical)	
			Renal	90				
			Endocr	90				
			Bd Wt	90				

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
116	Rat (CD)	13 wk (F)	Resp	750 F			IRDC 1977 OctaBDE (technical)	
			Cardio	750 F				
			Gastro	750 F				
			Hemato	70 F	750 F (reduced erythrocytes, hematocrit and hemoglobin)			
			Hepatic		5 M (cytomegaly with vacuolation and necrosis at higher doses)			
			Renal	50 M	600 M (minimal increase in tubular degenerative changes)			
			Endocr	7 F	50 M (increased thyroid weight with follicular epithelial changes at higher doses)			
			Dermal	750 F				
			Ocular	750 F				
			Bd Wt	70 F	600 M (12% reduced body weight gain)			
117	Rat (Long- Evans)	36 d Gd 6 - Pnd 21 (GO)	Endocr	1.7 F	10.2 F (reduced serum T4)		Kodavanti et al. 2010 PentaBDE (DE-71)	
			Bd Wt	30.6 F				

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
118	Rat (Sprague-Dawley)	28 d 1 x/d (GO)	Resp	250			Oberg et al. 2010 PentaBDE (Bromkal 70-5DE)	
			Cardio	250				
			Hemato	250				
			Hepatic	2.5	25 (centrilobular hypertrophy, reduced vitamin A content in liver)			
			Renal	250				
			Bd Wt	250				
			Metab	25	250 (hypercalcemia, magnesemia, and phosphatemia in males; hyperatremia and hypokalemia in females)			
119	Rat (Long- Evans)	~11 wk Pmd 28 - Pnd 21 (IN)	Bd Wt	11.4 F			Poon et al. 2011 PentaBDE (DE-71)	Rats were given pentaBDE-dosed vanilla wafers.
120	Rat (Wistar)	20 d Pnd 22-41 (GO)	Endocr	3 F	30 F (reduced serum T4)		Stoker et al. 2004 PentaBDE (DE-71)	
			Bd Wt	60 F				

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
121	Rat (Wistar)	31 d Pnd 23-53 (GO)	Endocr		3 M (reduced serum T4)		Stoker et al. 2004 PentaBDE (DE-71)	
			Bd Wt	60 M				
122	Rat (Wistar)	31 d Pnd 23-53 (GO)	Endocr		60 M (reduced serum T4)		Stoker et al. 2005 PentaBDE (DE-71)	
			Bd Wt	120 M				

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
123	Rat (Wistar)	28 d (G)	Resp	200			Van der Ven et al. 2008b PentaBDE (DE-71)	NOAEL values for Bd Wt, Hepatic, Endocr, and Metab effects are BMDL(RD10%) values for decreased Bd Wt, vitamin A in liver, serum T4 and glucose.
			Cardio	200				
			Gastro	200				
			Musc/skel	200				
			Hepatic	0.05 M				
			Renal	200				
			Endocr	1.1 M				
			Dermal	200				
			Bd Wt	9.7 M				
			Metab	66.7 M				
124	Rat (Wistar)	34 d Gd 1 - Pnd 14 (F)	Endocr		3.2 F (reduced serum T4)		Wang et al. 2011a TetraBDE (BDE47)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
125	Rat (Sprague-Dawley)	90 d (F)	Resp	100			WIL Research Laboratories 1984 PentaBDE (DE-71)	
			Cardio	100				
			Gastro	100				
			Hemato	100				
			Musc/skel	100				
			Hepatic		2	(hypertrophy, mild degeneration, and slight necrosis)		
			Renal	100				
			Endocr	2	10	(reduced serum T4)		
			Dermal	100				
			Ocular	100				
			Bd Wt	10	100	(reduced weight gain)		
126	Rat (Sprague-Dawley)	30 d 1 x/d (GO)	Bd Wt	1 M			Yan et al. 2012 TetraBDE (BDE47)	
127	Rat (Sprague-Dawley)	6 wk Gd 1 - Pnd 21 (GO)	Bd Wt	0.2 F			Zhao et al. 2014 PentaBDE (BDE99)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
128	Rat (Long-Evans)	36 d Gd 6 - Pnd 21 (GO)	Endocr	10 F	30 F (reduced maternal serum T4)		Zhou et al. 2002 PentaBDE (DE-71)	
			Bd Wt	30 F				
129	Mouse (CD-1)	36 d Gd 6 - Pnd 21	Bd Wt	18 F			Branchi et al. 2005 PentaBDE (BDE99)	Administered via "self-administration" from a modified syringe or gavage.
130	Mouse (C57BL/6J)	70-80 d Pmd 28 - Pnd 21 (IN)	Bd Wt	1 F			Koenig et al. 2012 TetraBDE (BDE47)	Mice were fed 1-2 tetraBDE dosed cornflakes.
131	Mouse (BALB/c)	28 d (F)	Hepatic		0.45 F (hepatocyte vacuolation, pyknotic nuclei in the hepatocytes, periportal lymphocytic infiltration)		Maranghi et al. 2013 TetraBDE (BDE47)	
			Endocr		0.45 F (cellular debris in the follicular lumen of thyroid; increased serum testosterone and E2)			
			Bd Wt	0.45 F				

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
132	Mouse (NS)	6 wk 5 d/wk (GO)	Hepatic	1 M			McIntyre et al. 2015 TetraBDE (BDE47)	No exposure-related changes in glucose tolerance, insulin resistance, lipogenesis, or liver weight or histology.
			Bd Wt	1 M				
			Metab	1 M				
133	Mouse (NMRI)	29 d Gd 4 - Pnd 17 ~every 3 d (GO)	Endocr	452 F			Skarman et al. 2005 PentaBDE (BDE99)	BDE-99; No change in maternal serum T4 levels.
			Bd Wt	452 F				
134	Mouse (NMRI)	29 d Gd 4 - Pnd 17 ~every 3 d (GO)	Endocr	452 F			Skarman et al. 2005 PentaBDE (Bromkal 70-5DE)	Bromkal 70-5DE; no change in maternal serum T4 levels.
			Bd Wt	452 F				
135	Mouse (C57BL/6J)	70-80 d Pmd 28 - Pnd 21 (IN)	Bd Wt	1 F			Ta et al. 2011 TetraBDE (BDE47)	Dams were fed tetraBDE-dosed cornflakes.
136	Mouse (C56BL/6)	30 d (GO)	Bd Wt	30 M			Wang et al. 2013 TetraBDE (BDE47)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
137	Mouse (ICR)	28 d 1 x/d (GO)	Hepatic		1.2 M (swollen hepatic cells)		Zhang et al. 2014 DiBDE (BDE15)	
			Renal		1.2 M (10% decrease in relative kidney weight)			
			Bd Wt	1.2 M				
138	Mouse (ICR)	12 wk 7 d/wk (GO)	Hepatic		150 M (increased relative liver weight, increased serum ALT, hepatocyte hypertrophy and vacuolization and inflammatory cell infiltration)		Zhang et al. 2015b TetraBDE (BDE47)	
			Bd Wt	150 M				
139	Mouse (ICR)	12 wk 7 d/wk (GO)	Hepatic		150 M (increased relative liver weight, increased serum ALT, hepatocyte hypertrophy and vacuolization and inflammatory cell infiltration)		Zhang et al. 2015a TetraBDE (BDE47)	
			Bd Wt	150 M				
140	Mink (NS)	9 wk (F)	Bd Wt	0.08 M	0.63 M (21% decrease in body weight)		Martin et al. 2007 PentaBDE (DE-71)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
141	Mink (NS)	16-17 wk Pmw 4 - Pnw 6 (F)	Bd Wt	0.31 F			Zhang et al. 2009 PentaBDE (DE-71)	
142	Mink (NS)	43-44 wk Pmw 4 - Pnw 33 (F)	Bd Wt	0.06			Zhang et al. 2009 PentaBDE (DE-71)	
Immuno/ Lymphoret								
143	Rat (Sprague-Dawley)	21 wk Pmd 70 - Pnd 42 (GO)		25			Bondy et al. 2011, 2013 PentaBDE (DE-71)	No exposure-related changes in spleen or thymus weight or histology.
144	Rat (CD)	28 d (F)		90			IRDC 1976 PentaBDE (technical)	No exposure-related changes in spleen weight or spleen or thymus histology.
145	Rat (CD)	28 d (F)		90			IRDC 1976 OctaBDE (technical)	No exposure-related changes in spleen weight or spleen or thymus histology.
146	Rat (CD)	28 d (F)		90			IRDC 1976 PentaBDE (technical)	No exposure-related changes in spleen weight or spleen or thymus histology.

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
147	Rat (CD)	13 wk (F)		750 F			IRDC 1977 OctaBDE (technical)	No exposure-related changes in spleen weight or spleen or thymus histology.
148	Rat (Sprague-Dawley)	28 d 1 x/d (GO)		250			Oberg et al. 2010 PentaBDE (Bromkal 70-5DE)	No exposure-related changes in spleen or thymus weight or histology.
149	Rat (Sprague-Dawley)	90 d (F)		100			WIL Research Laboratories 1984 PentaBDE (DE-71)	No exposure-related changes in thymus weight or spleen or thymus histology.
150	Mouse (BALB/c)	28 d (F)			0.45 F (follicular hyperplasia and lymphocytic infiltration in spleen; lymphocytic apoptosis and Hassal's bodies in thymus)		Maranghi et al. 2013 TetraBDE (BDE47)	
151	Mink (NS)	9 wk (F)		0.08 M	0.63 M (spleen hyperplasia)		Martin et al. 2007 PentaBDE (DE-71)	Immune function was not altered (KLH antibody induction, PHA skin challenge).

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Neurological								
152	Rat (Sprague-Dawley)	90 d 1 x/d (GO)		0.015 M			Daubie et al. 2011 PentaBDE (BDE99)	No exposure-related changes in elevated plus-maze, open-field, or Morris water maze test.
153	Rat (Long- Evans)	125 d Pnd 1-125 (F)		17.5 M	26.2 M (impaired attention and inhibitory control)		Driscoll et al. 2009 PentaBDE (DE-71)	
154	Rat (Sprague-Dawley)	28 d 1 x/d (GO)		250			Oberg et al. 2010 PentaBDE (Bromkal 70-5DE)	No exposure-related changes in brain weight or histology.
155	Rat (Wistar)	28 d (G)		200			Van der Ven et al. 2008b PentaBDE (DE-71)	No exposure-related changes in brain weight or histology.
156	Rat (Sprague-Dawley)	30 d 1 x/d (GO)			0.1 M (impaired learning and memory; decreased glutamate and receptor density in hippocampus)		Yan et al. 2012 TetraBDE (BDE47)	
157	Mink (NS)	19 wk Pmd 28 - Pnw 6 (F)		0.25 F			Bull et al. 2007 PentaBDE (DE-71)	No maternal cholinergic effects were observed.

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
158	Rat (Sprague-Dawley)	15 d 1 x/d (GO)		30 M	60 M (67% increase in serum prolactin; dose-related increase in serum testosterone and FSH)		Becker et al. 2012 PentaBDE (DE-71)	Study 1 (conducted at ILS)
159	Rat (Sprague-Dawley)	15 d 1 x/d (GO)		60 M			Becker et al. 2012 PentaBDE (DE-71)	Study 2 (conducted at RTI); no dose-related changes in reproductive organ weight, histopathology, or serum reproductive hormone levels.
160	Rat (Sprague-Dawley)	21 wk Pmd 70 - Pnd 42 (GO)		25			Bondy et al. 2011, 2013 PentaBDE (DE-71)	No treatment-related changes in the number of pregnant females, litters, or litter size.
161	Rat (Sprague-Dawley)	6 wk Gd 1 - Pnd 21 (IN)		30 F			Bowers et al. 2015 PentaBDE (DE-71)	Dams were given DE-71-dosed cookies. No treatment-related changes in no. of implants, no. of litters, litter size, or sex ratio.

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
162	Rat (Sprague-Dawley)	36 d Gd 6 - Pnd 21 (GO)		2 F			Cheng et al. 2009 PentaBDE (BDE99)	No exposure-related changes in number of litters, litter size, or sex ratio.
163	Rat (Sprague-Dawley)	70 d (F)		20 M			Ernest et al. 2012 52.1% penta-, 44.2% deca-, 0.4% octa-BDE	No exposure-related changes in reproductive organ weight, testicular histology, or sperm parameters.
164	Rat (Sprague-Dawley)	8 wk (GO)		0.001 M	0.03 M (increased epithelial thickness in testes, spermatocyte apoptosis)		Huang et al. 2015 TetraBDE (BDE47)	
165	Rat (CD)	28 d (F)		90			IRDC 1976 PentaBDE (technical)	No exposure-related changes in reproductive organ weight or histology.
166	Rat (CD)	28 d (F)		90			IRDC 1976 OctaBDE (technical)	No exposure-related changes in reproductive organ weight or histology.

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
167	Rat (CD)	13 wk (F)		600 M 750 F			IRDC 1977 OctaBDE (technical)	No exposure-related changes in reproductive organ weight or histology
168	Rat (Sprague-Dawley)	28 d 1 x/d (GO)		250			Oberg et al. 2010 PentaBDE (Bromkal 70-5DE)	No exposure-related changes in reproductive organ weight or histology.
169	Rat (Long- Evans)	~11 wk Pmd 28 - Pnd 21 (IN)		11.4 F			Poon et al. 2011 PentaBDE (DE-71)	Rats were given pentaBDE-dosed vanilla wafers; no change in number of pregnancies, implantation sites, or live pups, litter size, or sex ratio.
170	Rat (Wistar)	20 d Pnd 22-41 (GO)		30 F	60 F (delayed vaginal opening)		Stoker et al. 2004 PentaBDE (DE-71)	
171	Rat (Wistar)	31 d Pnd 23-53 (GO)		3 M	30 M (delayed preputial separation)		Stoker et al. 2004 PentaBDE (DE-71)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
172	Rat (Wistar)	31 d Pnd 23-53 (GO)			60 M (delayed PPS, 22-28% decrease in prostate and seminal vesicle weights)		Stoker et al. 2005 PentaBDE (DE-71)	
173	Rat (Wistar)	28 d (G)		9.6 M 200 F			Van der Ven et al. 2008b PentaBDE (DE-71)	Male NOAEL is a BMDL(RD10%) for increased % of deformed sperm heads; no exposure-related changes in female reproductive organ weights or histology.
174	Rat (Sprague-Dawley)	90 d (F)		100			WIL Research Laboratories 1984 PentaBDE (DE-71)	No exposure-related changes in reproductive organ weight or histology.
175	Rat (Sprague-Dawley)	8 wk 6 d/wk (GO)			0.001[c] M (34% reduction in serum testosterone)		Zhang et al. 2013b TetraBDE (BDE47)	
176	Rat (Sprague-Dawley)	6 wk Gd 1 - Pnd 21 (GO)		0.2 M			Zhao et al. 2014 PentaBDE (BDE99)	No exposure-related changes in gestational lengths, litter sizes, sex ratio, or live births.

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
177	Rat (Long-Evans)	36 d Gd 6 - Pnd 21 (GO)		30 F			Zhou et al. 2002 PentaBDE (DE-71)	No change in the gestation length, litter size, or sex ratio
178	Mouse (CD-1)	36 d Gd 6 - Pnd 21		18 F			Branchi et al. 2005 PentaBDE (BDE99)	Administered via "self-administration" from a modified syringe or gavage; No change in gestation length, litter size, # live pups, or sex ratio.
179	Mouse (C57BL/6J)	70-80 d Pmd 28 - Pnd 21 (IN)		1 F			Koenig et al. 2012 TetraBDE (BDE47)	Mice were fed 1-2 tetraBDE dosed cornflakes; no change in the number of pregnancies, size of litter, or sex ratio of pups.
180	Mouse (BALB/c)	28 d (F)			0.45 F (increased serum testosterone and E2)		Maranghi et al. 2013 TetraBDE (BDE47)	
181	Mouse (BALB/c)	34 d 1 x/d (GO)		50 F			Mercado-Feliciano and Bigsby 2008a PentaBDE (DE-71)	Mice were ovariectomized 3 weeks prior to exposure; no change in uterus weight or uterus/vaginal histology.

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
182	Mouse (NMRI)	29 d Gd 4 - Pnd 17 ~every 3 d (GO)		452 F			Skarman et al. 2005 PentaBDE (BDE99)	BDE-99; no change in maternal serum T4 levels.
183	Mouse (NMRI)	29 d Gd 4 - Pnd 17 ~every 3 d (GO)		452 F			Skarman et al. 2005 PentaBDE (Bromkal 70-5DE)	Bromkal 70-5DE; no changes in pregnancy rate, gestation length, or litter size.
184	Mouse (C57BL/6J)	70-80 d Pmd 28 - Pnd 21 (IN)		1 F			Ta et al. 2011 TetraBDE (BDE47)	Dams were fed tetraBDE-dosed cornflakes; no change in gestation length, litter size, or sex ratio.
185	Mouse (C56BL/6)	30 d (GO)		0.0015 M	0.045 M (germ cell loss and apoptosis in testes)		Wang et al. 2013 TetraBDE (BDE47)	
186	Mouse (C57BL/6)	10 wk Pmd 28 - Pnd 21 (GO)		0.03 F	0.1 F (58% decrease in litters surviving until Pnd 8)		Woods et al. 2012 TetraBDE (BDE47)	Females carrying on copy of a truncated Mecp2 gene were mated to unexposed wild-type males; LSE values are based on the wild-type offspring only.

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
187	Mink (NS)	19 wk Pmd 28 - Pnw 6 (F)		0.05 F	0.25 F (no litters produced)		Bull et al. 2007 PentaBDE (DE-71)	
188	Mink (NS)	16-17 wk Pmw 4 - Pnw 6 (F)		0.06 F		0.31 F (complete litter loss)	Zhang et al. 2009 PentaBDE (DE-71)	
Developmental								
189	Rat (Sprague-Dawley)	5 wk Gd 6 - Pnd 21 (IN)		1 M	10 M (reduced serum T4 in Pnd 21 offspring)		Bansal et al. 2014 PentaBDE (DE-71)	Dams were given DE-71-dosed vanilla wafers.
190	Rat (Sprague-Dawley)	36 d Gd 6 - Pnd 21 (GO)		1	2 (altered neurobehavior, decreased hippocampal BDNF, and decreased serum T3, T4, and free T4 in offspring at Pnd 21-23)		Blanco et al. 2013 PentaBDE (BDE99)	
191	Rat (Sprague-Dawley)	21 wk Pmd 70 - Pnd 42 (GO)		0.5 M	5 M (decreased serum T4 in male offspring on Pnd 43)		Bondy et al. 2011, 2013 PentaBDE (DE-71)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
192	Rat (Sprague-Dawley)	6 wk Gd 1 - Pnd 21 (IN)		0.3	3 (reduced serum T3 and T4 in Pnd 21 offspring)		Bowers et al. 2015 PentaBDE (DE-71)	Dams were given DE-71-dosed cookies.
193	Rat (Sprague-Dawley)	36 d Gd 6 - Pnd 21 (GO)			2 M (delayed appearance of reflexes, impaired learning/memory at Pnd 36-37, and oxidative stress in the hippocampus at Pnd 37)		Cheng et al. 2009 PentaBDE (BDE99)	
194	Rat (Long- Evans)	7 d Pnd 6-12 (GO)		15 M			Driscoll et al. 2012 PentaBDE (DE-71)	No learning or attention deficits at Pnd 40-95.
195	Rat (Sprague-Dawley)	33 d Gd 6 - Pnd 18 (GO)			18 (decreased serum T4 in offspring)		Ellis-Hutchings et al. 2006 PentaBDE (DE-71)	Half of the dams in each group were maintained on a vitamin A deficient diet.
196	Rat (Long- Evans)	36 d Gd 6 - Pnd 21 (GO)		1.7	10.2 (reduced serum T4 in offspring at Pnd 7 and 14, reduced mammary gland development at Pnd 21, reduced female body weight from Pnd 29-58)		Kodavanti et al. 2010 PentaBDE (DE-71)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
197	Rat (Long- Evans)	36 d Gd 6 - Pnd 21 (GO)		0.96 M	2.85 M (reduced serum T4 in male offspring at Pnd 7-21)		Miller et al. 2012 PentaBDE (DE-71)	
198	Rat (Long- Evans)	36 d Gd 6 - Pnd 21 (GO)			11.2 (reduced serum T4 in offspring at Pnd 7-21)		Miller et al. 2012 PentaBDE (DE-71)	
199	Rat (Long- Evans)	~11 wk Pmd 28 - Pnd 21 (IN)			5.7 (reduced serum T4)		Poon et al. 2011 PentaBDE (DE-71)	Rats were given pentaBDE-dosed vanilla wafers.
200	Rat (Long- Evans)	36 d Gd 6 - Pnd 21 (GO)			1.7 M (hypertensive reaction to hyperosmotic stress in adult male offspring)		Shah et al. 2011 PentaBDE (DE-71)	
201	Rat (Long- Evans)	36 d Gd 6 - Pnd 21 (GO)		1.7	10.2 (transient reduction in serum T4 in offspring at Pnd 4 and 21)		Szabo et al. 2009 PentaBDE (DE-71)	
202	Rat (Wistar)	34 d Gd 1 - Pnd 14 (F)			3.2 (reduced serum T4 in offspring at Pnd 7 and 14)		Wang et al. 2011a TetraBDE (BDE47)	

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
203	Rat (Sprague-Dawley)	6 wk Gd 1 - Pnd 21 (GO)		0.2 M			Zhao et al. 2014 PentaBDE (BDE99)	No exposure-related changes in reflex maturation, motor coordination, or spatial learning of Pnd 3-36 offspring; no offspring body weight effects.
204	Rat (Long-Evans)	36 d Gd 6 - Pnd 21 (GO)		1	10 (reduced serum T4 in offspring on Pnd 4 and 14)		Zhou et al. 2002 PentaBDE (DE-71)	
205	Mouse (CD-1)	36 d Gd 6 - Pnd 21			18 M (transient alterations in open-field behavior of offspring at Pnd 34)		Branchi et al. 2005 PentaBDE (BDE99)	Administered via "self-administration" from a modified syringe or gavage; data from both groups were pooled for neurodevelopmental endpoints.
206	Mouse (CD-1)	5 wk Gd 8 - Pnd 21 (GO)		0.2 M			Kim et al. 2015 TetraBDE (BDE47)	No exposure-related changes in offspring body weight, motor activity at Pnd 21, or social interaction at Pnd 70.
207	Mouse (C57BL/6J)	70-80 d Pmd 28 - Pnd 21 (IN)			0.03 (impaired learning in offspring at Pnw 8)		Koenig et al. 2012 TetraBDE (BDE47)	Mice were fed 1-2 tetraBDE dosed cornflakes.

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
208	Mouse (NMRI)	29 d Gd 4 - Pnd 17 ~every 3 d (GO)		452			Skarman et al. 2005 PentaBDE (BDE99)	BDE-99; no changes in offspring body weight or serum T4 levels
209	Mouse (NMRI)	29 d Gd 4 - Pnd 17 ~every 3 d (GO)			452 (reduced serum T4 in offspring at Pnd 11)		Skarman et al. 2005 PentaBDE (Bromkal 70-5DE)	Bromkal 70-5DE
210	Mouse (C57BL/6J)	70-80 d Pmd 28 - Pnd 21 (IN)			0.03 F (decreased center-field activity in open field in female offspring at Pnd 60)		Ta et al. 2011 TetraBDE (BDE47)	Dams were fed tetraBDE-dosed cornflakes.
211	Mouse (C57BL/6)	10 wk Pmd 28 - Pnd 21 (GO)			0.03 F (decreased pre-weaning weight; decreased pup vocalizations on Pnd 8-10, decreased sociability on Pnd 72)		Woods et al. 2012 TetraBDE (BDE47)	Females carrying on copy of a truncated Mecp2 gene were mated to unexposed wild-type males; LSE values are based on the wild-type offspring only.
212	Mink (NS)	19 wk Pmd 28 - Pnw 6 (F)		0.05			Bull et al. 2007 PentaBDE (DE-71)	No cholinergic effects in 6-week-old offspring.

Table 3-2 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
213	Mink (NS)	40 wk Pmd 28 - Pnw 27 (F)		0.05			Bull et al. 2007 PentaBDE (DE-71)	No cholinergic effects in 45-week-old offspring.
214	Mink (NS)	16-17 wk Pmw 4 - Pnw 6 (F)		0.06			Zhang et al. 2009 PentaBDE (DE-71)	No change in body weight, organ weights, plasma T3/T4, hepatic enzyme activity, or thyroid histology in offspring at weaning (Pnw 6).

a The number corresponds to entries in Figure 3-2.

b Three studies were used to derive an acute oral minimal risk level (MRL); concentration divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for animal to human, and 10 for human variability), resulting in an MRL of 0.00006 mg/kg/day.

c Used to derive an intermediate oral minimal risk level (MRL); concentration divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for animal to human, 10 for human variability), resulting in an MRL of 0.000003 mg/kg/day.

Note on chemical form: Mixtures are identified by composition or trade name (if reported); otherwise, they are reported as "technical". Trade names include Bromkal 70, Bromakal 70-5 DE, DE-71, and Saytex 115 for pentaBDE mixtures and DE-79, FR-1208, and Saytex 111 for octaBDE mixtures. For the studies by Bruchajzer (2011) and Bruchajzer et al. (2010, 2011, 2012), the mixtures were made to resemble formerly used commercial mixtures. The pentaBDE mixture was composed of 63.2% pentaBDE, 21.4% tetraBDE, 15.4% hexaBDE, and 0.04% heptaBDE; the octaBDE mixture was composed of 65.7% octaBDE, 14.8% heptaBDE, 1.7% hexaBDE, and 17.8% nona- and deca-BDE. Individual congeners are identified by IUPAC number: BDE 15 = 4,4'-diBDE; BDE 47 = 2,2',4,4'-tetraBDE; BDE 99 = 2,2',4,4',5-pentaBDE; BDE 153 = 2,2',4,4',5,5'-hexaBDE; BDE 183 = 2,2',3,4,4',5',6-heptaBDE; BDE 203 = 2,2',3,4,4',5,5',6-octaBDE; BDE 206 = 2,2',3,3',4,4',5,5',6-nonaBDE

BDNF = brain derived neurotrophic factor; Bd Wt = body weight; BMDL = benchmark dose lower confidence limit; Cardio = cardiovascular; d = day(s); E2 = estradiol; Endocr = endocrine; (F) = feed; F = Female; FSH = follicle stimulating hormone; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; (GO) = gavage in oil; Hemato = hematological; IgG = immunoglobulin G; Immuno/Lymphoret = immunological/lymphoreticular; IN = ingestion; LH = luteinizing hormone; KLH = keyhole limpet hemocyanin; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Metab = metabolism; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; PHA = phytohemagglutinin; Pmd = pre-mating day; Pmw = pre-mating week; Pnd = post-natal day; Pnw = post-natal week; PPS = preputial separation; Resp = respiratory; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone; x = time(s); wk = week(s)

Figure 3-2. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral

Acute (≤14 days)

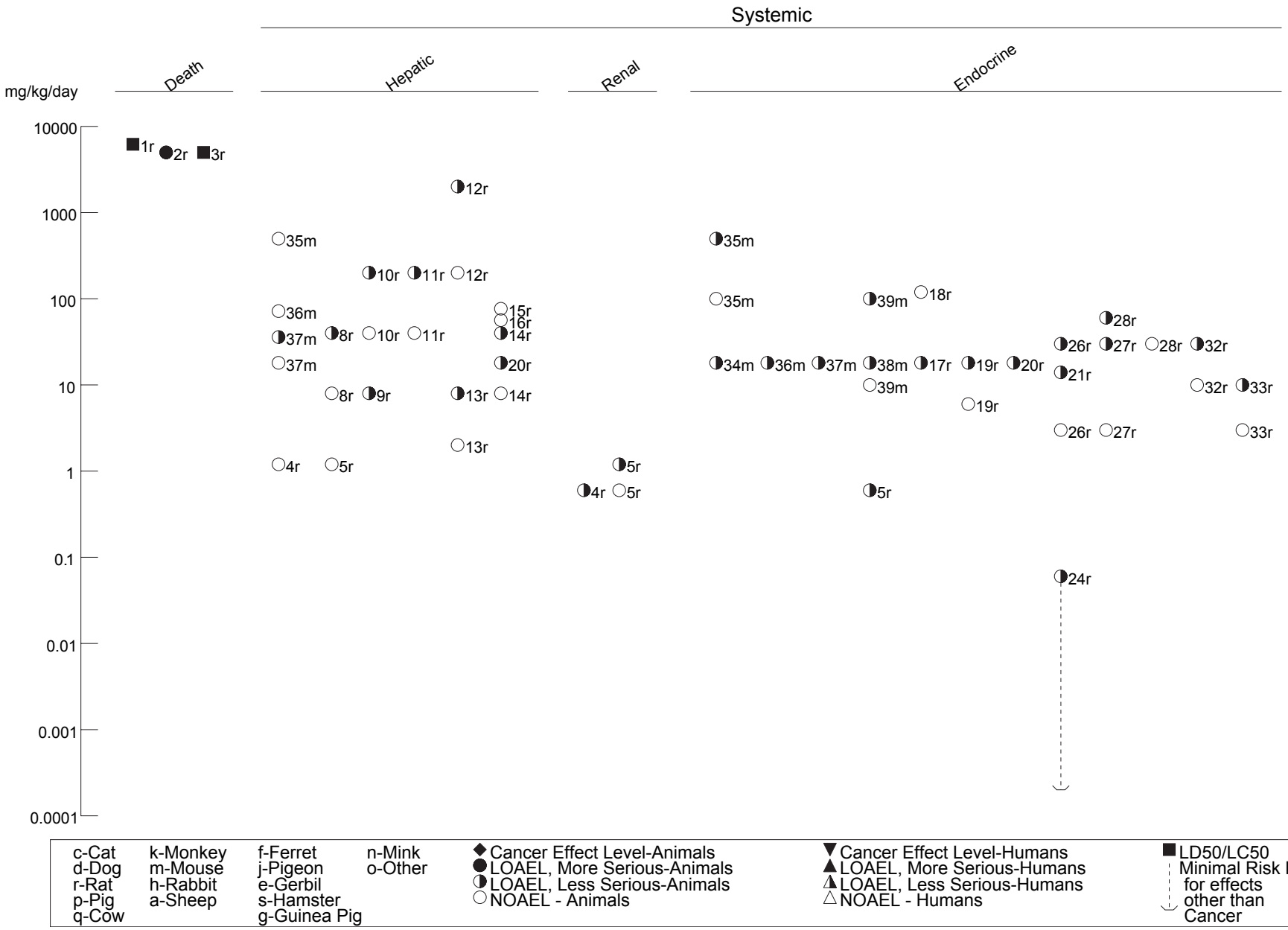


Figure 3-2. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral *(Continued)*

Acute (≥14 days)

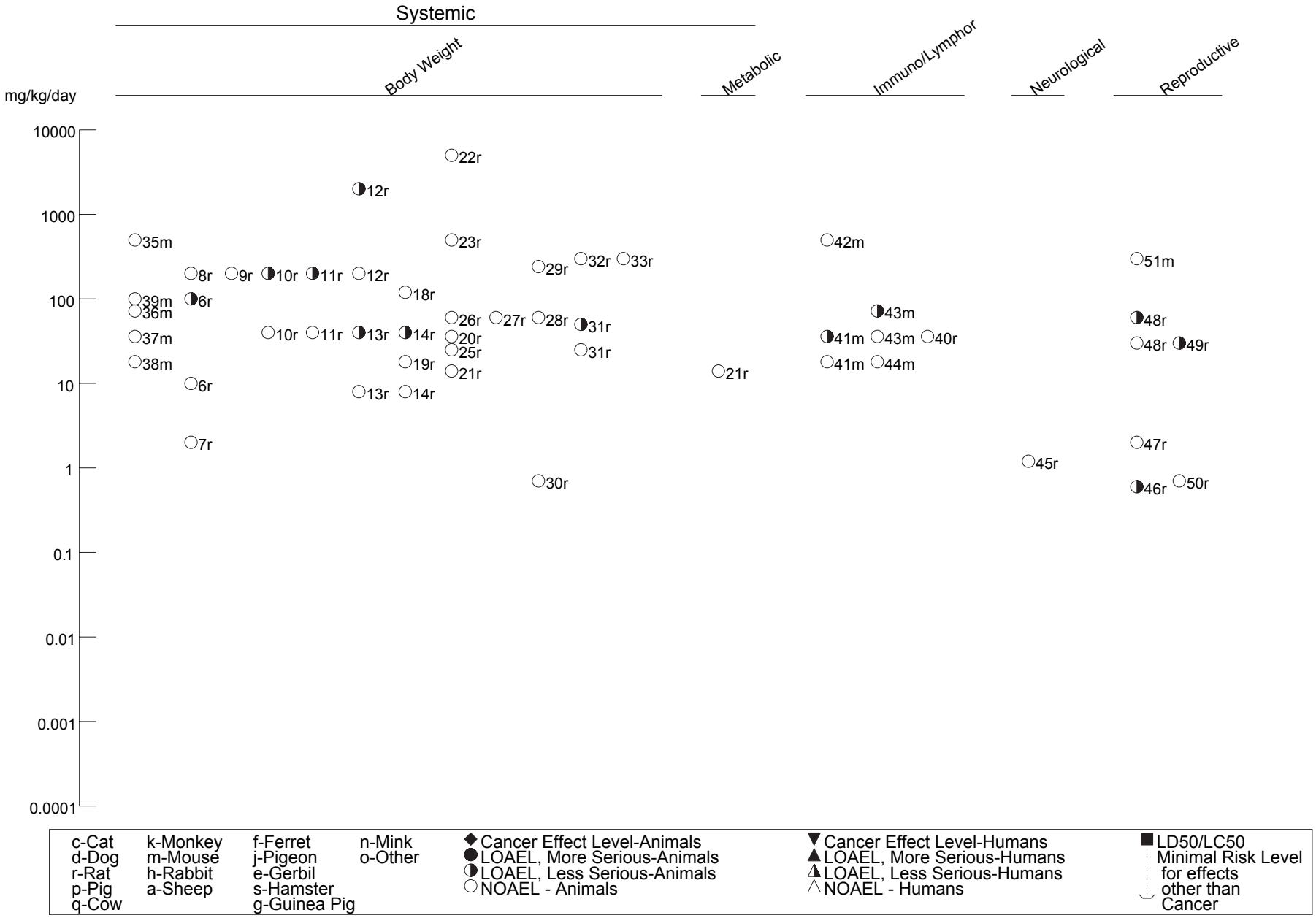


Figure 3-2. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral *(Continued)*

Acute (≤14 days)

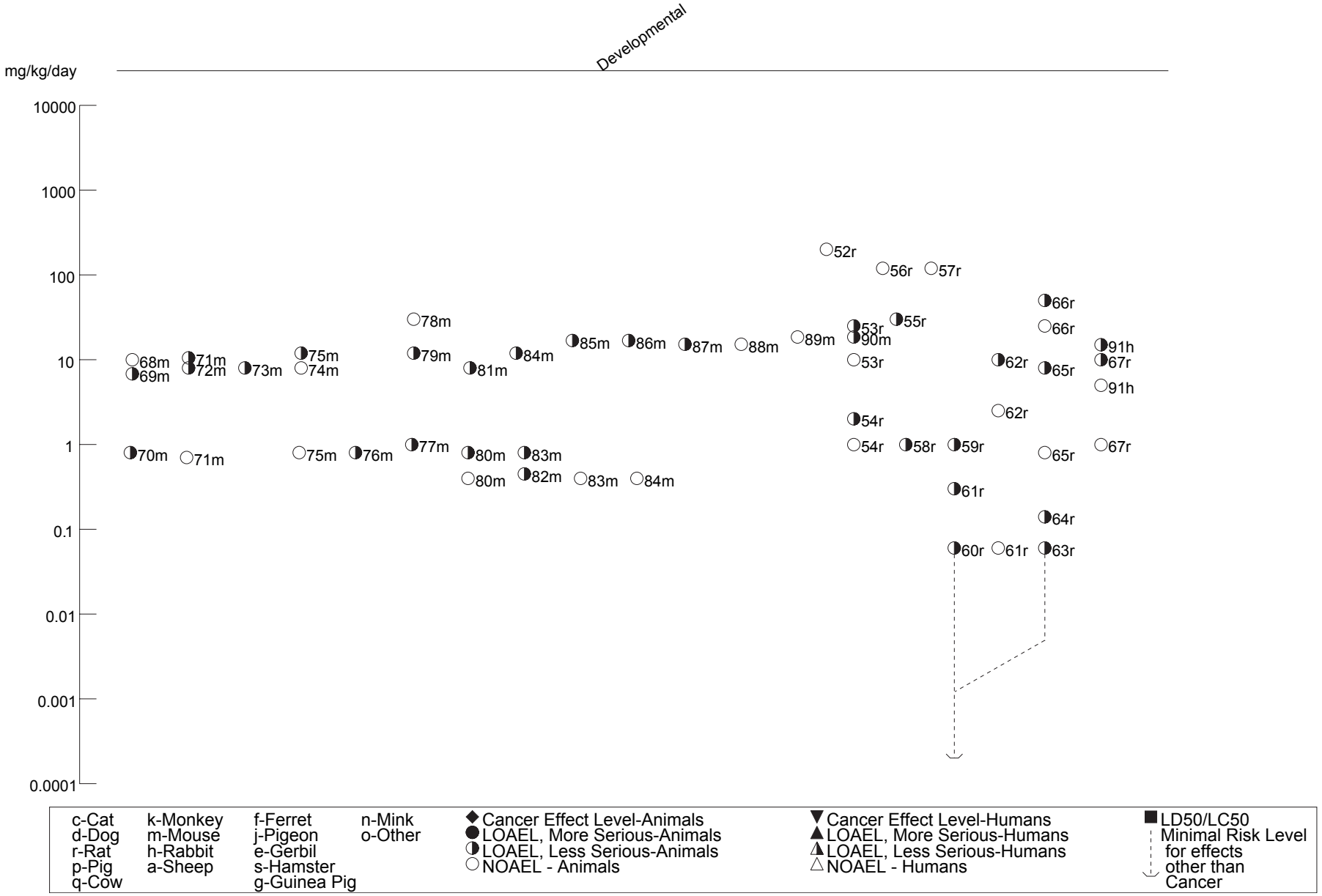


Figure 3-2. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral *(Continued)*

Intermediate (15-364 days)

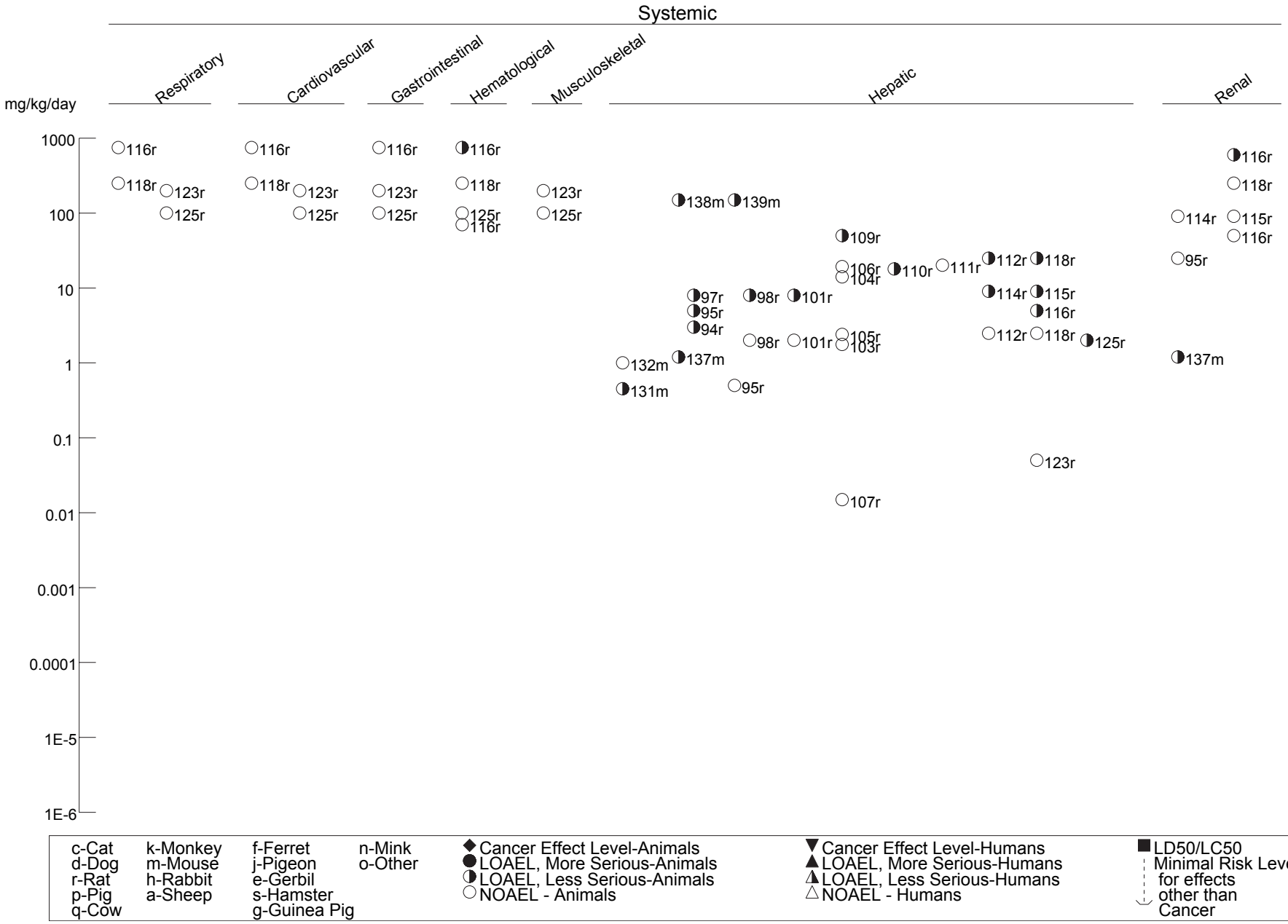


Figure 3-2. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral *(Continued)*

Intermediate (15-364 days)

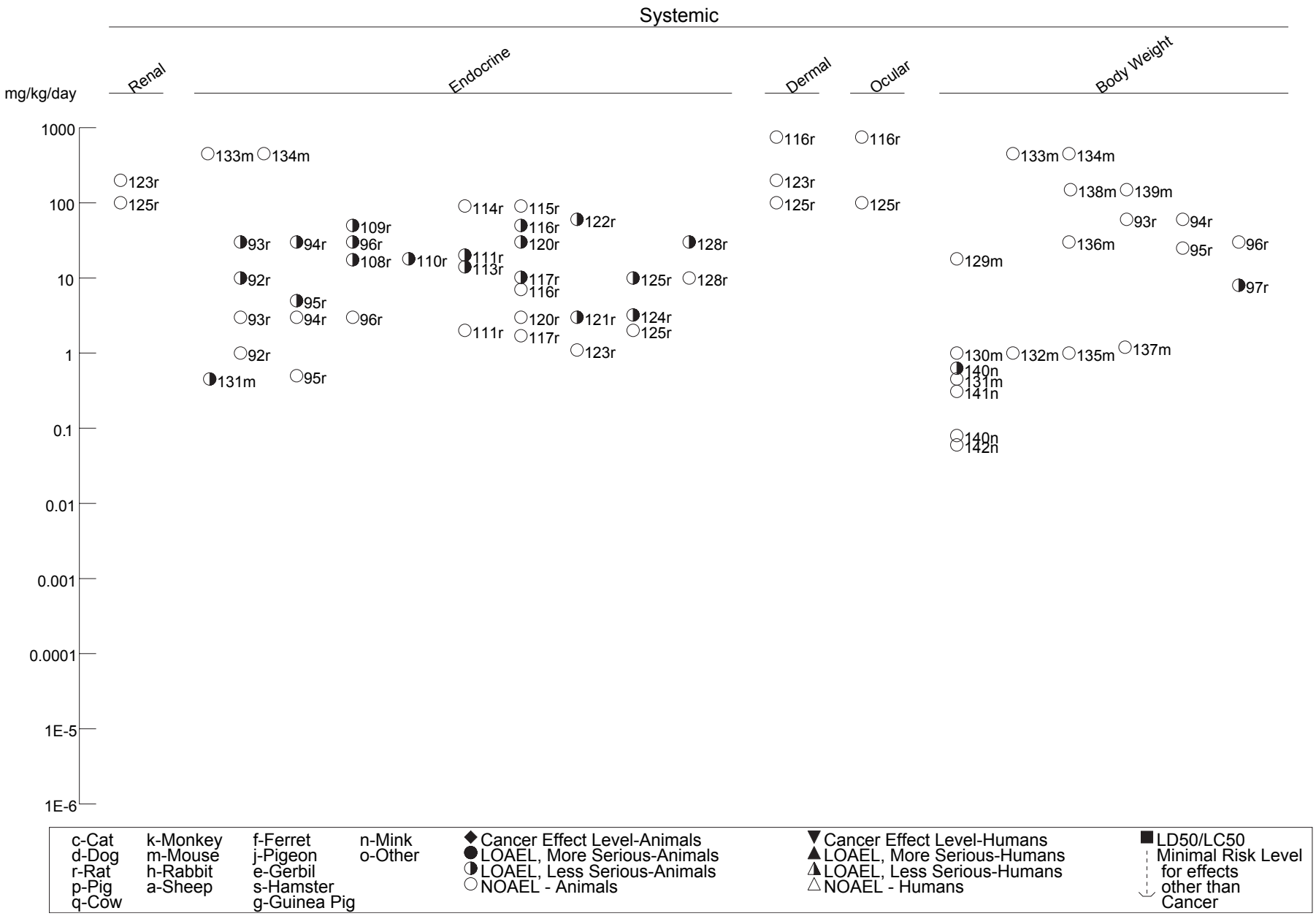


Figure 3-2. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral *(Continued)*

Intermediate (15-364 days)

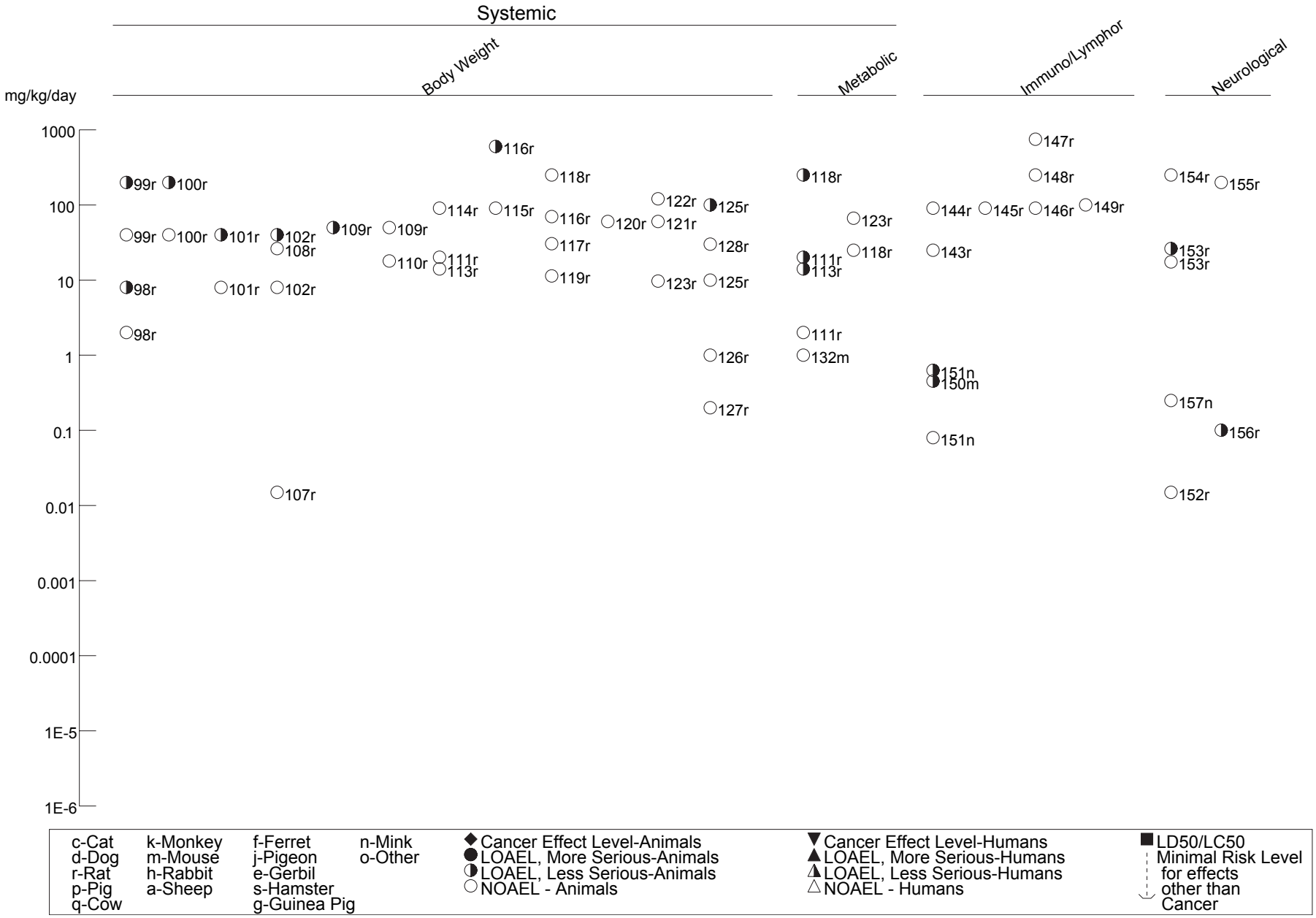


Figure 3-2. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral *(Continued)*

Intermediate (15-364 days)

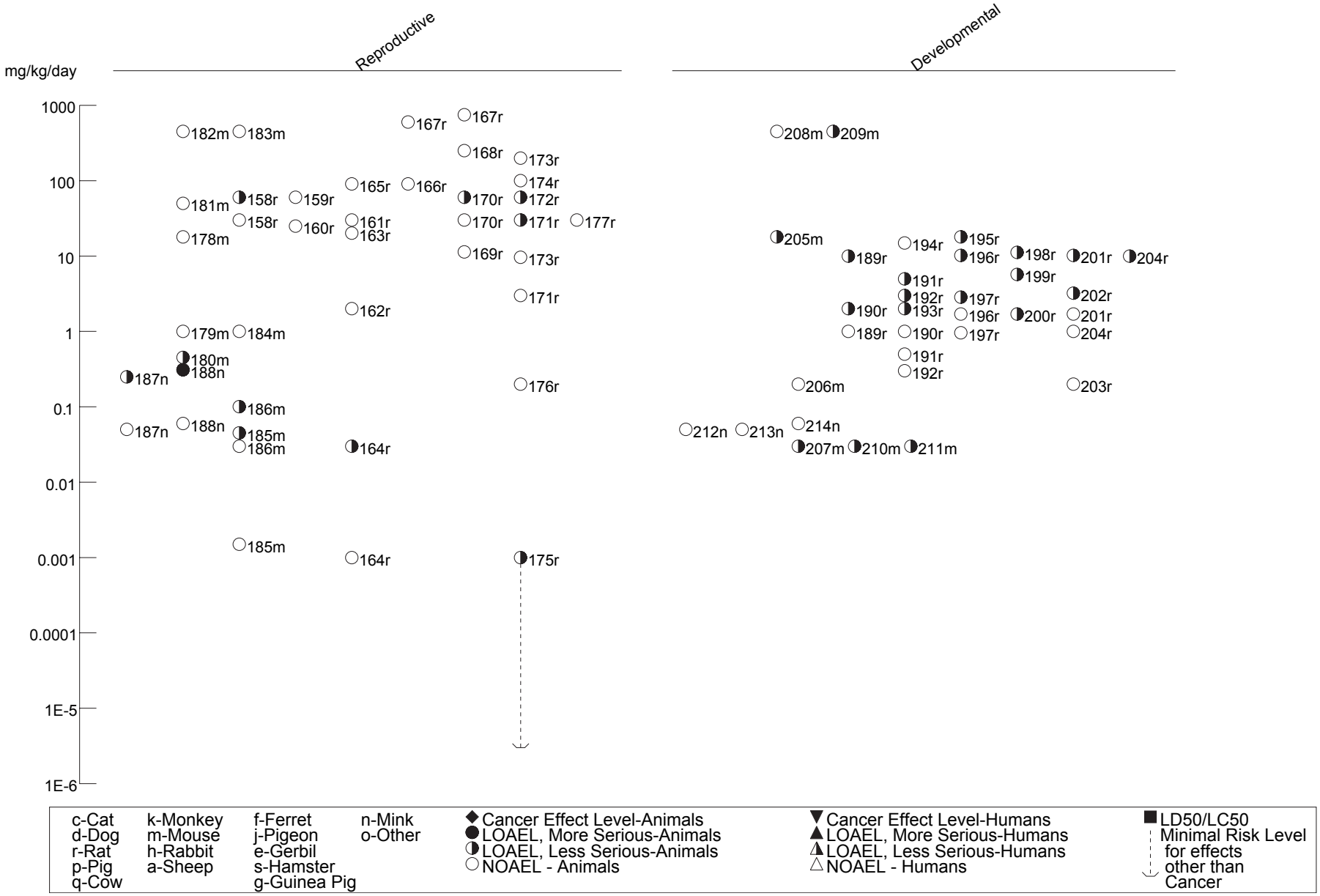


Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Systemic								
1	Rat (Wistar)	7 d 1 x/d	Hepatic	1000 F			Bruchajzer et al. 2010 DecaBDE (BDE209)	
			Bd Wt	1000 F				
2	Rat (Wistar)	14 d 1 x/d	Hepatic	1000 F			Bruchajzer et al. 2010 DecaBDE (BDE209)	
			Bd Wt	1000 F				
3	Rat (Sprague-Dawley)	14 d 1 x/d (GO)	Hepatic	95.9 M			Carlson 1980b DecaBDE (technical)	
4	Rat Spartan	once (GO)	Bd Wt	5000			IRDC 1974 DecaBDE (technical)	
5	Rat (Fischer- 344)	14 d 1 x/d (F)	Bd Wt	16000			NTP 1986 DecaBDE (technical, 94-97% pure)	
6	Rat (Long-Evans)	4 d 1 x/d (GO)	Endocr	100 F			Zhou et al. 2001 DecaBDE (DE-83R)	No exposure-related changes in serum thyroid hormone levels.
			Bd Wt		300 F			
7	Mouse (C57)	3 d Gd 7-9 (G)	Endocr	750 F	1500 F	(reduced maternal serum T4)	Chi et al. 2011 DecaBDE (BDE209)	
8	Mouse (B6C3F1)	14 d 1 x/d (F)	Bd Wt	19000			NTP 1986 DecaBDE (technical, 94-97% pure)	

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
9	Mouse (C3H/HeNCrlC(F)	1 wk	Bd Wt	9800 M			Sakamoto et al. 2013 DecaBDE (BDE209)	No change in liver weights were observed.
Developmental								
10	Rat (Sprague-Dawley)	2 wk Gd 1-14 (GO)		10 M	30 M (impaired learning in Pnd 25 offspring)		Chen et al. 2014 DecaBDE (BDE209)	
11	Rat (Sprague-Dawley)	once Pnd 3 (G)			6.7 M (decreased spontaneous activity at 2 months)		Viberg et al. 2007 DecaBDE (BDE209)	
12	Mouse (NMRI)	once Pnd 3 (GO)		1.34[b]	5.76 (decreased activity and impaired habituation at 2-4 months; impaired learning at 5-7 months)		Buratovic et al. 2014 DecaBDE	
13	Mouse (C57)	3 d Gd 7-9 (G)		150	750 (10% decrease in fetal weight, 3% increase in post-implantation loss)		Chi et al. 2011 DecaBDE (BDE209)	
14	Mouse (NMRI)	once Pnd 3 (G)		1.34[b] M	2.22 M (decreased activity and impaired habituation at 2 and 4 months)		Johansson et al. 2008 DecaBDE (BDE209)	

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
15	Mouse (C57BL/6J)	14 d Pnd 2-15 (IN)		6	20 (delayed ontogeny of reflexes in males and females, increased locomotion in Pnd 70 males, reduced serum T4 in Pnd 21 males)		Rice et al. 2007 DecaBDE (BDE209)	Compound administered via micropipette.
16	Mouse (C57BL/6J)	14 d Pnd 2-15 (IN)		6	20 (learning impairment and impulsivity at 16 months of age)		Rice et al. 2009 DecaBDE (BDE209)	Compound administered via micropipette.
17	Mouse (NMRI)	once Pnd 3 (G)			2.22 M (decreased spontaneous activity at 2 and 6 months)		Viberg et al. 2003b DecaBDE (BDE209)	
18	Mouse (NMRI)	once Pnd 10 (G)		20.1 M			Viberg et al. 2003b DecaBDE (BDE209)	No change in spontaneous activity or habituation at 2, 4, or 6 months.
19	Mouse (NMRI)	once Pnd 19 (G)		20.1 M			Viberg et al. 2003b DecaBDE (BDE209)	No change in spontaneous activity or habituation at 2, 4, or 6 months.
INTERMEDIATE EXPOSURE								
Systemic								
20	Rat (Sprague-Dawley)	36 d Gd 6 - Pnd 21 (GO)	Bd Wt	1000 F			Biesemeier et al. 2011 DecaBDE (BDE209)	

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
21	Rat (Wistar)	21 d 1 x/d	Hepatic	1000 F			Bruchajzer et al. 2010 DecaBDE (BDE209)	
			Bd Wt	1000 F				
22	Rat (Wistar)	28 d 1 x/d	Hepatic	1000 F			Bruchajzer et al. 2010 DecaBDE (BDE209)	
			Bd Wt	1000 F				
23	Rat (Sprague-Dawley)	31 d Gd 10 - Pnd 21 (F)	Bd Wt	146 F			Fujimoto et al. 2011 DecaBDE (BDE209)	
24	Rat (CD)	28 d (F)	Hepatic	90			IRDC 1976 DecaBDE (technical)	
			Renal	90				
			Endocr	90				
			Bd Wt	90				
25	Rat (Sprague-Dawley)	33 d (Pnd 10-42) 1 x/d (G)	Hepatic	100 M	300 M (fatty degeneration, inflammatory foci)		Lee et al. 2010 DecaBDE (BDE209)	
			Endocr		100 M (reduced serum T3)			
			Bd Wt	600 M				

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
26	Rat (Sprague-Dawley)	~11wk Pmd 21 - Pnd 21 (GO)	Hepatic		300 F (hepatocyte degeneration, eosinophilic changes)		Liu et al. 2012 DecaBDE (BDE209)	
			Bd Wt		300 F (12% decrease in maternal body weight)			
27	Rat (Fischer- 344)	13 wk (F)	Resp	8000			NTP 1986 DecaBDE (technical, 94-97% pure)	
			Cardio	8000				
			Gastro	8000				
			Hemato	8000				
			Musc/skel	8000				
			Hepatic	8000				
			Renal	8000				
			Endocr	8000				
			Bd Wt	8000				

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
28	Rat (Wistar)	28 d (G)	Hemato	60			Van der Ven et al. 2008a DecaBDE (BDE209)	
			Musc/skel	60				
			Renal	60				
			Endocr	30 F	60 F (increased serum T3)			
			Bd Wt	60				
29	Rat (Sprague-Dawley)	90 d 1 x/d (GO)	Hepatic	100 M			Wang et al. 2010 DecaBDE (BDE209)	No change in kidney weight, clinical chemistry parameters, or serum thyroid hormones.
			Renal	100 M				
			Endocr	100 M				
			Bd Wt	100 M				
30	Rat (Sprague-Dawley)	90 d 1 x/d (GO)	Endocr	50 M			Wang et al. 2011b DecaBDE (BDE209)	No exposure-related changes in serum thyroid hormone levels.
			Bd Wt	50 M				
31	Rat (Sprague-Dawley)	8 wk 7 d/wk (GO)	Endocr		0.05[c] M (12% increase in serum glucose)		Zhang et al. 2013a DecaBDE (BDE209)	
			Bd Wt	20 M				

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
32	Mouse (Tg2576)	15 d 1 x/d (GO)	Bd Wt	20 M			Heredia et al. 2012 DecaBDE (BDE209)	
33	Mouse (CD-1)	15 d 1 x/d (G)	Bd Wt	160			Liang et al. 2010 DecaBDE (BDE209)	
34	Mouse (CD-1)	30 d 1 x/d (G)	Bd Wt	160			Liang et al. 2010 DecaBDE (BDE209)	
35	Mouse (CD-1)	60 d 1 x/d (G)	Bd Wt	160			Liang et al. 2010 DecaBDE (BDE209)	
36	Mouse (B6C3F1)	13 wk (F)	Resp	9500			NTP 1986 DecaBDE (technical, 94-97% pure)	
			Cardio	9500				
			Gastro	9500				
			Hemato	9500				
			Musc/skel	9500				
			Hepatic	9500				
			Renal	9500				
			Endocr	9500				
			Bd Wt	9500				

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
37	Mouse (C3H/HeNCrlC	4 wk (F)	Hepatic		9400 M (moderate hepatocellular hypertrophy)		Sakamoto et al. 2013 DecaBDE (BDE209)	
			Bd Wt	9400 M				
38	Mouse (Parkes)	35 d (GO)	Endocr	750 M	950 M (reduced serum T3 and T4)		Sarkar et al. 2015 DecaBDE (BDE209)	
			Bd Wt	950 M				
39	Mouse (CD-1)	50 d 1 x/d (GO)	Bd Wt	1500 M			Tseng et al. 2006 DecaBDE (BDE209)	
40	Mouse (CD-1)	18 d Gd 0-17 (GO)	Bd Wt	1500 F			Tseng et al. 2008 DecaBDE (BDE209)	
41	Mouse (BALB/c)	31 d Gd 10 - Pnd 21 (F)	Bd Wt	260 F			Watanabe et al. 2008 DecaBDE (BDE209)	
42	Mouse (BALB/c)	28 d (F)	Bd Wt	1800 F			Watanabe et al. 2010a DecaBDE (BDE209)	

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
43	Rat (CD)	28 d (F)		90			IRDC 1976 DecaBDE (technical)	No exposure-related changes in spleen weight or spleen or thymus histology.
44	Rat (Sprague-Dawley)	~11wk Pmd 21 - Pnd 21 (NS)			300 F (increased spleen weight, lesions in spleen and thymus, altered T-cell distribution, decreased serum IgM, IgG, decreased lymphocyte proliferation)		Liu et al. 2012 DecaBDE (BDE209)	
45	Rat (Fischer- 344)	13 wk (F)		8000			NTP 1986 DecaBDE (technical, 94-97% pure)	
46	Rat (Wistar)	28 d (G)		60 M			Van der Ven et al. 2008a DecaBDE (BDE209)	No exposure-related changes in spleen or thymus weight or histology, no change in spleen cell subpopulations.
47	Mouse (C57BL/6)	10 mo every other day (GO)			800 F (impaired CD4 T cell immune function)		Feng et al. 2016b DecaBDE (BDE209)	

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
48	Mouse (B6C3F1)	13 wk (F)		9500			NTP 1986 DecaBDE (technical, 94-97% pure)	
49	Mouse (BALB/c)	28 d (F)		1800 F			Watanabe et al. 2010a DecaBDE (BDE209)	No change in pulmonary viral load after RSV infection 1 week after exposure period.
Neurological								
50	Rat (Sprague-Dawley)	90 d 1 x/d (GO)		50 M			Wang et al. 2011b DecaBDE (BDE209)	No changes in open-field behavior.
51	Rat (Wistar)	20 d Pnd 22-41 (G)			20.1 (decreased synaptic potency, short-term plasticity, and long-term potentiation on Pnd 60)		Xing et al. 2009 DecaBDE (BDE209)	
52	Mouse (Tg2576)	15 d 1 x/d (GO)			20 M (decreased anxiety behaviors)		Heredia et al. 2012 DecaBDE (BDE209)	
53	Mouse (CD-1)	15 d 1 x/d (G)		160			Liang et al. 2010 DecaBDE (BDE209)	No exposure-related changes in brain weight or AchE activity.

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
54	Mouse (CD-1)	30 d 1 x/d (G)		160			Liang et al. 2010 DecaBDE (BDE209)	No exposure-related changes in brain weight or AchE activity.
55	Mouse (CD-1)	60 d 1 x/d (G)		80	160 (transient reduction in brain AchE activity)		Liang et al. 2010 DecaBDE (BDE209)	
Reproductive								
56	Rat (Sprague-Dawley)	36 d Gd 6 - Pnd 21 (GO)		1000 F			Biesemeier et al. 2011 DecaBDE (BDE209)	No treatment-related changes in maternal toxicity, gestation length, or number of implantations.
57	Rat (Sprague-Dawley)	32 d Gd 10 - Pnd 21 (F)		146 F			Fujimoto et al. 2011 DecaBDE (BDE209)	No change in number of implantations, live pups, or sex ratio.
58	Rat (CD)	28 d (F)		90			IRDC 1976 DecaBDE (technical)	No exposure-related changes in reproductive organ weight or histology.
59	Rat (Sprague-Dawley)	Pnd 10-42 1 x/d (G)		600 M			Lee et al. 2010 DecaBDE (BDE209)	No exposure-related changes in reproductive organ weight or testicular histology.

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
60	Rat (Sprague-Dawley)	~11wk Pmd 21 - Pnd 21 (NS)			300 F (atrophic and fibrotic changes in ovary, decreased number of ovarian follicles)		Liu et al. 2012 DecaBDE (BDE209)	
61	Rat (Fischer- 344)	13 wk (F)		8000			NTP 1986 DecaBDE (technical, 94-97% pure)	No exposure-related changes in reproductive organ histology.
62	Mouse (B6C3F1)	13 wk (F)		9500			NTP 1986 DecaBDE (technical, 94-97% pure)	No exposure-related changes in reproductive organ histology.
63	Mouse (Parkes)	35 d (GO)		750 M	950 M (13-18% decrease in relative testis and epididymides weight, degenerative changes in seminiferous tubules, reduced sperm count/viability, reduced serum testosterone)		Sarkar et al. 2015 DecaBDE (BDE209)	
64	Mouse (CD-1)	50 d Pnd 21-70 (GO)		100 M	500 M (reduced amplitude of lateral head velocity of sperm; reduced sperm mitochondrial membrane potential)		Tseng et al. 2006 DecaBDE (BDE209)	

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
65	Mouse (CD-1)	18 d Gd 0-17 (GO)		1500 F			Tseng et al. 2008 DecaBDE (BDE209)	No change in gestational length or litter size, no change in ovary weight.
66	Mouse (BALB/c)	31 d Gd 10 - Pnd 21 (F)		260 F			Watanabe et al. 2008 DecaBDE (BDE209)	No change in the number of litters.
67	Mouse (BALB/c)	31 d Gd 10 - Pnd 21 (F)		2900 F			Watanabe et al. 2010b DecaBDE (BDE209)	No change in the number of litters.
Developmental								
68	Rat (Sprague-Dawley)	36 d Gd 6 - Pnd 21 (GO)		1000			Biesemeier et al. 2011 DecaBDE (BDE209)	No treatment-related changes in litter size, sex ratio, pup survival and development, or neurobehavior or neuropathology of offspring.
69	Rat (Sprague-Dawley)	32 d Gd 10 - Pnd 21 (F)			2 (diffuse liver cell hypertrophy with cytoplasmic eosinophilia in male offspring and cytoplasmic eosinophilia in the cortical proximal tubules of the kidney of female offspring at Pnd 20; recovered by Pnw 11)		Fujimoto et al. 2011 DecaBDE (BDE209)	

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
70	Rat (Sprague-Dawley)	20 d Gd 0-19 (GO)		1000 F			Hardy et al. 2002 97.34% deca-, 2.66% nona- and octa-BDE	
71	Rat (Wistar)	21 d Gd 1-21 (G)		20.1			Xing et al. 2009 DecaBDE (BDE209)	No change in hippocampal eletrophysiological readings in Pnd 60 offspring.
72	Rat (Wistar)	61 d Gd 1 - Pnd 41 (G)			20.1 (decreased synaptic potency, short-term plasticity, and long-term potentiation in hippocampus in Pnd 60 offspring)		Xing et al. 2009 DecaBDE (BDE209)	DecaBDE administered to dams Gd 1- Pnd 21 and to offspring Pnd 22-41.
73	Rat (Wistar)	21 d Pnd 1-21 (G)			20.1 (altered long-term potentiation in hippocampus of Pnd 60 offspring)		Xing et al. 2009 DecaBDE (BDE209)	DecaBDE administered to dams.
74	Rat (Wistar)	19 d Pnd 3-21 (G)			20.1 (decreased synaptic potency, short-term plasticity, and long-term potentiation in hippocampus on Pnd 60)		Xing et al. 2009 DecaBDE (BDE209)	DecaBDE administered to neonates.

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
75	Mouse (CD-1)	18 d Gd 0-17 (GO)			10 M (hepatocytic swelling in male offspring at Pnd 71)		Tseng et al. 2008 DecaBDE (BDE209)	
76	Mouse (CD-1)	18 d Gd 0-17 (GO)			10 M (vacuolization in interstitial cells of testes and sperm damage in male offspring at Pnd 71)		Tseng et al. 2013 DecaBDE (BDE209)	
77	Mouse (BALB/c)	31 d Gd 10 - Pnd 21 (F)		34	260 (15% decrease in pup weight on Pnd 21; increased viral load in lung and IFN-gamma in BALF in offspring on Pnd 33)		Watanabe et al. 2008 DecaBDE (BDE209)	Pups were infected with RSV on Pnd 28.
78	Mouse (BALB/c)	31 d Gd 10 - Pnd 21 (F)		290	2900 (increased viral load in lung and altered cytokine expression BALF in offspring on Pnd 29-33)		Watanabe et al. 2010b DecaBDE (BDE209)	Pups were infected with RSV on Pnd 28.
Cancer								
79	Mouse (C3H/HeNCrlC	27 wk (F)			9100 M (CEL: liver neoplastic nodules; altered foci)		Sakamoto et al. 2013 DecaBDE (BDE209)	
CHRONIC EXPOSURE								
Death								
80	Mouse (C57BL/6)	2 yr every other day (GO)				800 F (5/10 died vs. 1/10 controls)	Feng et al. 2015 DecaBDE (BDE209)	

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
81	Rat (Sprague-Dawley)	2 yr (F)	Resp	1			Kociba et al. 1975; Norris et al. 1975a 77% decaBDE, 22% nonaBDE	
			Cardio	1				
			Gastro	1				
			Hemato	1				
			Musc/skel	1				
			Hepatic	1				
			Renal	1				
			Endocr	1				
			Ocular	1				
			Bd Wt	1				

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
82	Rat (Fischer- 344)	103 wk (F)	Resp	2550 F			NTP 1986 DecaBDE (technical, 94-97% pure)	
			Cardio	2550 F				
			Gastro	1120 M	2240 M (acanthosis)			
			Hemato	2550 F				
			Musc/skel	2550 F				
			Hepatic		1120 M (precancerous neoplastic nodules)			
			Renal	2550 F				
			Endocr	2550 F				
			Bd Wt	2550 F				

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
83	Mouse (B6C3F1)	103 wk (F)	Resp	7780 F			NTP 1986 DecaBDE (technical, 94-97% pure)	
			Cardio	7780 F				
			Gastro	3760 F	7780 F (ulcers)			
			Hemato	7780 F				
			Musc/skel	7780 F				
			Hepatic		3200 M (centrilobular hypertrophy and granulomas)			
			Renal	7780 F				
			Endocr		3200 M (follicular cell hyperplasia)			
			Bd Wt	7780 F				
Immuno/ Lymphoret								
84	Rat (Sprague-Dawley)	2 yr (F)		1			Kociba et al. 1975; Norris et al. 1975a 77% decaBDE, 22% nonaBDE	No exposure-related changes in immune tissue histology.
85	Rat (Fischer- 344)	103 wk (F)			1200 F (splenic hematopoiesis)		NTP 1986 DecaBDE (technical, 94-97% pure)	
86	Mouse (B6C3F1)	103 wk (F)		7780 F			NTP 1986 DecaBDE (technical, 94-97% pure)	No exposure-related changes in immune tissue histology

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
87	Rat (Sprague-Dawley)	2 yr (F)		1 M 1 F			Kociba et al. 1975; Norris et al. 1975a 77% decaBDE, 22% nonaBDE	
88	Rat (Fischer- 344)	103 wk (F)		2240 M 2550 F			NTP 1986 DecaBDE (technical, 94-97% pure)	No exposure-related changes in reproductive organ histology.
89	Mouse (B6C3F1)	103 wk (F)		6650 M 7780 F			NTP 1986 DecaBDE (technical, 94-97% pure)	No exposure-related changes in reproductive organ histology.
Cancer								
90	Rat (Fischer- 344)	103 wk (F)				1120 M (CEL: liver neoplastic nodules)	NTP 1986 DecaBDE (technical, 94-97% pure)	

Table 3-3 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
91	Mouse (B6C3F1)	103 wk (F)				3200 M (CEL: hepatocellular adenomas and carcinomas)	NTP 1986 DecaBDE (technical, 94-97% pure)	

a The number corresponds to entries in Figure 3-3.

b Used to derive an acute oral minimal risk level (MRL); concentration divided by an uncertainty factor of 100 (10 for animal to human, and 10 for human variability), resulting in an MRL of 0.01 mg/kg/day.

c Used to derive an intermediate oral minimal risk level (MRL); concentration divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL,10 for animal to human, 10 for human variability), resulting in an MRL of 0.0002 mg/kg/day.

Note on chemical form: Mixtures are identified by composition or trade name (if reported); otherwise, they are reported as "technical". Trade names for decaBDE include DE-83R (98% decaBDE). The individual congener is identified by by IUPAC number: BDE 209 = 2,2',3,3',4,4',5,5',6,6'-decaBDE. Where study authors report use of decaBDE, without further compositional information, it is assumed the pure congener (BDE 209) was used.

AchE = acetylcholinesterase; BALF = brochoalveolar lavage fluid; Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; (GO) = gavage in oil; Hemato = hematological; IFN = interferon; IgG = immunoglobulin G; IgM = immunoglobulin M; Immuno/Lymphoret = immunological/lymphoreticular; IN = ingestion ; LOAEL = lowest-observed-adverse-effect level; M = male; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Pmd = pre-mating day; Pnd = post-natal day; Pnw = post-natal week; Resp = respiratory; RSV = respiratory syncytial virus; T3 = triiodothyronine; T4 = thyroxine; x = time(s); wk = week(s); yr = year(s)

Figure 3-3. Levels of Significant Exposure to Decabromodiphenyl Ether - Oral

Acute (≤14 days)

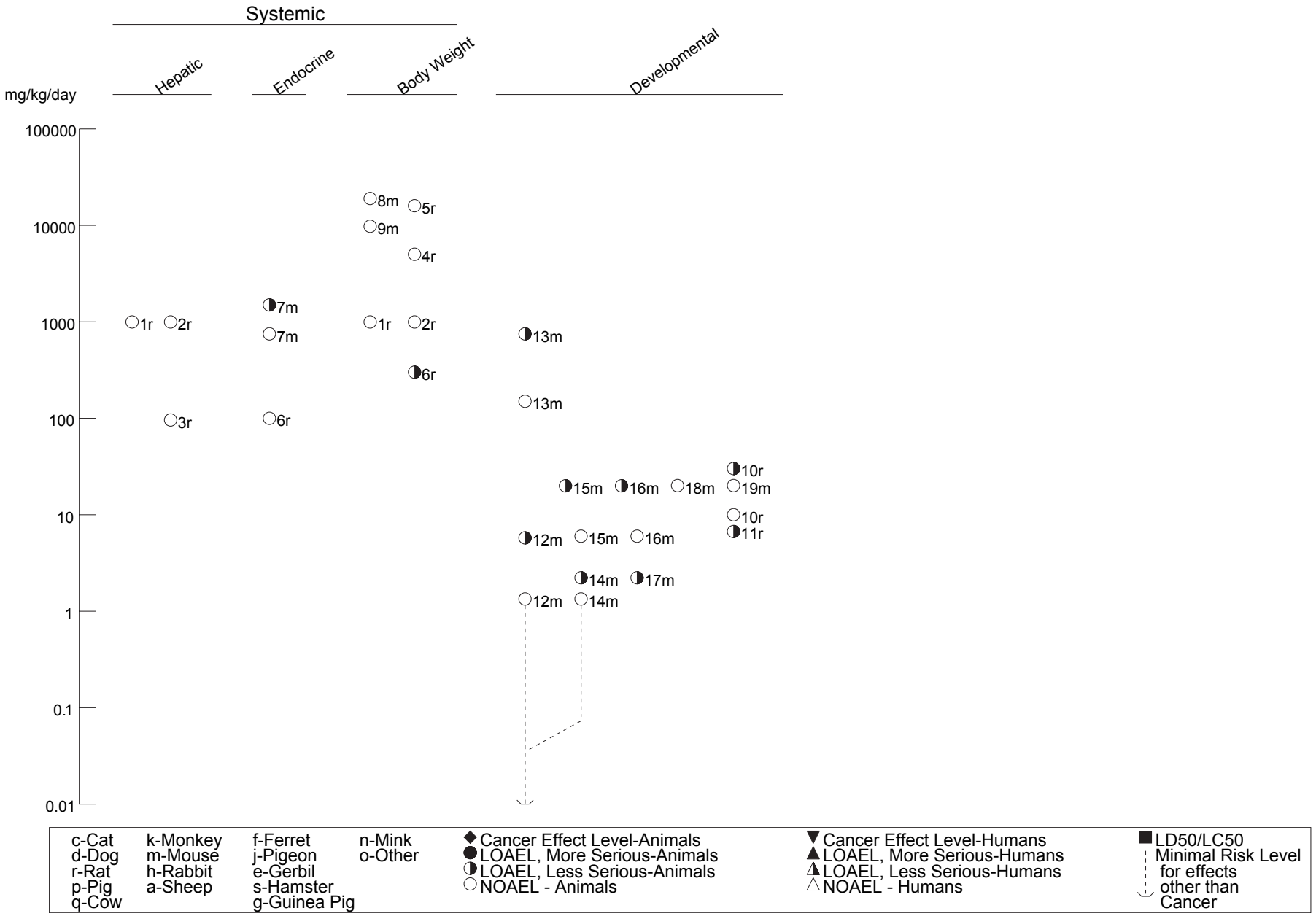


Figure 3-3. Levels of Significant Exposure to Decabromodiphenyl Ether - Oral *(Continued)*

Intermediate (15-364 days)

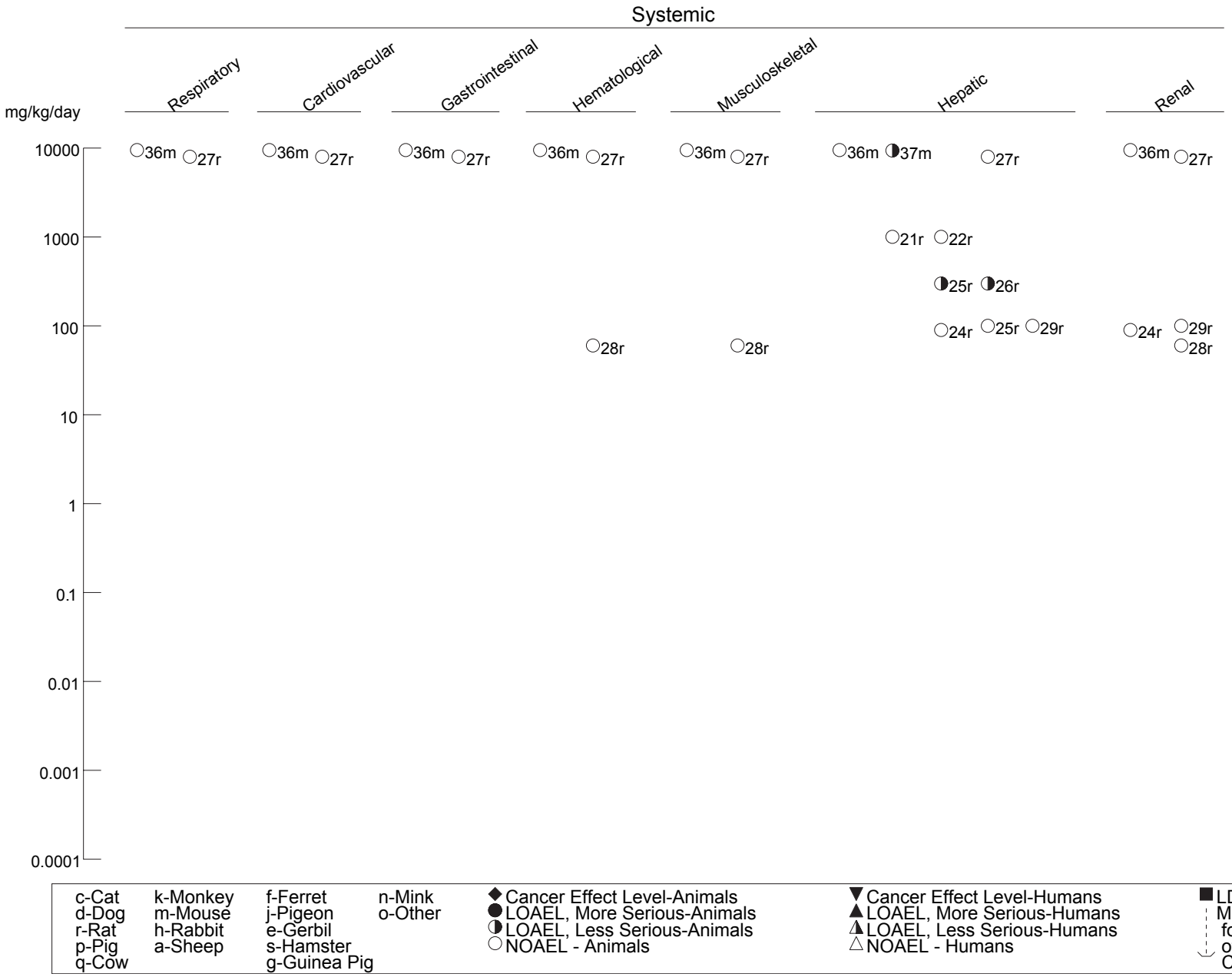


Figure 3-3. Levels of Significant Exposure to Decabromodiphenyl Ether - Oral *(Continued)*

Intermediate (15-364 days)

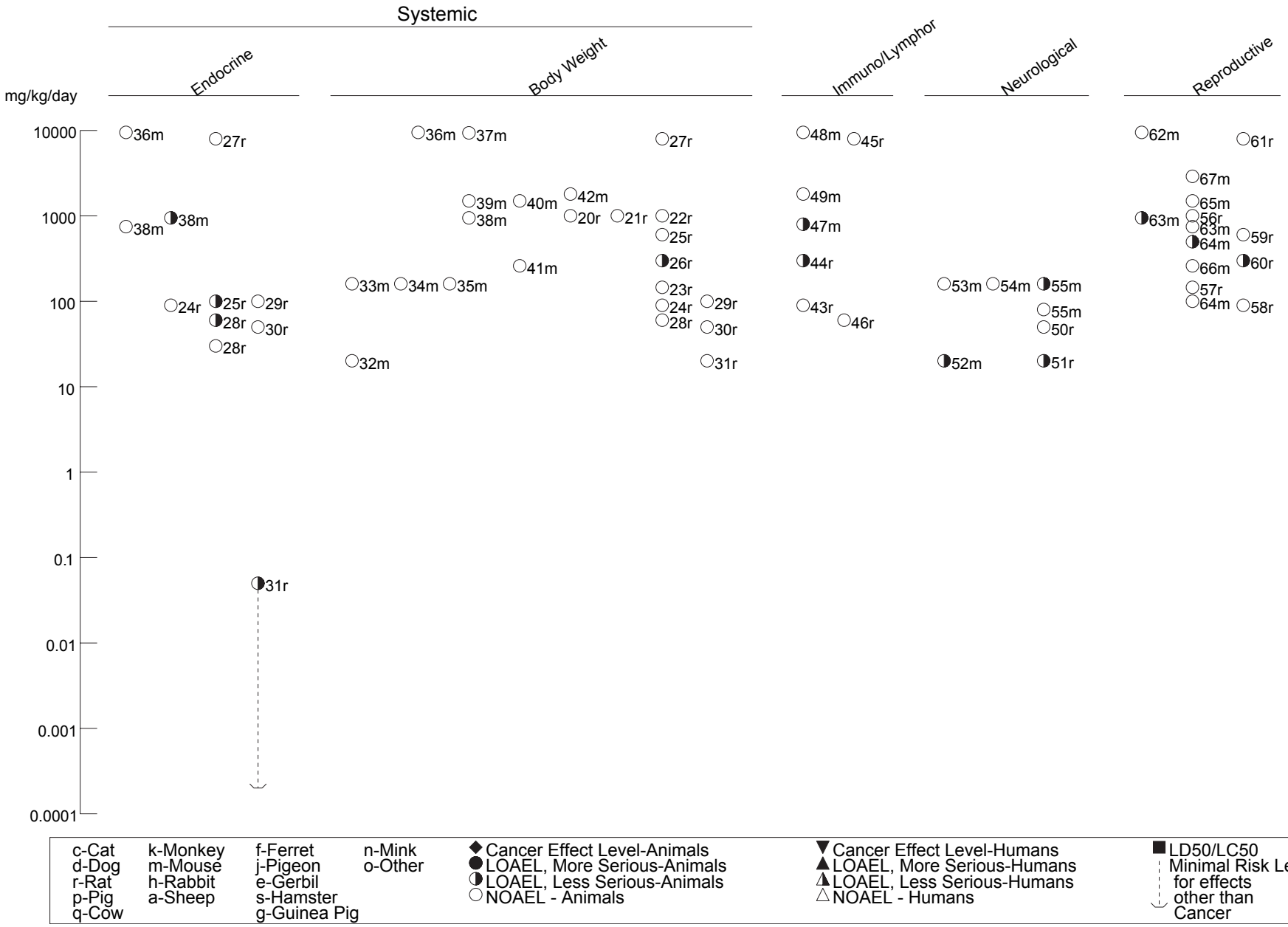
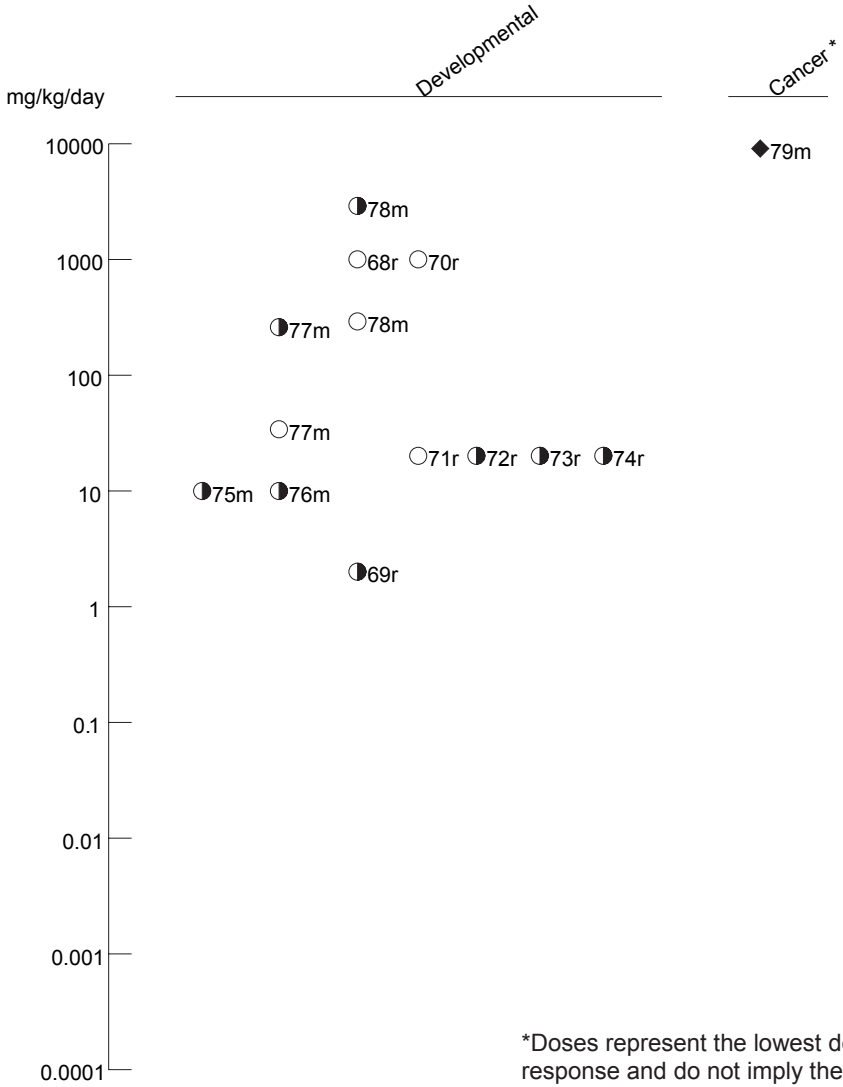


Figure 3-3. Levels of Significant Exposure to Decabromodiphenyl Ether - Oral *(Continued)*

Intermediate (15-364 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat, d-Dog, r-Rat, p-Pig, q-Cow; k-Monkey, m-Mouse, h-Rabbit, a-Sheep; f-Ferret, j-Pigeon, e-Gerbil, s-Hamster, g-Guinea Pig; n-Mink, o-Other

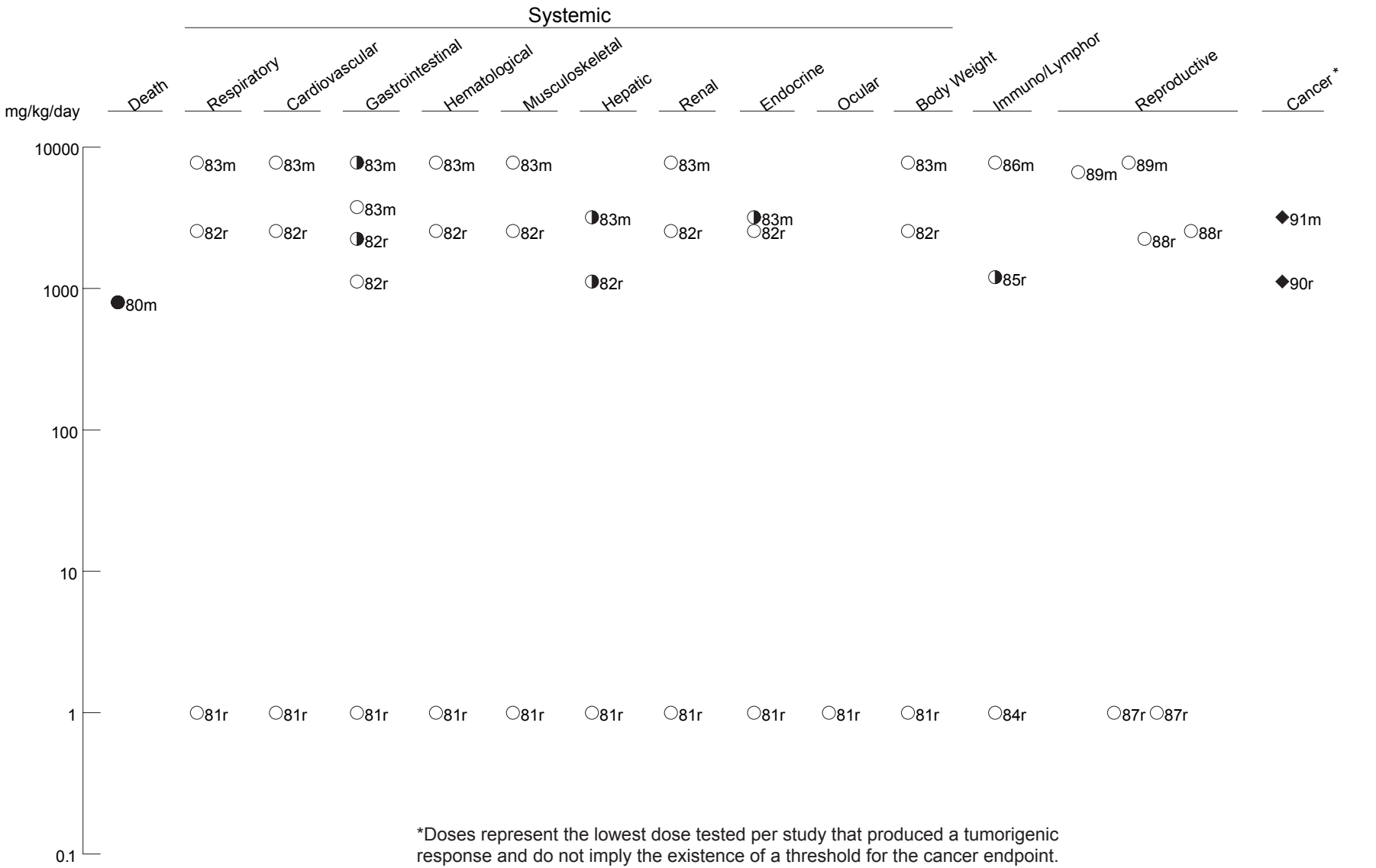
◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Figure 3-3. Levels of Significant Exposure to Decabromodiphenyl Ether - Oral *(Continued)*

Chronic (≥365 days)



In intermediate-duration dietary studies with decaBDE, there was no exposure-related mortality in rats that were exposed to estimated dietary doses of ≤90 mg/kg/day for 28 days (IRDC 1976) or rats and mice fed estimated doses of ≤8,000 and ≤9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986). In chronic studies, there were no effects on survival in rats that were fed 0.01–1.0 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaBDE) for 2 years (Kociba et al. 1975; Norris et al. 1975a), or in rats and mice fed decaBDE in estimated doses of ≤2,550 and ≤7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986). However, Feng et al. (2015) reported that 5/10 female mice died following exposure to decaBDE (98% purity) every other day via gavage for up to 2 years at a dose of 800 mg/kg/dose, compared with 1/10 vehicle control mice. No cause of death or gross or microscopic pathology was reported for animals that died. In surviving mice, various histopathological lesions were qualitatively described in the brain, heart, lung, liver, spleen, kidney, and ovaries of exposed animals, but incidence data were not provided. Due to the high mortality and lack of quantitative data, this chronic study is not discussed in the Systemic Effects section below.

The LD_{50} and LOAEL values for death are recorded in Tables 3-2 (lower BDEs) and 3-3 (decaBDE) and plotted in Figures 3-2 (lower BDEs) and 3-3 (decaBDE).

3.2.2.2 Systemic Effects

The systemic effects in humans and animals following oral exposure to PBDEs are described below. The highest NOAEL and all LOAEL values from each reliable study for systemic end points in each species and duration category are recorded in Tables 3-2 (lower-brominated PBDEs) or 3-3 (decaBDE) and plotted in Figures 3-2 (lower-brominated PBDEs) or 3-3 (decaBDE).

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to PBDEs. Effects of PBDEs on respiratory function have not been studied in orally exposed animals.

No histopathological changes in the respiratory tract or exposure-related changes in lung weight were observed in rats exposed to pentaBDE at doses up to 250 mg/kg/day for 28 days via gavage (Oberg et al. 2010; Van der ven et al. 2008a) or in rats exposed to dietary pentaBDE at doses up to 100 mg/kg/day for 90 days (WIL Research Laboratories 1984). Similarly, no changes in respiratory tract histology were observed in rats exposed to dietary octaBDE at doses up to 750 mg/kg/day for 13 weeks (IRDC 1977).

No exposure-related changes in lung weight were observed in rats exposed to a dietary penta-decaBDE mixture (52.1% pentaBDE, 44.2% decaBDE, 0.4% octaBDE) at 20 mg/kg/day for 70 days (Ernest et al. 2012) or in F0 or F1 rats exposed pentaBDE at 18 mg/kg/day from GD 6 to PND 18 via gavage (Ellis-Hutchings et al. 2006).

To determine if PBDE exposure alters vitamin A homeostasis in rats in a manner similar to the related PBB compounds (ATSDR 2004), vitamin A levels were measured in lung tissue following exposure to pentaBDE at 0, 2.5, 25, or 250 mg/kg/day via gavage (Oberg et al. 2010). No significant changes in lung vitamin A levels were observed at any dose.

No histopathological changes in respiratory tract tissues were found in rats and mice exposed to dietary decaBDE at estimated doses of ≤8,000 and ≤9,500 mg/kg/day, respectively, for 13 weeks or estimated doses of ≤2,550 and ≤7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986). Additionally, no histopathological changes in respiratory tract tissues were observed in rats that were fed ≤1.0 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaBDE) for 2 years (Kociba et al. 1975; Norris et al. 1975a).

Based on animal studies, respiratory effects are not likely to occur following oral exposure to PBDEs.

Cardiovascular Effects. No relationship was found between serum concentrations of tetraBDE (BDE 47) and carotid atherosclerosis (carotid artery plaques determined by ultrasound) or stroke in a population of 1,016 70-year-old volunteers (50.2% female) from Uppsala, Sweden (Lee et al. 2012; Lind et al. 2012). BDE 47 was detected in the serum of 77.2% of subjects with a median concentration of 12.6 pg/mL. No associations were observed between serum PBDE levels and gestational hypertension in 258 pregnant women from the Longitudinal Investigation of Fertility and the Environment (LIFE) cohort in Michigan and Texas (Smarr et al. 2016).

A case control study of 43 children (9–11 years old) from Upstate New York reported an association between higher levels of serum PBDEs and greater sympathetic activation during acute psychological stress and greater anger (Gump et al. 2014). Specifically, BDE 28 was associated with greater heart rate, lower pre-ejection period, and lower total peripheral resistance. BDE 47 and BDE 100 were associated with significantly lower diastolic blood pressure, and BDE 100 was also associated with a shorter pre-ejection period during acute stress. Lipid-adjusted mean blood levels of BDEs 28, 47, and 100 were 1.07, 8.53, and 0.86 ng/g, respectively. The investigators speculated that PBDE-induced increased levels of

calcium/calmodulin-dependent protein kinase II in the hippocampus may mediate the heart's response to β-adrenergic stimulation.

Effects of PBDE on cardiovascular function have not been studied in orally exposed animals.

No exposure-related changes in heart histology or weight were observed in rats exposed to pentaBDE at doses up to 250 mg/kg/day for 28 days via gavage (Oberg et al. 2010; Van der ven et al. 2008a), dietary pentaBDE at doses up to 100 mg/kg/day for 90 days (WIL Research Laboratories 1984), or dietary octaBDE at doses up to 750 mg/kg/day for 13 weeks (IRDC 1977). Additionally, no exposure-related changes in heart weight were observed in rats exposed to dietary penta- or octaBDE at doses up to 90 mg/kg/day for 28 days (IRDC 1976), in rats exposed to a dietary penta-decaBDE mixture (52.1% pentaBDE, 44.2% decaBDE, 0.4% octaBDE) at 20 mg/kg/day for 70 days (Ernest et al. 2012), in F0 or F1 rats exposed to dietary pentaBDE at doses up to 0.31 mg/kg/day for 4 weeks prior to mating through PNW 6 or 33 (Zhang et al. 2009), in F0 or F1 rats exposed pentaBDE at 18 mg/kg/day from GD 6 to PND 18 via gavage (Ellis-Hutchings et al. 2006), or in rats exposed to diBDE at 1.2 mg/kg/day for 28 days via gavage (Zhang et al. 2014).

No histopathological changes in the heart were found in rats and mice exposed to dietary decaBDE at estimated doses of ≤8,000 and ≤9,500 mg/kg/day, respectively, for 13 weeks or estimated doses of ≤2,550 and ≤7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986). No exposure-related changes in heart histology or weight were observed in rats that were fed ≤1.0 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaBDE) for 2 years (Kociba et al. 1975; Norris et al. 1975a). In addition, no exposure-related changes in heart weight were observed in rats exposed decaBDE at doses up to 20 mg/kg/day for 8 weeks via gavage (Zhang et al. 2013a) or to dietary decaBDE at doses up to 90 mg/kg/day for 28 days (IRDC 1976).

Based on human and animal studies, cardiovascular effects are not likely to occur following oral exposure to PBDEs.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after oral exposure to PBDEs.

No histopathological changes in the gastrointestinal tract were found in rats exposed to pentaBDE at doses up to 200 mg/kg/day for 28 days via gavage (Van der ven et al. 2008b), dietary pentaBDE at doses

up to 100 mg/kg/day for 90 days (WIL Research Laboratories 1984), or dietary octaBDE at doses up to 750 mg/kg/day for 13 weeks (IRDC 1977).

No histopathological changes in gastrointestinal tract tissues were found in rats and mice fed decaBDE in estimated doses of ≤8,000 and ≤9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986). In chronic dietary studies, there was no gastrointestinal tract histopathology in rats that were fed ≤1.0 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaBDE) for 2 years (Kociba et al. 1975; Norris et al. 1975a). Higher dietary doses of decaBDE for 103 weeks caused acanthosis of the forestomach in rats exposed to 2,240 mg/kg/day (no effects at ≤1,200 mg/kg/day) and stomach ulcers in mice exposed to 7,780 mg/kg/day (no effects at ≤3,760 mg/kg/day) (NTP 1986).

Based on animal studies, gastrointestinal effects are not likely to occur following oral exposure to PBDEs at environmentally-relevant exposure concentrations.

Hematological Effects.

Human Studies. Hematological end points were evaluated in a subset of 18 of a cohort of 33 children (18 girls and 15 boys) born in the Amsterdam/Zaandam area of the Netherlands and aged 14–19 years at the time of the study (Leijs et al. 2009). Serum PBDE concentrations (determined as the sum of congeners 28, 47, 85, 99, 100, 153, 154, and 183) ranged from 5 to 74 ng/g lipid with a mean of 13.9 ng/g lipid. Serum samples were used to assess hemoglobin, thrombocytes, and white blood cell count and differential. The researchers found a negative statistical association (not further described) between number of lymphocytes and PBDE concentrations in serum. Congener-specific analysis showed the main contributors to be BDE 183, BDE 154, and BDE 85. Although dioxins and PCBs were also assessed, no association with lymphocytes was found for these chemicals. The only other finding for PBDE was a positive association between serum hemoglobin and PBDE, primarily due to congeners 85 and 153. A Chinese study of 40 subjects exposed to PBDEs in an electronic waste dismantling area reported a significantly elevated neutrophil percentage among the workers compared to a group of 15 unexposed controls (Xu et al. 2015a). In addition, exposed subjects had significantly lower percentages of monocytes, lymphocytes, hemoglobin, and platelets than controls, while total white cell counts were not significantly different between the two groups. PBDEs assessed included congeners 15, 27, 85, 99, 100, 153, 154, and 184. Exposure was likely by multiple routes. PCBs also were found associated with lowered percentages of monocytes and lymphocytes.

Animal Studies

Lower-brominated PBDEs: Following exposure to dietary octaBDE at 750 mg/kg/day for 13 weeks, female rats showed 26, 22, and 22% decreases in erythrocytes, hematocrit, and hemoglobin, respectively; male rats showed a 10% decrease in erythrocytes at 600 mg/kg/day (IRDC 1977). No changes were observed in total or differential white blood cell counts (IRDC 1977). No changes in hematological parameters, including erythrocyte count, hemoglobin, hematocrit, full and differential leukocyte count, and platelet count, were observed in rats following pentaBDE exposure to dietary doses up to 100 mg/kg/day for 90 days (WIL Research Laboratories 1984) or gavage up to 250 mg/kg/day for 28 days (Oberg et al. 2010).

In another 28-day study, rats were exposed to pentaBDE at 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7, or 200 mg/kg/day via gavage (Van der ven et al. 2008b). The study authors reported minor dose-related changes in white blood cell differentials from femoral shaft bone marrow in male rats (blood hematology not evaluated); however, the doses at which these effects were observed were not reported. Instead, results were reported in terms of benchmark dose (BMD) analysis (Van der ven et al. 2008b). Statistically significant changes included increased number and percentage of monocytes (maximum increase of 69.5 and 66.7%, respectively; BMD/$BMDL_{RD20\%}$=11.2/0.7 and 31.8/3 mg/kg/day, respectively) and decreased percentage of eosinophilic granulocytes (maximum decrease of 20%; ($BMD_{RD20\%}$=28.6, BMDL not determined). The study authors also reported a significant, dose-related increase in the number and percentage of large unstained cells (maximum increase of 79.7 and 85.1%, respectively; BMD/$BMDL_{RD20\%}$=76.3/42.8 and 64.3/9.8 mg/kg/day, respectively), which they indicated may represent large or reactive lymphocytes, monocytes, or leukemic blasts (abnormal myeloblasts), typically associated with viral disease, leukemia, or endocrine active compounds. Since results were reported in terms of BMD analysis only, data and statistics for individual dose groups were not available for independent analysis. No data regarding other standard hematological end points were reported (Van der ven et al. 2008b).

Male mink exposed to dietary pentaBDE at doses of 0.63 or 0.78 mg/kg/day for 9 weeks showed significant 13 and 12% decreases in hematocrit, respectively; neither the number of red blood cells nor hemoglobin levels were evaluated (Martin et al. 2007). According to study authors, most of the ranges in the differential white blood cell counts fell within that expected for male mink of this age; however, the percentage of neutrophils was increased significantly by ~22% at 0.63 mg/kg/day and 37% at 0.78 mg/kg/day, the percentage of lymphocytes decreased significantly by ~33% at 0.78 mg/kg/day, and

the neutrophil:lymphocyte ratio increased significantly by ~90% at 0.78 mg/kg/day (data reported graphically) (Martin et al. 2007). Total white blood cell counts were not reported. No hematological effects were observed at 0.08 mg/kg/day (Martin et al. 2007). The minor hematological changes are of uncertain toxicological significance.

In a poorly-reported study, mouse dams exposed to pentaBDE from GD 6 to PND 21 via gavage did not show any hematological effects at doses up to 200 mg/kg/day (Hong et al. 2010).

DecaBDE: No hematological changes were observed in rats exposed to ≤60 mg/kg/day of decaBDE via gavage for 28 days (Van der ven et al. 2008a). In dietary studies with decaBDE, no hematological changes were found in rats exposed to ≤800 mg/kg/day for 30 days (Norris et al. 1973, 1975a), ≤8,000 mg/kg/day for 13 weeks (NTP 1986), or ≤2,550 mg/kg/day for 103 weeks (NTP 1986), or in mice exposed to ≤9,500 mg/kg/day for 13 weeks or ≤7,780 mg/kg/day for 103 weeks (NTP 1986). There also were no hematological effects in rats exposed by diet to ≤1.0 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaBDE) for 2 years (Kociba et al. 1975; Norris et al. 1975a).

In a poorly-reported study, mouse dams exposed to decaBDE from GD 6 to PND 21 via gavage showed significant increases in the number of white blood cells and neutrophils at 500 mg/kg/day, compared with controls, but not at 2,500 or 12,500 mg/kg/day (Hong et al. 2010).

Summary. Minor hematological changes observed in humans and animal are of uncertain toxicological significance. Based on the available information, it is unlikely that adverse effects would occur in the human hematological system following oral PBDE exposure.

Musculoskeletal Effects. No association was found between serum PBDE concentrations (BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 209, and their sum) and bone mineral density (measured by x-ray in the forearm) or serum concentrations of biochemical markers of bone metabolism (osteocalcin and crosslaps [a peptide of type I collagen]) in a population of 50 post-menopausal Swedish women married or previously married to professional fisherman on the east coast of the country and born before 1954 (Weiss et al. 2006). Median serum ∑PBDE concentration in this population was 3.6 ng/g fat.

No musculoskeletal changes were seen in rats following exposure to pentaBDE at doses up to 200 mg/kg/day for 28 days via gavage (Van der ven et al. 2008b) or dietary pentaBDE at doses up to 100 mg/kg/day for 90 days (WIL Research Laboratories 1984).

No changes in bone parameters were observed in rats exposed to decaBDE at doses up to 60 mg/kg/day for 28 days via gavage (Van der ven et al. 2008a). Dietary studies with decaBDE found no histopathological changes in musculoskeletal tissues in rats exposed to ≤8,000 mg/kg/day for 13 weeks (NTP 1986), ≤1.0 mg/kg/day (77.4% containing 21.8% nonaBDE and 0.8% octaBDE) for 2 years (Kociba et al. 1975; Norris et al. 1975a), or ≤2,550 mg/kg/day for 103 weeks (NTP 1986), or in mice exposed to ≤9,500 mg/kg/day for 13 weeks or ≤7,780 mg/kg/day for 103 weeks (NTP 1986).

Based on human and animal studies, musculoskeletal effects are not likely to occur following oral exposure to PBDEs.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to PBDEs.

Acute-Duration Animal Studies

Lower-brominated PBDEs: A series of gavage studies evaluated liver histology following a single exposure to pentaBDE doses of 0, 25, 200, or 2,000 mg/kg or repeat exposures to pentaBDE doses of 0, 8, 40, or 200 mg/kg/day for 7 or 14 days (Bruchajzer et al. 2010, 2011). Fatty degeneration of the liver was observed in female rats following pentaBDE exposure via gavage at 2,000 mg/kg for 1 day (Bruchajzer et al. 2011) or 200 mg/kg/day for 7 or 14 days (Bruchajzer et al. 2010). In the single-exposure study, rats from the 2,000 mg/kg group showed steatosis of the microvesicular type, which was most frequently observed in the central and intermediate zones of lobules; however, animal incidence numbers were not reported (Bruchajzer et al. 2011). At 4 and 12 hours after pentaBDE administration, these changes were noted in no more than 25% of hepatocytes; after 24–120 hours, fatty degeneration embraced 26–75% of hepatocytes; and after 120 hours, a mixed type of change (micro- and macrovesicular) was observed (Bruchajzer et al. 2011). In the repeated-exposure studies, rats from the 200 mg/kg/day group showed steatosis of the microvesicular type in 3–25% of hepatocytes after 7 days and steatosis of the microvesicular and mascrovesicular type in 26–75% of hepatocytes in the central and intermediate zones of lobules (Bruchazjer et al. 2010). Again, animal incidence data were not reported. No exposure-related histological changes in the liver were reported for single doses ≤200 mg/kg or repeated doses ≤40 mg/kg/day (Bruchazjer et al. 2010, 2011). In the only other study that included histopathological examination, histological changes were not observed in male rats 45 days after a single administration of pentaBDE at doses up to 1.2 mg/kg (Albina et al. 2010).

In the Bruchazjer et al. (2010, 2011) studies, no biologically relevant changes were observed in serum clinical chemistry values. Serum alanine transaminase (ALT) and aspartate transaminase (AST) activity levels were slightly increased to 130 and 170–190% of control values following a single gavage exposure to 2,000 mg/kg, respectively, after 72–120 hours; no changes were reported for doses ≤200 mg/kg (Bruchazjer et al. 2011). The study authors did not report statistics; however, these serum chemistry changes are not considered biologically relevant since the magnitude of change, compared with control, is <2-fold. Similarly, no exposure-related changes were observed in serum ALT or AST at doses up to 200 mg/kg/day for 7 or 14 days (Bruchajzer et al. 2010). Another 14-day gavage study found no exposure-related changes in serum sorbital dehydrogenase (SDH) levels in rats exposed to pentaBDE at 56.4 mg/kg/day or octaBDE at 76.6 mg/kg/day (equimolar doses) (Carlson 1980b). In male rats exposed once to pentaBDE at doses of 0, 0.6, or 1.2 mg/kg, serum ALT activity was significantly increased by ~72% in the 1.2 mg/kg group, compared with control, when evaluated 45 days after exposure (Alonso et al. 2010). However, this change is not considered biologically relevant because the magnitude of change, compared with control, is <2-fold, the measured ALT activity of 31 U/l was well within reference value ranges for adult male rats (20–81 U/l) (Charles River Laboratories 1998), and no changes were observed in serum AST, alkaline phosphatase (ALP), or lactate dehydrogenase (LDH) (Alonso et al. 2010).

Bruchajzer et al. (2010, 2011) also reported altered markers of oxidative stress in the liver following exposure to pentaBDE. Liver malondialdehyde (MDA) levels were significantly elevated after exposure to ≥200 mg/kg/day for 1–14 days (Bruchajzer et al. 2010, 2011). Levels of reduced glutathione (GSH) in the liver were not increased following a single exposure; however, GSH concentration was elevated at ≥40 mg/kg/day after 7 days and ≥8 mg/kg/day after 14 days (Bruchajzer et al. 2010, 2011). Oxidized glutathione (GSSG) was significantly elevated after single exposures to ≥25 mg/kg, but no changes in glutathione S-transferase (GST) activity were observed (Bruchajzer et al. 2010). Liver GSSG levels and GST activity were not evaluated following 7- or 14-day exposures. In another study, liver GSH levels were significantly decreased and liver SOD activity, GSSG levels, and GSSG/GSH ratio were significantly increased in male rats 45 days after a single gavage administration of pentaBDE at 0.6 or 1.2 mg/kg (Albina et al. 2010).

In two other studies by Bruchazjer and colleagues (Bruchazjer 2011; Bruchazjer et al. 2012), female rats were examined for hepatic porphyria following exposure to pentaBDE at doses of 0, 8, 40, or 200 mg/kg/day or octaBDE at doses of 0, 2, 8, 40, or 200 mg/kg/day via gavage for 7 or 14 days. PentaBDE caused significant dose-related elevations in total porphyrin levels in the liver following

exposure to ≥40 mg/kg/day for 7 days (~2–6-fold) or ≥8 mg/kg/day for 14 days (~3–11-fold). Following gavage exposure to octaBDE for 7 days, liver concentrations of high carboxylated porphyrins (octa- and hepta-) were significantly elevated at 8, 40, and 200 mg/kg/day by ~4-, 4-, and 7-fold, respectively, compared with vehicle controls (Bruchajzer et al. 2012). Lower carboxylated porphyrins were <1% of total liver porphyrins measured, and were not further analyzed. Following exposure to octaBDE for 14-days, total liver concentrations of high carboxylated porphyrins were increased by ~3-fold in the 200 mg/kg/day group only, compared with vehicle controls (Bruchajzer et al. 2012). However, the vehicle controls at this duration had an unusually high hepatic porphyrin levels (4-fold increase compared with untreated control). When compared with the untreated controls, porphyrin levels were significantly increased by ~2-, 4-, 6-, and 7-fold in the 2, 8, 40, and 200 mg/kg/day groups, respectively, supporting that exposure to octaBDE for 14 days causes porphyria as observed in the 7-day study. These studies also measured hepatic delta-aminolevulinate synthase (ALA-S) activity and delta-aminolevilinate dehydratase (ALA-D) activity, two enzymes involved in heme biosynthesis. The results were mixed. PentaBDE exposure caused a significant elevation in ALA-S activity at ≥40 mg/kg/day after 7 or 14 days (ALA-D was not measured), while octaBDE exposure caused a significant increase in ALA-S activity at ≥8 mg/kg/day after 7 days and significant decreases in ALA-S activity at 200 mg/kg/day after 14 days and ALA-D activity at ≥40 mg/kg/day after 7 or 14 days (Bruchajzer 2011; Bruchajzer et al. 2012).

The gavage studies by Bruchajzer and colleagues (Bruchajzer 2011; Bruchajzer et al. 2012) also reported elevated liver weights accompanied by hepatic microsomal enzyme induction. Significant relative liver weight increases (data reported graphically) were about ≥30% after a single exposure to pentaBDE at ≥200 mg/kg (Bruchajzer et al. 2011), ≥13% after exposure to pentaBDE at 8 or 200 mg/kg/day for 7 days (Bruchajzer et al. 2010), or ≥25% after exposure to pentaBDE at ≥40 mg/kg/day for 14 days (Bruchazjer et al. 2010). Hepatic enzyme induction (e.g., increased CYP1A and CYP2B activity and/or CYP1A1, CYP4A, and total cytochrome P450 protein levels) was observed at lower doses: ≥25 mg/kg in the single dose study and ≥8 mg/kg/day in the 7- and 14-day studies (Bruchazjer et al. 2010, 2011).

Consistent with the findings of Bruchazjer and colleagues (Bruchajzer 2011; Bruchajzer et al. 2012), elevated liver weights have been reported following acute exposure to penta-, octa-, or tetraBDE in several other animal studies. Significantly increased liver weights were reported in rats and mice exposed to penta-, tetra-, or octaBDE via gavage at ≥10 and ≥18 mg/kg/day, respectively, for 1–14 days (Carlson 1980b; Fowles et al. 1994; Hallgren et al. 2001; Mercado-Feliciano and Bigsby 2008a; Richardson et al. 2008; Stoker et al. 2004, 2005; Zhou et al. 2001). However, no exposure-related changes in relative liver weight were observed in female rats exposed to tetraBDE at doses up to18 mg/kg/day for 14 days via

gavage (Hallgren and Darnerud 2002). In the studies that evaluated hepatic enzyme induction (e.g., elevated EROD, MROD, PROD, and UDPGT activity and/or increased CYP protein levels), significantly increased enzyme levels and activities were observed in rats and mice at gavage doses of ≥6 and ≥3 mg/kg/day, respectively, and were always observed at doses at or below the dose causing elevated liver weights in the same study (Carlson 1980b; Fowles et al. 1994; Hallgren and Darnerud 2002; Hallgren et al. 2001; Richardson et al. 2008; Stoker et al. 2004; Zhou et al. 2001). Exposure to a single pentaBDE dose of 0.03 or 0.6 mg/kg on GD 6 via gavage caused significant induction of hepatic enzymes (EROD, UDPGT) in dams and pups from the 0.6 mg/kg group on PND 22; liver weight was not evaluated (Kuriyama et al. 2007). Collectively, these findings support that elevated liver weight following exposure to lower-brominated PBDEs is associated with hepatic enzyme induction.

Richardson et al. (2008) evaluated genomic changes in mice following exposure to tetraBDE at 0, 3, 10, or 100 mg/kg/day for 4 days via gavage. The mRNA levels of several hepatic enzymes were significantly elevated at ≥20 mg/kg/day (Cyp2b10, Ugt1a1, Ugt1a7, Ugt2b2). In addition, several exposure-related changes were observed in hepatic efflux transporter (Mrp3, Mdr1a) and thyroid hormone transporter (Ttr, Mct8) mRNA expression levels in hepatic tissue (Richarson et al. 2008).

To determine if PBDE exposure alters vitamin A homeostasis in a manner similar to the related PBBs (ATSDR 2004), hepatic vitamin A levels were measured in mice and rats exposed to pentaBDE at 0, 18, or 36 mg/kg/day and mice exposed to tetraBDE at 0 or 18 mg/kg/day for 14 days via gavage (Hallgren et al. 2001). In pentaBDE-exposed animals, hepatic vitamin A levels were significantly decreased by 24 and 26% in rats in the 18 and 36 mg/kg/day groups, respectively, and 22% in mice in the 36 mg/kg/day group. No changes in hepatic vitamin A levels were observed in mice exposed to penta- or tetraBDE at 18 mg/kg/day.

DecaBDE: Unlike the lower-brominated PBDEs, there is no evidence of hepatic toxicity following acute exposure to decaBDE. Exposure to decaBDE at doses up to 1,000 mg/kg/day for 7 or 14 days via gavage did not produce liver damage, as indicated by liver histology or clinical chemistry, nor did it produce increased liver weight or hepatic enzyme induction in female rats (Bruchajzer et al. 2010). Carlson (1980b) observed no changes in serum SDH activity in rats exposed to decaBDE at 95.9 mg/kg/day for 14 days via gavage; significantly elevated liver weights were found, but in the absence of hepatic enzyme induction. In a shorter-duration study, exposure to decaBDE at doses up to 100 mg/kg/day for 4 days via gavage did not cause changes in liver weight or hepatic enzyme induction in female rats; clinical chemistry and histology were not examined (Zhou et al. 2001).

Sakamoto et al. (2013) evaluated both liver weight and hepatocyte proliferation in female rats following exposure to decaBDE at 980 mg/kg/day for 1 week. Consistent with other studies, no exposure-related findings in absolute or relative liver weight were observed. Additionally, no exposure-related changes in hepatocyte proliferation were observed, as evaluated by labeling indices of proliferating cell nuclear antigen (PCNA) in paraffin-embedded liver sections.

Intermediate-Duration Animal Studies

Lower-brominated PBDEs: Histopathological effects in the liver have been consistently observed in animals exposed to lower-brominated PBDEs for 15–90 days at doses as low as 2 mg/kg/day. Hepatocytomegaly (hepatocyte hypertrophy) was observed in rats exposed to dietary pentaBDE at doses ≥2 mg/kg/day (lowest dose tested) for 13 weeks (WIL Research Laboratories 1984). The hepatocytomegaly was dose-related with respect to severity (some affected hepatocytes at higher doses had vacuoles that likely contained lipid) and was not completely reversible, as it was still evident in ≥10 mg/kg/day males and 100 mg/kg/day females at 24 weeks postexposure in lessened severity and incidence. Females exposed to 2 or 100 mg/kg/day pentaBDE for 90 days also had an increased incidence of degeneration and necrosis of individual liver parenchymal cells at 24 weeks postexposure; the investigators concluded that this may represent the final loss of previously damaged cells and probably should be considered compound-related (WIL Research Laboratories 1984). Similarly, dietary exposure to octaBDE at 0, 100, 1,000, or 10,000 ppm (0, 5, 50, or 600 mg/kg/day in males and 0, 7, 70, or 750 mg/kg/day in females) for 13 weeks caused liver lesions in 40% of males at 5 mg/kg/day and 100% of both sexes at ≥50–70 mg/kg/day (IRDC 1977). The lesions were dose-related in severity as well as incidence and characterized by cytomegaly, change in hepatocytic cytoplasm to a finely granular, homogeneous type, and cytoplasmic vacuolation. At 600–750 mg/kg/day, many of the livers had vacuolation of centrolobular hepatocytes and some had hepatocyte necrosis. Examinations performed at 8 weeks and 6 months postexposure showed that the liver effects persisted in the rats exposed to ≥50–70 mg/kg/day for 13 weeks (IRDC 1977).

Hepatocellular hypertrophy was also observed in rats exposed to dietary penta- or octaBDE at ≥9 mg/kg/day for 28 days (IRDC 1976), in rats exposed to pentaBDE at ≥3 mg/kg/day for 15–28 days via gavage (Becker et al. 2012; Fattore et al. 2001; Oberg et al. 2010), and in rats exposed to tetraBDE at 150 mg/kg/day via gavage for 12 weeks (only dose tested) (Zhang et al. 2015a, 2015b). No histopathological changes were observed in mice exposed to tetraBDE at 1 mg/kg/day via gavage for

6 weeks (only dose tested) (McIntyre et al. 2015). In another gavage study, rats were exposed to pentaBDE at doses of 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7, or 200 mg/kg/day for 28 days (Van der ven et al. 2008b). The study authors reported centrilobular hypertrophy and an increased ratio of binucleated hepatocytes; however, the incidence data and dose(s) at which effects were observed were not reported (Van der ven et al. 2008b). Van der ven et al. (2008b) also reported a near-significant 5-fold increase in the number of apoptotic cells in males exposed to 200 mg/kg/day (p=0.067). Rats exposed to pentaBDE at 200 mg/kg/day for 21or 28 days via gavage showed "minor lesions" in the liver, but no further information regarding the type or incidence of the lesion was reported (Bruchajzer et al. 2010). In mice, exposure to dietary tetraBDE at 0.45 mg/kg/day for 28 days induced hepatocyte vacuolation, pyknotic nuclei in the hepatocytes, and periportal lymphocytic infiltration; no lesions were observed in control animals (Maranghi et al. 2013).

In rats exposed to pentaBDE via gavage for 70 days prior to mating until PND 42, significantly increased incidences of hepatocellular hypertrophy were observed in F0 males at ≥5 mg/kg/day and F0 females and F1 offspring at 25 mg/kg/day; no exposure-related effects were observed at 0.5 mg/kg/day (Bondy et al. 2013). Similarly, all F1 rats exposed to pentaBDE at 50 mg/kg/day from GD 6 to PNW 16 via gavage showed hepatocellular hypertrophy characterized by enlarged hepatocytes with an increased amount of cytoplasm, enlarged nuclei, and pale eosinophilic and granular cytoplasm. Hepatocyte vacuolization was also significantly increased in exposed F1 males, but not F1 females (Dunnick et al. 2012).

No exposure-related changes were observed in serum chemistry markers (ALT, AST, ALP, LDH, cholesterol, triglycerides, albumin, globulin) in rats exposed to dietary pentaBDE at doses up to 100 mg/kg/day for 13 weeks (WIL Research Laboratories 1984), octaBDE at doses up to 750 mg/kg/day for 90 days (IRDC 1977), or a penta-decaBDE mixture (52.1% pentaBDE, 44.2% decaBDE, 0.4% octaBDE) at 20 mg/kg/day for 70 days (Ernest et al. 2012). Similarly, no exposure-related changes were observed in serum ALP, ALT, AST, or gamma-glutamyl transferase (GGT) following exposure to pentaBDE at low doses up to 0.015 mg/kg/day via gavage for 90 days (Daubie et al. 2011). Marginal changes (<2-fold) were observed in shorter-duration rat gavage studies with higher doses. A statistically significant 51% increase in serum ALT level was observed in male rats exposed to pentaBDE at 250 mg/kg/day via gavage for 28 days; no changes were observed in males at 2.5 or 25 mg/kg/day or females at any dose (Oberg et al. 2010). No exposure-related changes were observed in serum ALP (Oberg et al. 2010). Similarly, in female rats exposed to pentaBDE at 200 mg/kg/day via gavage for 21 or 28 days, serum ALT levels were increased to 190% of control values at 21 or 28 days and serum AST levels were increased to 185% of control values at 28 days; no changes were observed at ≤40 mg/kg/day

(Bruchajzer et al. 2010). Increases in serum cholesterol, total protein, and ALT and decreases in the albumin/globulin ratio were observed in rats exposed to pentaBDE at 250 mg/kg/day, but the magnitudes of these effects were not reported (Fattore et al. 2001). In another gavage study, rats were exposed to pentaBDE at doses of 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7, or 200 mg/kg/day for 28 days (Van der ven et al. 2008b). The study authors reported a dose-related increase in serum ALT in male rats (maximal increase of 148.1%); however, the lowest dose at which the effect was observed was not reported. Instead, results were reported in terms of BMD analysis (BMD/$BMDL_{RD10\%}$=61.4/15.5 mg/kg/day). No changes in serum ALT were observed in female rats, and no changes in serum ALP were observed in either sex, although the authors noted procedural problems with their ALP assay (Van der ven et al. 2008b). The study authors reported dose-related increases in serum cholesterol in male and female rats (maximal increases in males and females were 257 and 144%, respectively). Again, the lowest dose at which the effect was observed was not reported (BMD/$BMDL_{RD10\%}$=15.4/8.4 mg/kg/day in males and 22.4/11.6 mg/kg/day in females) (Van der ven et al. 2008b). In mice, serum ALT levels were significantly elevated by approximately 2-fold in males exposed to tetraBDE at gavage doses of 150 mg/kg/day for 12 weeks, compared with control (only dose tested); no other serum biochemistry endpoints were evaluated (Zhang et al. 2015a, 2015b).

Significant changes in hepatic oxidative stress parameters were observed in rats following exposure to pentaBDE at doses ≥8 mg/kg/day via gavage for 21 or 28 days, including increased hepatic GSH and MDA levels (Bruchajzer et al. 2010). Oxidative stress markers were also significantly altered in the livers of rat offspring following exposure to pentaBDE from GD 6 to PND 21, including significantly increased activities of catalase (CAT) activity at ≥1 mg/kg/day and SOD at 2 mg/kg/day (Blanco et al. 2014). There was no change in total levels of thiobarbituric acid reactive substances (TBARS) in offspring (Blanco et al. 2014). In mice, exposure to diBDE at 1.2 mg/kg/day via gavage for 28 days caused significantly decreased levels of GSH, decreased activities of SOD and glutathione peroxidase (GPx), and increased levels of MDA in the liver (Zhang et al. 2014).

Female rats were examined for hepatic porphyria following exposure to pentaBDE or octaBDE at doses of 0, 2, 8, 40, or 200 mg/kg/day via gavage for 21 or 28 days (Bruchajzer 2011; Bruchajzer et al. 2012). PentaBDE caused significant dose-related elevations in total porphyrin levels in the liver following exposure to ≥8 mg/kg/day for 21 days (~3–8-fold) or 28 days (~3–19-fold), compared to vehicle controls (Bruchajzer 2011), OctaBDE cause significant elevations in liver concentrations of high carboxylated porphyrins (octa- and hepta-) following exposure to ≥8 mg/kg/day for 21 days (~2–3-fold) or 2 or 8 mg/kg/day for 28 days (~3–4-fold) (Bruchajzer et al. 2012). At 28 days, high carboxylated porphyrin

levels in the 40 and 200 mg/kg/day groups were not were not significantly elevated compared to the vehicle control. However, the vehicle controls at this duration had an unusually high hepatic porphyrin levels (4-fold increase compared with untreated control). When compared with the untreated controls, porphyrin levels were significantly increased by ~10-, 14-, 4-, and 5-fold in the 2, 8, 40, and 200 mg/kg/day groups, respectively. Lower carboxylated porphyrins were <1% of total liver porphyrins measured, and were not further analyzed. These studies also measured ALA-S and ALA-D activity, two enzymes involved in heme biosynthesis. The results were mixed. PentaBDE exposure caused a significant elevation in ALA-S activity at ≥8 mg/kg/day after 21 days and at ≥2 mg/kg/day after 28 days, while octaBDE exposure caused a significant decrease in ALA-S activity at 200 mg/kg/day after 21 days and at ≥40 mg/kg/day after 28 days. ALA-D activity was also significantly decreased in rats exposed to octaBDE at ≥40 mg/kg/day for 21 or 28 days (ALA-D activity was not assessed in pentaBDE-exposed rats) (Bruchajzer 2011; Bruchajzer et al. 2012). Hepatic porphyria was also observed in rats after exposure to dietary pentaBDE for 90 days (WIL Research Laboratories 1984). Liver porphyrins were significantly elevated by 8.5- and 390-fold in males and females from the 100 mg/kg/day group, respectively, and 3-fold in females from the 20 mg/kg/day group; no significant changes were observed in the 2 mg/kg/day group (WIL Research Laboratories 1984).

Elevated liver weights have been reported following intermediate-duration exposure to penta-, octa-, tetra-, or diBDE in several animal studies. Significantly increased liver weights were reported in rats and mice exposed to penta-, di-, or tetraBDE via gavage at ≥1.2, ≥50, and 150 mg/kg/day, respectively, for 15–84 days (Becker et al. 2014; Bruchajzer et al. 2010; Fattore et al. 2001; Mercado-Feliciano and Bigsby 2008a; Oberg et al. 2010; Stoker et al. 2004, 2005; Van der Ven et al. 2008b; Zhang et al. 2014, 2015a, 2015b). No exposure-related changes in liver weight were observed in mice exposed to tetraBDE at 1 mg/kg/day via gavage for 6 weeks (only dose tested) (McIntyre et al. 2015). In dietary studies, significantly increased liver weights were reported in rats exposed to penta- or octaBDE at ≥5 mg/kg/day for 28–90 days (IRDC 1976, 1977; WIL Research Laboratories 1984) or a penta-decaBDE mixture (52.1% pentaBDE, 44.2% decaBDE, 0.4% octaBDE) at 20 mg/kg/day for 70 days (Ernest et al. 2012). In mink exposed to dietary pentaBDE at 0, 0.08, 0.63, or 0.78 mg/kg/day for 90 days, increased absolute liver weight was observed at 0.78 mg/kg/day and increased relative liver weight was observed at ≥0.08 mg/kg/day; however, these findings are confounded by significant body weight loss at ≥0.63 mg/kg/day (Martin et al. 2007). No exposure-related changes in liver weight were observed in mice exposed to dietary tetraBDE at 0.45 mg/kg/day for 28 days (Maranghi et al. 2013).

In studies that evaluated hepatic enzyme induction (e.g., elevated EROD, MROD, PROD, and UDPGT activity), significantly increased enzyme activities were observed in rats, mice, and mink at doses of ≥2.5, ≥50, and ≥0.08 mg/kg/day, respectively, and were always observed at doses at or below the dose causing elevated liver weights in the same study (Bruchajzer et al. 2010; Ernest et al. 2012; Fattore et al. 2001; Martin et al. 2007; Mercado-Feliciano and Bigsby 2008a; Oberg et al. 2010; Stoker et al. 2004). Additionally, microsomal enzyme activity was induced in rats exposed by gavage to doses as low as 0.6 mg/kg/day of octaBDE and 0.4 mg/kg/day of pentaBDE for 90 days as indicated by increases in O-ethyl O-*p*-nitrophenyl phenylphosphonothioate (EPN) detoxification, *p*-nitroanisole demethylation, and cytochrome c reductase and cytochrome P-450 levels (Carlson 1980a). Some of these changes were persistent, lasting for 30–60 days after cessation of treatment.

Maternal and pup liver weights were significantly elevated in rats exposed to pentaBDE from GD 1 or GD 6 to PND 21 via gavage or dosed cookies at 30 and ≥3 mg/kg/day, respectively (Bowers et al. 2015; Ellis-Hutchings et al. 2006; Zhou et al. 2002). In mice, elevated maternal liver weights were observed in dams exposed to 452 mg/kg/day of the pentaBDE congener BDE 99, but not the pentaBDE commercial mixture Bromkal 70-5DE, from GD 6 to PND 17 via gavage (Skarman et al. 2005). Elevated liver weights were not observed in mouse pups (Skarman et al. 2005). In a one-generation study in rats (pre-mating day 70 to PND 42), F0 males and F1 offspring showed significantly increased liver weights on PND 43 following exposure to 25 and ≥5 mg/kg/day via gavage (Bondy et al. 2011, 2013). In other one-generation studies, no changes were observed in maternal or pup liver weight in rats exposed to pentaBDE-dose vanilla wafers at doses up to 11.4 mg/kg/day from pre-mating day 28 to PND 21 (Poon et al. 2011) or adult F1 liver weight in rats exposed to pentaBDE at 50 mg/kg/day from GD 6 to PNW 16 via gavage (Dunnick et al. 2012). In mink, exposure to dietary pentaBDE from 4 weeks premating until PNW 6 or 33 led to elevated liver weights in sows at 0.31 mg/kg/day (highest dose tested) and F1 offspring at 0.06 mg/kg/day (Zhang et al. 2009). In the studies that evaluated hepatic enzyme levels (e.g., EROD, PROD, UDPGT), significantly increased enzyme activities were always observed at doses at or below the dose causing elevated liver weights in the same study, with significant changes observed in F0 and F1 rats at ≥0.3 mg/kg/day, F0 and F1 mice at 450 mg/kg/day, and F0 and F1 mink at ≥0.06 and 0.01 mg/kg/day, respectively (Bowers et al. 2015; Skarman et al. 2005; Zhang et al. 2009; Zhou et al. 2002). Additionally, significant induction of hepatic enzymes was observed in male rat pups following exposure to pentaBDE at ≥1.7 mg/kg/day from GD 6 to PND 21 via gavage (Szabo et al. 2009).

Szabo et al. (2009) also evaluated genomic changes in F1 rats following exposure to pentaBDE at 0, 1.7, 10.2, or 30.6 mg/kg/day from GD 6 to PND 4 or 21 via gavage. Significant dose-related increases were

observed in hepatic mRNA expression levels of phase I enzymes (Cyp1a1, Cyp2b1, Cyp2b2, Cyp3a1), phase II enzymes (Ugt1a6, Ugt1a7, Ugt2b, Sult1b1), phase III influx transporters (Oatp1a4), phase II efflux transporters (Mdr1, Mrp2, Mrp3), the serum binding protein transthyretin (Ttr), and deiodinase 1 (d1) during exposure (PND 4 and 21); the majority of which no longer differed from control at PND 60. Genomic changes were also evaluated in F1 rats following exposure to pentaBDE at 0, 1, or 2 mg/kg/day via gavage (Blanco et al. 2014). Various cytochrome isoforms were evaluated, but mRNA levels were only significantly elevated for CYP2B1 in pup liver tissue from the 2 mg/kg/day group. Additionally, significant changes were observed in the thyroid hormone receptor, including decreased TRα1 mRNA in both dose groups, decreased TRβ1 mRNA at 2 mg/kg/day, and decreased TRα1 protein levels at 2 mg/kg/day. Cylcin D1 protein expression was significantly decreased in both groups and the phosphorylation levels of Akt and GSK3β were significantly decreased at 2 mg/kg/day.

To determine if PBDE exposure alters vitamin A homeostasis in a manner similar to the related PBBs (ATSDR 2004), hepatic vitamin A levels were measured in rats following exposure to pentaBDE at 0, 2.5, 25, or 250 mg/kg/day via gavage for 28 days (Oberg et al. 2010). Hepatic liver vitamin A content was significantly decreased in females at 25 mg/kg/day and males and females at 250 mg/kg/day by up to 36 and 47%, respectively (Oberg et al. 2010). Hepatic vitamin A levels were also significantly decreased by 13% in dams and 50% in F1 pups on PND 18 in rats exposed to pentaBDE at 0 or 18 mg/kg/day from GD 6 to PND 18 via gavage (Ellis-Hutchings et al. 2006). Additional pup sacrifices on PNDs 12 and 31 showed significant 59 and 25% decreases in hepatic vitamin A level, respectively (Ellis-Hutchings et al. 2006).

DecaBDE: Moderate hepatocellular hypertrophy was observed in all mice exposed to dietary decaBDE at 9,400 mg/kg/day for 28 days, but in none of the controls (Sakamoto et al. 2013). Similarly, slight centrilobular hypertrophy was “occasionally” observed in rats exposed to decaBDE at doses ranging from 1.87 to 60 mg/kg/day for 28 days via gavage, which was most obvious in “some” of the 60 mg/kg/day males (incidence data not reported) (Van der ven et al. 2008a). In an older study using an impure decaBDE compound (77% purity), centrilobular cytoplasmic enlargement and vacuolation were observed in male rats exposed to dietary doses of 800 mg/kg/day for 30 days (incidences not reported); no changes were observed at 8 or 80 mg/kg/day (Norris et al. 1973, 1975a). However, no exposure-related changes in liver histology were observed in rats and mice exposed to dietary decaBDE at estimated doses as high as 2,000–8,000 and 2,375–9,500 mg/kg/day for 13 weeks, respectively (NTP 1986), or in rats exposed to gavage doses up to 1,000 mg/kg/day for 21 or 28 days (Bruchajzer et al. 2010).

Pregnant dams and developing rats and mice appear to be more susceptible to liver damage following exposure to decaBDE than adult animals. Hepatocyte degeneration and eosinophil changes were observed in the livers of rat dams exposed to decaBDE at 300 mg/kg/day for 21 days prior to mating, through mating, gestation, and lactation (PND 21) (Liu et al. 2012). Compared with control, the exposed group had significantly increased "histological scores;" however, the method of histological scoring and incidences of lesions were not reported (Liu et al. 2012). Following exposure to dietary decaBDE from GD 10 to PND 21, male and female rat pups showed significantly increased incidence of follicular cell hypertrophy in the liver at ≥2 and 146 mg/kg/day, respectively (Fujimoto et al. 2011). These findings were transient, as they were no longer evident in male and female offspring evaluated at PNW 11 (Fujimoto et al. 2011). Male offspring from mouse dams exposed to decaBDE from GD 0 to 17 via gavage showed dose-related histopathological changes in the liver on PND 71 at doses ≥10 mg/kg/day (lowest dose tested) (Tseng et al. 2008). Histological changes observed in exposed groups included acute cell swelling of hepatocytes associated with pressure occlusion of hepatic sinusoids (Tseng et al. 2008). Young male rats exposed to decaBDE at doses of 0, 100, 300, or 600 mg/kg/day from PND 10 to 42 via gavage showed fatty degeneration at ≥300 mg/kg/day (incidence data were not reported) (Lee et al. 2010).

Wang et al. (2010) found no exposure-related changes in serum ALT, AST, or ALP in male rats following gavage exposure to decaBDE at 100 mg/kg/day for 90 days, but serum total cholesterol, high density lipid-cholesterol, and total bile acid levels were significantly increased by 23, 26, and 98%, respectively. No biologically relevant, exposure-related changes were observed in either serum ALT or AST, or total cholesterol or triglyceride levels, in rats exposed to decaBDE at doses up to 1,000 mg/kg/day for 21–56 days (Bruchazjer et al. 2010; Van der ven et al. 2008a; Zhang et al. 2013a).

No exposure-related changes were observed in hepatic markers of oxidative stress (GSH, MDA) in female rats exposed to decaBDE up to doses of 1,000 mg/kg/day for 21 or 28 days (Bruchazjer et al. 2010).

Significantly elevated liver weights were observed in male rats after exposure to decaBDE at ≥1 mg/kg/day for 8 weeks via gavage (Zhang et al. 2013a) and male mice after exposure to dietary decaBDE at 9,400 mg/kg/day for 28 days (Sakamoto et al. 2013). However, no exposure-related changes in liver weight were observed in rats exposed to decaBDE up to doses of 1,000 mg/kg/day via gavage for 21–90 days (Bruchazjer et al. 2010; Van der ven et al. 2008a; Wang et al. 2010, 2011b). In an older study using an impure decaBDE compound (77% purity), increased liver weights were observed at dietary doses ≥80 mg/kg/day in male rats exposed for 30 days (Norris et al. 1973, 1975a). Following exposure to

dietary decaBDE from GD 10 to PND 21, male and female rat pups showed significantly increased liver weights at ≥2 and 146 mg/kg/day, respectively (Fujimoto et al. 2011). These findings were transient, as they were no longer evident in male and female offspring evaluated at PNW 11 (Fujimoto et al. 2011). No liver weight changes were observed in dams or PND 71 male offspring exposed to decaBDE from GD 0 to 17 via gavage at doses up to 1,500 mg/kg/day (Tseng et al. 2008). Young male rats exposed to decaBDE at doses of 0, 100, 300, or 600 mg/kg/day from PND 10 to 42 via gavage showed elevated liver weights at ≥300 mg/kg/day (Lee et al. 2010).

Hepatic enzyme induction (e.g., EROD, PRO activity, CYP protein levels) was significantly elevated in a dose-related manner in adult male and female rats exposed to decaBDE at 1.87–60 mg/kg/day for 28 days via gavage (Van der ven et al. 2008a) and young male rats exposed to decaBDE at 100–600 mg/kg/day from PND 10 to 42 via gavage (Lee et al. 2010). EROD activity was also significantly elevated in PND 71 male mice exposed to 1,500 mg/kg/day during gestation (Tseng et al. 2008). However, no exposure-related changes were observed in hepatic enzyme induction in female rats exposed to decaBDE up to doses of 1,000 mg/kg/day for 21 or 28 days (Bruchazjer et al. 2010). Genomic analyses of liver tissue reported elevated mRNA levels of CYP proteins in male mice exposed to dietary decaBDE at 9,400 mg/kg/day for 28 days (Sakamoto et al. 2013) and a dose-dependent elevation of Cyp2b2 mRNA in male rats exposed to decaBDE at doses of 1.87–60 mg/kg/day for 28 days via gavage (Van der ven et al. 2008a).

To determine if PBDE exposure alters vitamin A homeostasis in a manner similar to the related PBBs (ATSDR 2004), hepatic vitamin A levels were measured in rats following exposure to decaBDE at 0, 1.87, 3.75, 7.5, 15, 30, or 60 mg/kg/day for 28 days via gavage (Van der ven et al. 2008a). In females, but not males, hepatic vitamin A levels were increased in a dose-dependent manner by up to 14.6%, compared with controls; however, the lowest dose at which the effect was observed was not reported. The study authors conclude that the relevance of the effect is uncertain due to high variation in the data, as evidenced by a high BMD/BMDL ratio ($BMD/BMDL_{RD10\%}$ =13.8/1.2 mg/kg/day).

Chronic-Duration Animal Studies

Lower-brominated PBDEs: No chronic-duration studies analyzing hepatic effects were located for lower-brominated PBDEs.

DecaBDE: In chronic studies, exposure to 94–97% decaBDE for 103 weeks caused liver lesions that included neoplastic nodules in rats at ≥1,120 mg/kg/day, thrombosis and degeneration in rats at 2,240 mg/kg/day, and centrilobular hypertrophy and granulomas in mice at ≥3,200 mg/kg/day (NTP 1986). The thrombosis in the rats was characterized by a near total occlusion of a major hepatic blood vessel by a dense fibrin coagulum. A NOAEL was not identified in the rats or mice. The only other chronic study of decaBDE found that exposure to 1 mg/kg/day of a 77% pure mixture for 2 years caused no liver effects in rats; higher doses were not tested, precluding identification of a LOAEL (Kociba et al. 1975; Norris et al. 1975a).

Summary. No studies are available on hepatic effects of PBDEs in humans. Based on the evidence in animals, including enzyme induction, liver enlargement, histological lesions, hepatic porphyria, and markers of hepatic oxidative stress, PBDEs are potentially hepatotoxic in humans, especially lower-brominated PBDEs.

Renal Effects. Only one study was located that provides information on renal effects in humans following exposure to PBDEs. A pilot study measured serum creatinine and urinary β_2-microglobulin, as indices of renal function, in 40 Chinese residents exposed to PBDEs in an electronic waste dismantling area (Xu et al. 2015a). Mean ΣPBDEs in serum from exposed subjects was almost twice that in a control group, but the difference was not statistically significant. The results showed that neither serum creatinine nor β_2-microglobulin were correlated with PBDEs. However, congener analyses showed that BDE 28, BDE 47, BDE 85, and BDE 153 were positively correlated with urinary levels of β_2-microglobulin, and that BDE 28 and BDE 85 were positively correlated with serum creatinine levels. PCBs also played a role in the findings reported.

Acute-Duration Animal Studies

Lower-brominated PBDEs: Two studies examined renal effects in male rats 45 days after a single gavage administration of pentaBDE at doses of 0, 0.06, or 1.2 mg/kg (Albina et al. 2010; Alonso et al. 2010). In urine, a 4-fold increase in total protein levels was observed at 1.2 mg/kg (Alonso et al. 2010). Although this might suggest possible tubular damage, histopathological examination of the kidneys found no abnormalities other than a dose-related increase in phagolysosomes (incidence data not reported) (Albina et al. 2010). No other changes were observed in urinalysis or serum chemistry parameters (urea, creatinine, uric acid) (Alonso et al. 2010). Altered oxidative stress markers were found in the kidney after

exposure to 1.2 mg/kg/day, including significantly decreased CAT activity and increased GSSG and GSSG/GSH ratio (Albina et al. 2010).

No exposure-related changes in kidney weight were observed in male rats exposed to pentaBDE at doses up to 240 mg/kg/day for 9 days via gavage (Stoker et al. 2005) or female mice exposed to tetraBDE at doses up to 100 mg/kg/day for 4 days via gavage (Richardson et al. 2008).

DecaBDE: No acute-duration studies analyzing renal effects were located for decaBDE.

Intermediate-Duration Animal Studies

Lower-brominated PBDEs: Renal effects induced by dietary octaBDE included non-inflammatory kidney changes in male rats exposed to 600 mg/kg/day for 13 weeks, but not females exposed to doses up to 750 mg/kg/day (IRDC 1977). The incidence and severity of the kidney lesions (tubule regeneration, intratubular casts, and cellular debris occurred in most 600 mg/kg/day males) suggested a compound-related effect (IRDC 1977). However, no histopathological lesions of the kidney were observed in male or female rats exposed to pentaBDE at doses up to 250 mg/kg/day for 28 days via gavage (Oberg et al. 2010; Van der ven et al. 2008b), dietary penta- or octaBDE at doses up to 90 mg/kg/day for 28 days (IRDC 1976), or dietary pentaBDE doses up to 100 mg/kg/day for 90 days (WIL Research Laboratories 1984). Additionally, no histopathological kidney lesions were observed in F0 or F1 rats exposed to pentaBDE at doses up to 25 mg/kg/day from pre-mating through PND 42 (Bondy et al. 2013).

Statistically significant changes in blood urea and urea nitrogen levels were reported in some intermediate-duration studies; however, none of the changes were considered biologically relevant due to the small magnitude of change (<2-fold) compared with control. Following exposure to pentaBDE at 0, 2.5, 25, or 250 mg/kg/day via gavage for 28 days, blood urea levels in male rats from the 25 or 250 mg/kg/day groups were significantly increased by 1.5- and 1.2-fold, respectively; no changes were observed in blood urea levels in females or blood creatinine in either sex (Oberg et al. 2010). In another 28-day study, rats were exposed to pentaBDE at 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7, or 200 mg/kg/day via gavage (Van der ven et al. 2008b). Study authors reported dose-related increases in blood urea levels in male and female rats (maximal increases in males and females were 61.2 and 33.1%, respectively); however, the lowest dose at which the effect was observed was not reported. Instead, results were reported in terms of BMD analysis ($BMD/BMDL_{RD10\%}$=64.2/30.2 mg/kg/day in males and 65.1/22.2 mg/kg/day in females) (Van der ven et al. 2008b). Again, no changes in serum creatinine were

reported in either sex at doses up to 200 mg/kg/day (Van der ven et al. 2008b). Blood urea nitrogen (BUN) levels were elevated by 35% in male rats and 59% in female rats exposed to dietary octaBDE for 13 weeks at 600 and 750 mg/kg/day, respectively (IRDC 1977). No changes in BUN levels were observed in male or female rats exposed to dietary pentaBDE doses up to 100 mg/kg/day for 90 days (WIL Research Laboratories 1984).

Kidney weight changes following intermediate-duration exposure are inconsistent. A significant 10% decrease in relative kidney weight was observed in mice exposed to diBDE at 1.2 mg/kg/day for 28 days via gavage (Zhang et al. 2014). Absolute organ weights were not reported; however, no body weight effects were observed. In contrast, no change in kidney weight was reported in rats following exposure to pentaBDE at gavage doses up to 120 mg/kg/day for 20–90 days (Daubie et al. 2011; Stoker et al. 2004, 2005) or dietary penta- or octaBDE at doses up to 750 mg/kg/day for 28–90 days (IRDC 1976, 1977; WIL Research Laboratories 1984). Similarly, no change in F0 or F1 kidney weight was observed in rats exposed to pentaBDE at doses up to 25 mg/kg/day via gavage from 70 days prior to mating through PND 42 (Bondy et al. 2013) or mink exposed to dietary pentaBDE at doses up to 0.31 mg/kg/day from 4 weeks prior to mating to PNW 6 or 33 (Zhang et al. 2009). In other studies, significantly increased kidney weights have been reported, including a dose-related increase in absolute liver weight in rats exposed to pentaBDE doses up to 200 mg/kg/day for 28 days via gavage (dose at which effect was first observed was not reported; maximum increase of 11.5% in males and 15.6% in females) (Van der ven et al. 2008b), a significant 15% increase in relative, but not absolute, kidney weight following exposure to pentaBDE at 250 mg/kg/day for 28 days via gavage (Oberg et al. 2010), and a significant 18% increase in relative kidney weight following exposure to a dietary penta-decaBDE mixture (52.1% pentaBDE, 44.2% decaBDE, 0.4% octaBDE) at 20 mg/kg/day for 70 days (absolute kidney weight not reported) (Ernest et al. 2012).

To determine if PBDE exposure alters vitamin A homeostasis in rats in a manner similar to the related PBBs (ATSDR 2004), vitamin A levels were measured in kidney tissue following exposure to pentaBDE at 0, 2.5, 25, or 250 mg/kg/day via gavage (Oberg et al. 2010). No significant changes in kidney vitamin A levels were observed at any dose.

DecaBDE: No renal histopathological changes were observed in rats or mice exposed to dietary decaBDE at doses up to 8,000 or 9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986). A 28-day study that evaluated histology of “major organs” did not report any exposure-related kidney effects in rats exposed to decaBDE at doses up to 60 mg/kg/day via gavage (Van der ven et al. 2008a). Studies of low

purity (≈77%) commercial decaBDE mixtures found kidney pathology (hyaline degenerative cytoplasmic changes) in male rats exposed to dietary levels of 800 mg/kg/day for 30 days (Norris et al. 1973,1975a), but not in rats exposed to ≤90 mg/kg/day for 28–30 days (IRDC 1976; Norris et al. 1973, 1975a). Interpretation of this finding is complicated by the fact that hyaline degenerative cytoplasmic changes are not uncommon in adult male rats and might be induced by a mechanism specific to certain aged male rats.

No exposure-related changes were observed in serum urea or creatinine levels in rats exposed to decaBDE at doses up to 100 mg/kg/day for 28–90 days via gavage (Van der ven et al. 2008a; Wang et al. 2010). Additionally, no exposure-related changes were observed in kidney weights in rats exposed to decaBDE at doses up to 600 mg/kg/day for 28–90 days via gavage (Lee et al. 2010; Van der ven et al. 2008a; Wang et al. 2010, 2011b; Zhang et al. 2013b), F0 or F1 mice exposed to decaBDE at doses up to 1,500 mg/kg/day from GD 0 to 17 via gavage (Tseng et al. 2008), or dietary decaBDE at doses up to 800 mg/kg/day for 28–30 days (IRDC 1976; Norris et al. 1973, 1975a).

Chronic-Duration Animal Studies

Lower-brominated PBDEs: No chronic-duration studies analyzing renal effects were located for lower-brominated PBDEs.

DecaBDE: No renal histopathological changes were observed in rats or mice exposed to dietary decaBDE at doses up to 2,550 or 7,780 mg/kg/day, respectively, for 2 years (NTP 1986). The only other chronic study of decaBDE found that exposure to dietary doses up to 1 mg/kg/day of the 77% pure mixture for 2 years caused no exposure-related changes in kidney histology or weight in rats (Kociba et al. 1975; Norris et al. 1975a).

Summary. No studies are available on hepatic effects of PBDEs in humans. While there is limited evidence from animal studies that lower-brominated PBDEs can cause kidney damage at high exposure levels, data are inconsistent and there is no evidence of impaired renal function. Animal studies do not indicate that decaBDE causes renal toxicity. Taken together, animals studies indicate that renal effects are not likely to occur in humans at environmentally-relevant exposure concentrations.

Endocrine Effects.

Human Studies. Numerous studies have been performed to evaluate the relationship between concentrations of PBDE in body tissues and circulating hormone levels in human populations. While these studies have demonstrated that PBDE can perturb the human endocrine system and affect hormone levels, the specific findings are not consistent across studies. For example, even limiting the discussion to studies that evaluated both PBDE concentrations and thyroid hormone levels in serum samples collected only from adult men, studies have reported positive associations with T_4 (Turyk et al. 2008), negative associations with T_4 (Abdelouahab et al. 2011), and no association with T_4 (Hagmar et al. 2001). These studies also reported either negative association with T_3 (Turyk et al. 2008) or no association with T_3 (Abdelouahab et al. 2011; Hagmar et al. 2001), and either negative association with TSH (Hagmar et al. 2001; Turyk et al. 2008) or no association with TSH (Abdelouahab et al. 2011). Populations in these studies were 308 adult male consumers of sport fish from U.S. Great Lakes with serum ∑PBDE ranging from 15.8 to 1,360 ng/g lipid (0.13–10.15 ng/g) with a median of 38.4 ng/g lipid (0.26 ng/g) (Turyk et al. 2008), 48 adult men recruited through an infertility clinic in Quebec with median serum ∑PBDE of 0.302 ng/mL and maximum of 2.250 ng/mL (Abdelouahab et al. 2011), and 110 adult men from Latvia and Sweden having varying consumption of Baltic Sea fish with serum BDE 47 ranging from 0.10 ng/g lipid (10^{th} percentile) to 5.16 ng/g lipid (90^{th} percentile) and a median of 1.04 ng/g lipid (Hagmar et al. 2001).

Similarly, studies of serum PBDE and serum thyroid hormones in populations including adults of both sexes found: (1) a negative association with free T_4 and no association with T_4, T_3, or TSH in 114 elderly residents of the upper Hudson River area of New York State (∑PBDE range of 0.04–9.80 µg/L and median of 0.19 µg/L in 48 women; range of 0.04–4.74 µg/L and median of 0.16 µg/L in 66 men) (Bloom et al. 2014); (2) a negative association with total T_4 (BDE 47, BDE 99, and BDE 100) and a positive association with TSH (BDE 153 only), but no association with free T_4 or total T_3, in a longitudinal study of 51 healthy adult office workers from Boston with serum ∑PBDE geometric means ranging from 19 to 23 ng/g lipid over 18 months (Makey et al. 2016); (3) no association with free or total T_4, T_3, or TSH in 36 New York anglers living in counties adjacent to Lakes Erie or Ontario with median serum ∑PBDE of 15 ng/g lipid and maximum of 2,303 ng/g lipid (Bloom et al. 2008); (4) a positive association with T_3 (for BDE 47 only) and no association with free T_4 or TSH in 623 Nunavik Inuits with geometric mean BDE 47=2.16 and BDE 153=2.05 µg/kg lipid (Dallaire et al. 2009); (5) no association with T_4, T_3, or TSH in 11 Swedish electronics recycling workers sampled repeatedly over 1.5 years (∑PBDE median=7.2 pmol/g lipid weight at start of employment and 9.7 pmol/g lipid weight at the conclusion of the study) (Julander

et al. 2005); (6) a positive association with TSH in 23 Chinese e-waste workers (∑PBDE median=382 ng/g lipid with range of 77–8,452 ng/g lipid) versus 26 controls (∑PBDE median=158 ng/g lipid with range of 18–436 ng/g lipid) (Yuan et al. 2008); (7) a positive association with T_4 (for BDE 126 and BDE 205 only) and no association with free T_4, T_3, free T_3, or TSH in another group of 239 Chinese e-waste workers (∑PBDE median=189.79 ng/g lipid with range of 0–6,016 ng/g lipid) and 93 farmers from the e-waste area (∑PBDE median=164.64 ng/g lipid with range of 0–8,600 ng/g lipid) versus 116 controls (∑PBDE median=122.37 ng/g lipid with range of 0–1,398 ng/g lipid) (Wang et al. 2010); (8) a negative association with T_3 (for BDE 17 and BDE 153 only) and no association with free T_4 or TSH in 124 residents of northern China with serum ∑PBDE median=7.16 ng/g lipid with range of 2.09–160.3 ng/g lipid (Huang et al. 2014); (9) no associations between serum PBDEs or their methyoxylated or hydroxylated metabolites and thyroid hormones in 77 residents living near an e-waste recycling site in Vui Dau, Vietnam or 34 residents living in rural Doung Quang, Vietnam (median ∑PBDE serum concentrations of 290 and 230 pg/g wet weight, respectively) (Eguchi et al. 2015); and (10) no associations between serum PBDEs and thyroid hormones in 40 residents living near an e-waste recycling site in Luqiao, China or 15 residents living in rural Yunhe, China (median ∑PBDE serum concentrations of 51.61 and 66.45 ng/g, respectively) (Xu et al. 2015a). Similar studies performed in children found: (1) a positive association with serum PBDE for serum TSH in 195 6–8-year-old children from an e-waste recycling area in China (∑PBDE mean=664.28 ng/g lipid) or 174 children from a control area (∑PBDE mean=375.81 ng/g lipid) (Han et al. 2011); (2) a negative association with serum PBDE for free T_3 and a positive association with serum PBDE for TSH in 162 4–6-year-old children living in an e-waste area in China (serum ∑PBDE median=189.99 ng/g lipid) (Xu et al. 2014b); (3) no association between free and total T_3 and T_4, TSH, and serum PBDE in 21 8-year-old children from an e-waste recycling area in China (∑PBDE median=31.86 ng/g lipid) or 24 children from a control area (∑PBDE median=6.97 ng/g lipid) (Xu et al.2014a); (4) positive associations with T_3 and free T_4 (for BDE 99 only) and no association with T_4 or TSH in 17 Dutch teenagers with serum ∑PBDE ranging from 4.9–22.1 ng/g lipid and a mean of 10.5 ng/g lipid (Leijs et al. 2012); and (5) and negative or no association with free T_3 (depending on type of analysis), positive or no association with TSH, and no association with free T_4 in 515 Flemish teenagers with a median serum ∑PBDE of 7 ng/L and maximum of 125 ng/L (Kicinski et al. 2012).

Oulhote et al. (2016) examined the potential association between PBDE exposure and hypothyroidism in Canadian women aged 30–79 years. PBDE levels were not significantly different in women diagnosed with hypothyroidism (n=90; geometric mean serum ∑PBDE=15.4 ng/g lipid) compared with women without hypothyroidism (n=655; geometric mean serum ∑PBDE=20.5 ng/g lipid). In a model adjusted for age, income, education, alcohol consumption, race/ethnicity, and history of breast-feeding, the

prevalence ratio of hypothyroidism was marginally increased by 1.7 per 10-fold increase in ∑PBDEs (95% CI 1.0, 3.0) or BDE 47 (95% CI 1.0, 2.9) and marginally increased by 1.8 in individuals with detectable BDE 100 compared with those without detectable BDE 100 (95% CI 1.0, 3.3). When models were adjusted for age income, education, alcohol consumption, and lipid-standardized PCB-153, these marginal associations were no longer observed.

A couple of studies were located in which exposure was estimated from concentrations of PBDE in house dust, rather than serum samples. In the earlier study, Meeker et al. (2009) found a positive association between PBDE in house dust and serum levels of free T_4, but no association with T_3 or TSH in 24 men recruited through an infertility clinic. Median and maximum concentrations of PBDE in dust collected from houses of study subjects were 500 and 7,620 ng/g dust for BDE 47, 838,and 9,220 ng/g dust for BDE 99, and 180 and 2,830 ng/g dust for BDE 100. A later study by these same researchers found positive associations between dust concentrations of pentaBDE (sum of BDE 47, BDE 99, and BDE 100; median and maximum concentrations of 1,049 and 22,300 ng/g in dust) and octaBDE (sum of BDE 183 and BDE 201; median and maximum concentrations of 30.5 and 1,181 ng/g in dust) and serum free T_4, and also between pentaBDE and T_3 and octaBDE and TSH in 38 additional men recruited through the infertility clinic (Johnson et al. 2013). There were no significant associations between thyroid hormones and exposure to decaBDE (sum of BDE 206, BDE 207, BDE 208, and BDE 209; median and maximum concentrations of 1,800 and 38,483 ng/g in dust) in this study.

As in other populations, findings in pregnant women differed across studies. Both free and total T_4 were significantly and positively associated with PBDE (several individual congeners and their sum) in serum collected during the 3rd trimester (>34 weeks) of pregnancy in a North Carolina cohort of 137 primarily African-American expectant mothers with serum ∑PBDE ranging from 3.59 to 693.95 ng/g lipid and a median of 36.56 ng/g lipid (Stapleton et al. 2011). Associations for free and total T_3 and TSH were not significant in this cohort. In contrast, a study of a California cohort of 270 mostly Latina women tested at the 27th week of pregnancy (serum ∑PBDE range of 3.6–1338.6 ng/g lipid, median of 25.2 ng/g lipid) found no association between PBDE and free or total T_4 (Chevrier et al. 2010). However, all PBDE congeners identified and their sum were significantly negatively associated with TSH in this study. Further analysis showed that women in the highest quartile of PBDE exposure had significantly increased odds of subclinical hyperthyroidism (defined as low TSH and normal free T_4) relative to women in the first quartile. A study of 105 pregnant women in South Korean that looked at blood samples collected the day before delivery (serum ∑PBDE median=2.13 ng/g lipid with 25th–75th percentile range of 1.35–4.34 ng/g lipid) found significant negative associations for PBDE with free and total T_3, a significant

positive association with free T_4, and no association with T_4 or TSH (Kim et al. 2013a). There were no correlations between PBDE and free and total T_4 and free and total T_3 in maternal serum samples (∑PBDE range from 15 to 580 ng/g lipid, median=37 ng/g lipid) collected just prior to delivery in another study of 12 patients from Indiana (Mazdai et al. 2003).

Abdelouahab et al. (2013) compared maternal serum concentrations of PBDE collected at <20 weeks of pregnancy (for ∑PBDE, median=30.92 ng/g lipid, maximum=726.09 ng/g lipid, n=380) with thyroid hormone levels in maternal blood at <20 weeks of pregnancy and in maternal blood collected at delivery for 260 pregnancies in Quebec. At <20 weeks of pregnancy, they found significant negative associations between PBDE and T_3 and T_4, but significant positive associations between PBDE and free T_3 and free T_4. However, using the thyroid hormone levels from the maternal samples at delivery, they found negative associations between maternal serum PBDE at <20 weeks and maternal free and total T_3 and T_4 at delivery. There was no significant association with TSH for either of the samples. In a Swedish cohort (n=166), a significant inverse relationship was observed between first trimester total T_3 levels and maternal body burden of BDE 153 (median breast milk concentration, 0.48 ng/g lipid); this relationship was not significant for third trimester total T_3 levels (Lignell et al. 2016). No significant associations were observed between breast milk BDE 153 levels and free T_4 or TSH in first or third trimester serum samples. No associations were observed between serum thyroid hormone levels and breast milk concentrations of tetra-pentaBDE (BDE 47, BDE 99, BDE 100; median concentration, 2.3 ng/g lipid).

A few studies included analysis of maternal serum samples collected after delivery. Kim et al. (2011d, 2012b) found significant positive relationships between serum PBDE (BDE 49 only) and free T_4, and between PBDE (BDE 154 and BDE 153 only) and TSH, and a significant negative relationship between PBDE (BDE 153 only) and T_3 in maternal blood samples collected from 12 South Korean mothers after delivery (for ∑PBDE, range=1.88–53.54 ng/g lipid, mean=18.79 ng/g lipid). There were no such correlations between serum PBDE and thyroid hormone levels in post-delivery blood samples collected from 26 mothers of infants born with congenital hypothyroidism (for ∑PBDE, range=3.81–1563 ng/g lipid, mean=65.16 ng/g lipid) (Kim et al. 2011d, 2012b). In a second study in which maternal blood samples were collected after delivery (for 21 South Korean mothers undergoing Cesarean section), there was no correlation between PBDE concentrations and thyroid hormone (free T_4, T_3, and TSH) levels in the maternal serum (Kim et al. 2012a). In this study, ∑PBDE in maternal blood ranged from 1.8 to 17.66 ng/g lipid, with a median of 7.81 ng/g lipid. Kim et al. (2011a) reported a significant positive correlation between concentrations of BDE 153 in breast milk (mean ≈0.25 ng/g lipid) and serum TSH collected post-delivery in another group of South Korean mothers.

In addition to the maternal effects, several of these studies and others reported on thyroid hormone changes in developing offspring, as identified by umbilical cord or neonatal blood samples in relation to PBDE concentrations in maternal serum, cord serum, or breast milk. The data on thyroid hormone effects in developing offspring are presented in Section 3.2.2.6 on Developmental Effects.

Few human data were located on endocrine end points other than thyroid (discussed above) or sex hormones (discussed in Section 3.2.2.5, Reproductive Effects). Lim et al. (2008) performed an analysis of cross-sectional U.S. NHANES 2003–2004 data that showed significant increases in risk of diabetes and metabolic syndrome associated with serum concentrations of BDE 153 (but not BDE 28, BDE 47, BDE 99, or BDE 100) based on 156 and 237 cases, respectively, in a population of 1,367 adults examined for diabetes status and, for metabolic syndrome, a subset of 637 participants with a morning fasting blood sample. In both analyses, the risk of disease was higher with exposure to 25th–50th percentile BDE 153 concentrations (median=3.6 ng/g lipid) and 50–75th percentile BDE 153 concentrations (median=6.6 ng/g lipid) than >75th percentile BDE 153 concentrations (median=24.6 ng/g lipid). Adjusted ORs were 2.6, 2.7, and 1.8 for diabetes and 2.5, 2.4, and 1.7 for metabolic syndrome in the respective quartiles. Serum BDE 153 concentrations (0.04 ng/g serum) were also shown to be significantly associated with increased odds of developing gestational diabetes in 258 pregnant women from the LIFE cohort in Michigan and Texas; no associations were observed for BDE 28, BDE 47, BDE 85, BDE 99, BDE 100, or BDE 154 (Smarr et al. 2016). A report from the Great Lakes Fish Consumption Study in Wisconsin found no significant difference in total serum PBDE or BDE 47 concentrations between individuals who were diagnosed with (n=64; Ln∑PBDEs reported as 0.31 ng/g) compared with non-diabetics (n=349; Ln∑PBDEs reported as 0.30 ng/g) (Turyk et al. 2015). In the Great Lakes cohort, established biomarkers of diabetes (C-reactive protein [CRP], gamma glutamyl transferase [GGT] and adiponectin) were also not associated with PBDE exposure; however, in stratified models, Ln∑PBDE and LnBDE47 were significantly associated with LnGGT and Lnadiponectin in persons above the median age.

In elderly populations, cross-sectional and prospective studies have found no relationship between serum PBDE (BDE 47 and BDE 153) concentrations and diabetes in cohorts from Finland (Airaksinen et al. 2011) or Sweden (Lee et al. 2011). In the Finnish study, 308 participants with diabetes had median serum concentrations of 2.7 ng/g lipid BDE 47 and 1.5 ng/g lipid BDE 153, while 1,680 nondiabetic participants had median serum concentrations of 2.9 ng/g lipid BDE 47 and 1.7 ng/g lipid BDE 153 (Airaksinen et al. 2011). PBDE exposure levels for the Swedish study (n=725) were not available (Lee et al. 2011).

In conclusion, although numerous studies have attempted to evaluate the relationship between tissue concentrations of PBDE and endocrine changes in humans, no clear pattern has emerged. Studies have found different results even in similar populations (e.g., adult men or pregnant women) from the same part of the world (e.g., North America or Asia) exposed to similar concentrations of PBDE. Although the specific findings are not consistent across studies, the bulk of the data have demonstrated that PBDE can interact with the human endocrine system to affect hormone levels.

Acute-Duration Animal Studies

Lower-brominated PBDEs: No exposure-related changes in thyroid weight or histology were observed in female rats exposed to penta- or tetraBDE doses up to 36 mg/kg/day for 14 days via gavage (Darnerud and Sinjari 1996; Hallgren and Darnerud 1998; Hallgren et al. 2001). No other acute studies evaluating thyroid weight or histology were identified.

Altered thyroid hormone levels have been reported following acute exposure to lower-brominated PBDEs. Significant reductions in serum T_4 of 19–92% have been reported following gavage exposure to penta-, octa-, or tetraBDE at doses ≥10 and ≥0.8 mg/kg/day in rats and mice, respectively, for 1–14 days (Darnerud and Sinjari 1996; Fowles et al. 1994; Hallgren and Darnerud 1998, 2002; Hallgren et al. 2001; Hoppe and Carey 2007; Richardson et al. 2008; Stoker et al. 2004, 2005; Zhou et al. 2001). The decreases in serum T_4 were associated with reduced *ex vivo* binding of T_4 to the plasma thyroid hormone transporter protein TTR (Hallgren and Darnerud 1998). Significant reductions in serum T_3 were observed in rats exposed to penta- and octaBDE at doses ≥100 and ≥60 mg/kg/day, with maximum reductions up to 25–30% at 300 and 100 mg/kg/day, respectively, for 4 days via gavage (Zhou et al. 2001). No changes in serum T_3 were observed in rats exposed to pentaBDE at lower doses (≤60 mg/kg/day) for 5 days via gavage (Stoker et al. 2004). No compound-related changes were observed in serum TSH levels in rats or mice exposed to penta- or octaBDE at doses up to 300 mg/kg/day for 4–14 days via gavage (Darnerud and Sinjari 1996; Hallgren and Darnerud 1998, 2002; Hallgren et al. 2001; Stoker et al. 2004; Zhou et al. 2001). In a low-dose study, male rats did not show exposure-related changes in serum T_4, T_3, or TSH levels measured 45 days after a single exposure pentaBDE at doses up to 1.2 mg/kg/day via gavage (Alonso et al. 2010).

Following a single gavage exposure to pentaBDE on GD 6, reductions in serum T_4 levels were observed at doses ≥0.06 mg/kg in rat dams on PND 1 (23–33%), but not PND 22, and F1 males and females at 0.3 mg/kg/day on PND 22 (19–23%), but not PND 1 or 14; no changes in serum T_3 were observed in

dams or F1 rats at any time-point at doses up to 1.2 mg/kg (Kuriyama et al. 2007). Maternal serum T_4 was also reduced on GD 20 in rat dams exposed to pentaBDE doses ≥60 mg/kg/day from GD 6.5 to 19.5 via gavage, but not on GD 12 in rat dams exposed to up to 120 mg/kg/day on GDs 6.5–11.5 (Ellis-Hutchings et al. 2009). Exposure to pentaBDE at doses up to 120 mg/kg/day on GDs 6.5–11.5 or 6.5–19.5 did not significantly alter maternal serum T_3, TSH, or TTR levels (Ellis-Hutchings et al. 2009).

A limited amount of information is available on hormonal effects of PBDEs other than thyroid. There were no clear chemical-related changes in serum corticosterone levels in female mice that were exposed to pentaBDE at doses up to 72 mg/kg/day via gavage for 14 days (Fowles et al. 1994). Limited data regarding serum reproductive hormone levels after acute-duration exposure to lower-brominated PBDEs are discussed in Section 3.2.2.5 (Reproductive Effects).

DecaBDE: No exposure-related changes in serum T_4, T_3, or TSH were observed in female rats exposed to decaBDE at doses up to 100 mg/kg/day via gavage for 4 days (Zhou et al. 2001). In neonatal male rats exposed to doses of 0, 6, or 20 mg/kg/day from PND 2 to 15, serum T_4 was reduced by ~8 and 22% at 6 and 20 mg/kg/day, respectively (Rice et al. 2007). This finding was reported as a dose-related trend; however, pair-wise statistics were not reported. No exposure-related changes in serum T_4 levels were observed in similarly exposed neonatal females (Rice et al. 2007). In pregnant mice exposed to decaBDE at 0, 150, 750, 1,500, or 2,500 from GD 7 to 9 via gavage, maternal serum T_4 was significantly reduced by ~11 and 14% on GD 16 in the 1,500 and 2,500 mg/kg/day groups, respectively (data reported graphically) (Chi et al. 2011). In the 2,500 mg/kg/day group, serum T_3 levels were also significantly reduced by ~40% (Chi et al. 2011).

Intermediate-Duration Animal Studies

Lower-brominated PBDEs: In a comprehensive 90-day study in rats, incidences of thyroid follicular cell hyperplasia were 0/10, 2/10, 2/10, and 5/10 in males and 0/10, 0/10, 1/10, and 4/10 in females exposed to dietary pentaBDE at 0, 2, 10, and 100 mg/kg/day, respectively (WIL Research Laboratories 1984). The thyroid hyperplasia was mild and transient, as it was characterized as very slight in severity at all doses and was no longer observed at 24 weeks postexposure in any animals. In a 28-day dietary study, thyroid hyperplasia was equivocally increased in male rats that were exposed to 90 mg/kg/day of penta- or octaBDE (IRDC 1976). Incidences of slight or moderate hyperplasia in the 0, 9, or 90 mg/kg/day dose groups were 0/5, 1/5, and 3/5 in pentaBDE-exposed males and 0/5, 0/5, and 3/5 in the octaBDE-exposed males, respectively; no increases were seen in females (IRDC 1976). In males rats exposed to pentaBDE

at doses of 0, 3, 30, or 60 mg/kg/day for 15 days via gavage, follicular cell hypertrophy and hyperplasia were observed in the 30 and 60 mg/kg/day groups (13/15 and 10/15, respectively); however, incidences in control and low-dose groups were not reported (Becker et al. 2012). Additionally, when this study was repeated in a different laboratory, no treatment-related histological changes were observed in the thyroid from the 60 mg/kg/day group (animals from 3- and 30-mg/kg/day group were not evaluated) (Becker et al. 2012). Follicular cell hypertrophy was observed in 50% of F1 rats exposed to pentaBDE at 50 mg/kg/day for 18 weeks (GD 6 to PNW 16) via gavage; hypertrophy was not observed in any controls (Dunnick et al. 2012).

Other histological and morphological changes observed in the thyroid included increased epithelial thickness of inner follicles of the thyroid in male rats exposed to a dietary penta-decaBDE mixture (52.1% pentaBDE, 44.2 decaBDE, and 0.4% octaBDE) at 20 mg/kg/day for 70 days (incidence data not reported) (Ernest et al. 2012); altered morphology of the epithelium (tall columnar rather than the normal cuboidal type) in 4/35 male and 1/35 female rats exposed to estimated dietary octaBDE doses of 600 and 750 mg/kg/day, respectively, for 28 days (IRDC 1977); an increased incidence of cellular debris in the follicular lumen of the thyroid in female mice exposed to dietary tetraBDE at 0.45 mg/kg/day for 28 days (Maranghi et al. 2013); a significant 60–66% increase in the follicular epithelial height score, a 23–44% decrease in the colloid area, and a 12.5–13.3% increased incidence of follicular degeneration in male and female rats exposed to pentaBDE at 60 mg/kg/day via gavage for 31 or 20 days, respectively (Stoker et al. 2004); an increase in the observed degree of vacuolation in the thyroid of female mink exposed to 0.31 mg/kg/day for 16–17 weeks (4 weeks pre-mating through PNW 6) (Zhang et al. 2009); and a borderline significant (p=0.057) increase in thyroid follicular epithelial cell height in F1 mink exposed to 0.06 mg/kg/day via gavage during a one-generation study (4 weeks pre-mating through PNW 33) (Zhang et al. 2009). Additionally, the epithelial height of the inner follicles of the thyroids showed a biphasic response in rats following dietary exposure to a penta-decaBDE mixture (52.1% pentaBDE, 44.2 decaBDE, and 0.4% octaBDE) at 0. 02, 0.2, 2, or 20 mg/kg/day for 70 days (Ernest et al. 2012). Compared with controls, the epithelial height was significantly decreased at 0.02 mg/kg/day and significantly increased at 20 mg/kg/day (other doses were not significantly different from controls; quantitative data not reported) (Ernest et al. 2012). In contrast to findings in other studies, no exposure-related changes in thyroid histology were observed in male or female rats exposed to pentaBDE doses up to 200 mg/kg/day for 28 days via gavage (Van der ven et al. 2008b).

Relative, but not absolute, thyroid weights were significantly elevated by 50% in male and females exposed to dietary pentaBDE at 100 mg/kg/day for 90 days (WIL Research Laboratories 1984). Eight

weeks postexposure, thyroid weights were still significantly elevated by 40% in female, but not male, rats; no exposure-related changes were observed 24 weeks postexposure (WIL Research Laboratories 1984). Rats that were exposed to octaBDE in estimated dietary doses of 5, 50, or 600 mg/kg/day (males) or 7, 70, or 750 mg/kg/day (females) for 13 weeks had increased absolute and relative thyroid weights of 15–31 and 32–56%, respectively, at ≥50/70 mg/kg/day (IRDC 1977). The thyroid weight increases were still observed at 8 weeks postexposure in the 600/750 mg/kg/day groups (increased 67 and 13% in males and females, respectively). However, no exposure-related thyroid weight changes were observed in rats exposed to pentaBDE at doses up to 200 mg/kg/day for 15–28 days via gavage (Becker et al. 2012; Van der ven et al. 2008b), in rats exposed to dietary penta- or octaBDE at doses up to 90 mg/kg/day for 28 days (IRDC 1976), or in F0 or F1 mink exposed to dietary pentaBDE at doses up to 0.31 mg/kg/day in one generation studies (4 weeks pre-mating through PNW 6 or 33) (Zhang et al. 2009).

Following exposure to pentaBDE doses of 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7, or 200 mg/kg/day for 28 days via gavage, hyperemia in the zona reticularis of the adrenal gland was observed in ~50% of exposed male rats across all dose groups and occasionally in exposed female rats, but not in any control rats (incidence data for exposed animals not reported) (Van der ven et al. 2008b). In females, there was a dose-related increase in necrotic lesions in the zona reticularis, with pyknosis at 67 mg/kg/day and widespread necrosis at 200 mg/kg/day (Van der ven et al. 2008b). The authors reported that the combined incidence of hyperemia and zona reticularis necrosis was statistically significantly increased at the higher doses (precise doses not specified). No histological changes were observed in the adrenal gland of female mice exposed to dietary tetraBDE at 0.45 mg/kg/day for 28 days (Maranghi et al. 2013).

Mink that were exposed to dietary pentaBDE for 9 weeks had increased absolute and relative adrenal weights at 0.78 mg/kg/day (25 and 67%, respectively); no changes were observed at ≤0.63 mg/kg/day (Martin et al. 2007). No exposure-related adrenal weight changes were observed in rats exposed to pentaBDE at doses up to 240 mg/kg/day for 28–31 days via gavage (Stoker et al. 2005; Van der ven et al. 2008b), in rats exposed to dietary penta- or octaBDE at doses up to 90 mg/kg/day for 28 days (IRDC 1976), or in male or female rats exposed to dietary octaBDE doses up to 600 and 750 mg/kg/day, respectively, for 13 weeks (IRDC 1977). Adrenal weight was not assessed in any other intermediate-duration studies identified.

Serum T_4 levels were significantly reduced by 22–91% in rats exposed to pentaBDE at gavage doses of ≥3 mg/kg/day for 15–125 days (Becker et al. 2012; Driscol et al. 2009; Hoppe and Carey 2007; Stoker et al. 2004) or dietary pentaBDE at doses ≥20 mg/kg/day for 90 days (WIL Research Laboratories 1984).

Similarly, serum T_4 levels were significantly reduced by 22% in rats exposed to a dietary penta-decaBDE mixture (52.1% pentaBDE, 44.2 decaBDE, and 0.4% octaBDE) at 20 mg/kg/day for 70 days (Ernest et al. 2012). In a 28-day study, rats were exposed to pentaBDE at 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7, or 200 mg/kg/day via gavage (Van der ven et al. 2008b). Study authors report dose-related decreases in serum T_4 levels (maximal reduction of 88–89%); however, the doses at which these effects were observed were not reported. Instead, results were reported in terms of BMD analysis ($BMD/BMDL_{RD10\%}$= 1.4/1.1 mg/kg/day in males and 2.7/1.8 mg/kg/day in females) (Van der ven et al. 2008b). Serum T_3 levels were significantly reduced by 14–25% and serum TSH levels were significantly increased by 63–144% in male rats exposed to pentaBDE via gavage at ≥30 mg/kg/day for 15–31 days (Becker et al. 2012; Stoker et al. 2004). However, other studies reported no significant changes in serum T_3 and/or TSH levels in rats exposed to pentaBDE at doses up to 200 mg/kg/day for 20–28 days via gavage (Stoker et al. 2004; Van der ven et al. 2008b), dietary pentaBDE at doses up to 100 mg/kg/day for 90 days (WIL Research Laboratories 1984), or to a dietary penta-decaBDE mixture (52.1% pentaBDE, 44.2 decaBDE, and 0.4% octaBDE) at doses up to 20 mg/kg/day for 70 days (Ernest et al. 2012).

Altered thyroid hormone levels have also been reported in F0 animals following exposure to lower-brominated PBDEs in one-generation and gestational/lactation exposure studies. Following exposure to pentaBDE at 0, 0.5, 5, or 25 mg/kg/day via gavage for 70 days prior to mating through PND 42, serum T_4 levels were significantly reduced in F0 males at ≥5 mg/kg/day (50–87%) and females at 25 mg/kg/day (67%) (Bondy et al. 2011, 2013). In rat dams exposed to pentaBDE at 18 mg/kg/day via gavage from GD 6 to PND 18, serum T_4 levels were significantly decreased by 45%; no changes were observed in serum T_3, TSH, or TTR (Ellis-Hutchings et al. 2006). Following exposure to pentaBDE via gavage from GD 6 to PND 21, maternal serum T_4 levels were significantly reduced by ≥31% at ≥10.2 mg/kg/day, and maternal serum TSH levels were significantly increased by 127% at 30.6 mg/kg/day (Kodavanti et al. 2010; Zhou et al. 2002). No exposure-related changes were observed in serum T_3 (Kodavanti et al. 2010; Zhou et al. 2002). In dams exposed to 30 mg/kg/day via pentaBDE-dosed cookies from GD 1 to PND 21 via dose-cookies, significant decreases were observed in serum T_3 and T_4 levels (quantitative data not reported); no significant changes were observed in T_3 and T_4 levels at ≤3 mg/kg/day or TSH levels at doses up to 30 mg/kg/day (Bowers et al. 2015). In a similar study, a significant 25–50% decrease in maternal free and total T_4 levels was observed in dams exposed to ≥10 mg/kg/day via pentaBDE-dosed cookie from GD 6 to PND 21; no exposure-related changes were observed in T_3 or TSH levels at doses up to 30 mg/kg/day (Bansal et al. 2014). In rat dams exposed to tetraBDE via gavage from GD 1 to PND 14, maternal T_4 levels were significantly reduced on PND 1 and 7 by 28–31% at doses ≥3.2 mg/kg/day; no changes were observed in serum T_3 levels (Wang et al. 2011a). In mice, no exposure-related changes

were observed in maternal serum T_4 levels after exposure to pentaBDE at 452 mg/kg/day via gavage from GD 4 to PND 17 (Skarman et al. 2005). In one-generation dietary pentaBDE studies in mink (4 weeks premating through PNW 6), significant reductions in plasma T_3, but not T_4, were observed in F0 females exposed to 0.31 mg/kg/day (Zhang et al. 2009).

Numerous studies have also reported decreased serum T_3 and/or T_4 levels in pups after gestational and lactational exposure to penta- or tetraBDE at doses as low as 0.3 mg/kg/day in rats and at 452 mg/kg/day in mice (Bansal et al. 2014; Blanco et al. 2013; Bondy et al. 2011, 2013; Bowers et al. 2015; Ellis-Hutchings et al. 2006; Kodavanti et al. 2010; Miller et al. 2012; Poon et al. 2011; Shah et al. 2011; Skarman et al. 2005; Szabo et al. 2009; Wang et al. 2011a; Zhou et al. 2002). Changes observed in mink juveniles included decreases in serum T_3, as in the adults, but also an increase in serum T_4 in juvenile females (Zhang et al. 2009). See Section 3.2.2.6 (Developmental Effects) for more details.

Consistent, exposure-related changes were not observed between studies that evaluated serum reproductive hormone levels after intermediate-duration exposure to lower-brominated PBDEs; see Section 3.2.2.5 (Reproductive Effects) for more details.

DecaBDE: Dose-related increases in thyroid hyperplasia were reported for male Sprague-Dawley rats exposed to dietary decaBDE at 80 or 800 mg/kg/day for 30 days (Norris et al. 1973, 1975a), although not in rats exposed to ≤90 mg/kg/day for 90 days, rats exposed to ≤8,000 mg/kg/day for 13 weeks, or mice exposed to ≤9,500 mg/kg/day for 13 weeks (IRDC 1976; NTP 1986). The occurrence of thyroid hyperplasia in the rats exposed to ≥80 mg/kg/day for 30 days could be related to the low purity composition of the older commercial decaBDE mixture tested by Norris et al. (1973, 1975a) (i.e., 77.4% decaBDE, 21.8% nonaBDE, and 0.8% octaBDE, compared to the ≥94% decaBDE composition used in the NTP studies). Similarly, observed incidences of diffuse follicular cell hypertrophy were not significantly elevated in rat dams exposed to dietary decaBDE from GD 10 to PND 21 at doses up to 146 mg/kg/day, compared with control (Fujimoto et al. 2011). However, in young male rats exposed to decaBDE at doses of 0, 100, 300, or 600 mg/kg/day via gavage for 33 days (PNDs 10–42), multiple areas of degenerated follicular epithelium and slight attenuation of the follicular epithelium were observed in the thyroid glands of rat exposed to 300 or 600 mg/kg/day (incidence data not reported) (Lee et al. 2010).

In young male rats exposed to decaBDE at doses of 0, 100, 300, or 600 mg/kg/day via gavage for 33 days (PND 10–42), absolute and relative thyroid weights were significantly increased by 60 and 40%, respectively, in the 600 mg/kg/day group (Lee et al. 2010). Increased absolute and relative thyroid

weights were also reported in rat dams exposed to dietary decaBDE at 2, 15, or 146 mg/kg/day from GD 10 to PND 21; findings were significant at 2 mg/kg/day (21–22% decrease) and 146 mg/kg/day (21% decrease) (Fujimoto et al. 2011). No exposure-related changes in thyroid weight were observed in rats exposed to dietary decaBDE at doses up to 90 mg/kg/day for 28 days (IRDC 1976).

Unlike the lower-brominated PBDEs, serum T_4 levels were not altered in rats exposed to decaBDE at gavage doses up to 600 mg/kg/day for 28–90 days (Lee et al. 2010; Van der ven et al. 2008a; Wang et al. 2010, 2011b). Serum T_3 levels were significantly reduced by up to 25% in female, but not male, rats exposed to decaBDE at 60 mg/kg/day via gavage for 28 days (Van der ven et al. 2008a), and no changes in serum T_3 levels were observed in male rats exposed to decaBDE at doses up to 100 mg/kg/day via gavage for 90 days (Wang et al. 2010, 2011b). In young male rats exposed to decaBDE for 33 days (PNDs 10–45) via gavage, serum T_3 was significantly reduced by up to 45% at doses ≥100 mg/kg/day and serum TSH was significantly increased by ~70% following exposure to ≥300 mg/kg/day decaBDE for 33 days (PNDs 10–42) (Lee et al. 2010). In male mice, serum T_4 and T_3 were significantly reduced by 22 and 44%, respectively, following exposure to decaBDE at gavage doses of 950 mg/kg/day for 35 days, compared with controls; no exposure-related changes in serum thyroid hormone levels were observed at 750 mg/kg/day (Sarkar et al. 2015). In developing animals, significant reductions in serum T_3 levels were observed following gestational and lactational exposure to decaBDE at 146 mg/kg/day in rats and 1,500 mg/kg/day in mice; no changes were observed in serum T_4 or TSH (Fujimoto et al. 2011; Tseng et al. 2008); see Section 3.2.2.6 (Developmental Effects) for more details.

In young male rats exposed to decaBDE at doses of 0, 100, 300, or 600 mg/kg/day via gavage for 33 days (PNDs 10–42), absolute and relative adrenal weights were significantly increased by 14 and 20%, respectively, in the 600 mg/kg/day group (Lee et al. 2010). No exposure-related changes in adrenal weight were observed in rats exposed to dietary decaBDE at doses up to 90 mg/kg/day for 28 days (IRDC 1976). In mice, no significant changes in adrenal weight were observed in dams exposed to decaBDE at doses up to 1,500 mg/kg/day from GD 0 to 17 (Tseng et al. 2008).

Pancreatic effects were evaluated in rats exposed to decaBDE at doses of 0, 0.05, 1 or 20 mg/kg/day via gavage for 8 weeks (Zhang et al. 2013a). Serum insulin levels were significantly reduced by 50–60% at ≥1 mg/kg/day, and blood glucose levels were elevated by 12–21% at ≥0.05 mg/kg/day (Zhang et al. 2013a). Consistent with these findings, morphological changes in the pancreas were observed at ≥1 mg/kg/day, including blurred boundaries among pancreatic islet cells (incidence not reported).

Microarray analysis of liver tissue indicated significant alterations in genes from the canonical pathway for type I diabetes mellitus (Zhang et al. 2013a).

Chronic-Duration Animal Studies

Lower-brominated PBDEs: No chronic-duration studies analyzing endocrine effects were located for lower-brominated PBDEs,

DecaBDE: Hyperplasia of the thyroid was observed in rats and mice following repeated dietary exposures to decaBDE. Thyroid follicular cell hyperplasia was increased in male B6C3F1 mice that were exposed to ≥94% pure commercial decaBDE for 103 weeks (NTP 1986). Incidences of the lesion were 2/50 (4%), 10/50 (20%), and 19/50 (38%) in the 0, 3,200, and 6,650 mg/kg/day dose groups of this study. Slight increases in follicular cell tumors that were considered to be equivocal evidence of thyroid carcinogenicity were also observed in the male mice (see Section 3.2.2.7, Cancer). No decaBDE-related histopathological changes in the thyroid were found after 103 weeks of exposure to ≤7,780 mg/kg/day in female mice, ≤2,240 mg/kg/day in male Sprague-Dawley rats, or ≤2,550 mg/kg/day in female rats (NTP 1986).

Summary. While human data are inconsistent, they suggest that PBDEs can interact with thyroid hormone homeostasis. These data, along with available animal studies, indicate that the thyroid is a target of concern for PBDE exposure, especially lower-brominated PBDEs. One study reported pancreatic effects, including altered insulin regulation and pancreatic lesions, following intermediate decaBDE exposure; however, no other animal studies evaluated these end points. Limited human evidence is inconclusive regarding potential associations between diabetes and PBDE exposure; however, considering the animal data, the pancreas may be a target of concern for oral PBDE exposure. There is little evidence for endocrine effects other than those mediated by the thyroid and pancreas; data for altered reproductive hormones in humans and animals exposed to PBDEs are inconclusive (see Section 3.2.2.5, Reproductive Effects).

Dermal Effects. No studies were located regarding dermal effects in humans after oral exposure to PBDEs.

Histopathological examinations showed no dermal changes in rats following gavage exposure to ≤200 mg/kg/day of pentaBDE for 28 days (Van der ven et al. 2008b), dietary exposure to

≤100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984), or dietary exposure to ≤750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977). No studies examining dermal effects after exposure to decaBDE were located.

Based on animal studies, dermal effects are unlikely with oral exposure to PBDEs.

Ocular Effects. No studies were located regarding ocular effects in humans after oral exposure to PBDEs.

Histopathological examinations showed no ocular effects in rats following dietary exposure to ≤100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984) or ≤750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977). Similarly, histopathological examinations showed no ocular effects in rats following dietary exposure to ≤1.0 mg/kg/day of 77.4% decaBDE (containing 21.8% nonaBDE and 0.8% octaBDE) for 2 years (Kociba et al. 1975; Norris et al. 1975a).

Based on animal studies, ocular effects are unlikely with oral exposure to PBDEs.

Body Weight Effects. The only information located was that serum concentrations of BDE 47 in a group of 36 metabolically healthy but obese women (MHO) were not significantly different than in a group of 40 metabolically abnormal obese women (p=33 for comparison of the medians) (Gauthier et al. 2014). The significance of this isolated finding is unknown

Acute-Duration Animal Studies

Lower-brominated PBDEs: Percent body weight gain was significantly decreased by 2–19% in female rats following gavage exposure to pentaBDE at 2000 mg/kg for 1 day, pentaBDE at 200 mg/kg/day for 7 days, pentaBDE at 8–200 mg/kg/day for 14 days, or octaBDE at 40–200 mg/kg/day for 7 or 14 days (Bruchajzer et al. 2010, 2011, 2012). However, no changes in percent body weight gain were observed in female rats exposed to pentaBDE at doses up to 200 mg/kg/day for 7 or 14 days via gavage in another study by the same investigators (Bruchajzer 2011). Additionally, body weight effects were not reported in any other acute-duration study. No changes in body weight or body weight gain were observed in rats exposed to penta- or tetraBDE at doses up to 240 mg/kg/day for 3–14 days via gavage (Bruchajzer 2011; Hallgren and Darnerud 2002; Hallgren et al. 2001; Hoppe and Carey 2007; Stoker et al. 2004; Stoker et al. 2005; Zhou et al. 2001) or in mice exposed to penta-, tetra-, or octaBDE at doses up to 100 mg/kg/day

for 4–14 days via gavage (Hallgren and Darnerud 2002; Fowles et al. 1994; Hallgren et al. 2001; Richardson et al. 2008; Zhou et al. 2001).

In pregnant rats exposed to pentaBDE at 0, 10, 100, or 200 mg/kg/day via gavage from GD 6 to 15, maternal body weight gain was significantly decreased by 20 and 30% in the 100 and 200 mg/kg/day groups (Argus Research Laboratories 1985a). Pregnant rats exposed to octaBDE at 0, 2.5, 10, 15, 25, or 50 mg/kg/day via gavage from GD 6 to 15 showed a significantly 40% reduction in maternal body weight gain at 50 mg/kg/day (WIL Research Laboratories 1986). In pregnant rabbits, exposure to octaBDE at 15 mg/kg/day via gavage from GD 7 to 19 also resulted in a 7% decreased in maternal body weight gain (statistics not provided); no body weight effects were observed at ≤5 mg/kg/day (Breslin et al. 1989). In other studies, maternal body weight was not affected in rats by gestational exposure via gavage to pentaBDE at doses up to 120 mg/kg/day (Blanco et al. 2012, Ellis-Hutchings et al. 2009; Zhao et al. 2014) or tetraBDE at 0.7 mg/kg/day (Talness et al. 2008).

DecaBDE: No change in body weight or body weight gains were observed in rats exposed to decaBDE at doses up to 1,000 mg/kg/day for 4–14 days via gavage (Bruchajzer et al. 2010; Zhou et al. 2001) or in rats and mice exposed to dietary decaBDE doses up to 16,000 and 19,000 mg/kg/day, respectively, for 7–14 days (NTP 1986; Sakamoto et al. 2013). DecaBDE did not alter maternal body weight in mice exposed to doses up to 1500 mg/kg/day from GD 0 to 17 via gavage (Tseng et al. 2006).

Intermediate-Duration Animal Studies

Lower-brominated PBDEs: Percent body weight gain was significantly decreased by 8–19% in rats exposed to pentaBDE at 8–200 mg/kg/day or octaBDE at 40–200 mg/kg/day for 21 or 28 days via gavage (Bruchajzer 2011; Bruchajzer et al. 2012). However, in another study by the same investigators that exposed female rats to 0, 8, 40, or 200 mg/kg/day for 21 or 28 days via gavage, percent body weight gain was only significantly decreased at 200 mg/kg/day (Bruchajzer et al. 2010). Another 28-day gavage study reported no body weight gain changes in female rats at pentaBDE doses up to 200 mg/kg/day; however, significant changes were observed in male rats after the 28-day exposure to 0.27–200 mg/kg/day (Van der ven et al. 2008b). The $BMD_{RD10\%}$ and $BMDL_{RD10\%}$ for decreased body weight gain in male rats were 61.3 and 9.7 mg/kg/day, respectively (data were reported in terms of BMD analysis only; raw data were not reported). At higher doses (≥600 mg/kg/day), rats exposed to dietary octaBDE for 13 weeks showed ≥12% decreases in weight gain (IRDC 1977). In mink, dietary pentaBDE exposure at doses of 0.63 and 0.78 mg/kg/day for 9 weeks showed significant body weight decreases of 21 and

28%, respectively; no body weight effects were observed in mink exposed to dietary concentrations of 0.08 mg/kg/day (Martin et al. 2007).

Body weight effects were not reported in other intermediate-duration studies. No changes in body weight or body weight gain were observed in rats exposed to pentaBDE at doses up to 250 mg/kg/day for 15–90 days via gavage (Becker et al. 2012; Daubie et al. 2011; Hoppe and Carey 2007; IRDC 1976; Oberg et al. 2010; Stoker et al. 2004, 2005; WIL Research Laboratories 1984), in mice exposed to tetra- or diBDE at doses up to 30 mg/kg/day for 28–30 days via gavage (Wang et al. 2013; Zhang et al. 2014), in mice exposed to tetraBDE at doses up to 150 mg/kg/day for 6–12 weeks via gavage (McIntyre et al. 2015; Zhang et al. 2015a, 2015b), in rats exposed to dietary penta- or octaBDE or a penta-decaBDE mixture (52.1% pentaBDE, 44.2% decaBDE, and 0.4% octaBDE) at doses up to 90 mg/kg/day for 28–125 days (Driscol et al. 2009; Ernest et al. 2012; IRDC 1976), or in mice exposed to dietary tetraBDE at doses up to 0.45 mg/kg/day (Maranghi et al. 2013).

Maternal body weight effects were not observed following gavage exposure to pentaBDE during gestation and lactation in rats at doses up to 30.6 mg/kg/day (Bowers et al. 2015; Ellis-Hutchings et al. 2006; Kodavanti et al. 2010; Zhou et al. 2002) or in mice at doses up to 452 mg/kg/day (Branchi et al. 2005; Skarman et al. 2005). In one-generation studies, no body weight effects were observed in F0 or F1 rats following pentaBDE exposure to gavage doses up to ≤50 mg/kg/day (Bondy et al. 2011, 2013; Dunnick et al. 2012), in F0 dams following administration of doses up to 11.4 mg/kg/day via pentaBDE-dosed vanilla wafers (Poon et al. 2011), in F0 or F1 mink exposed to dietary pentaBDE doses of ≤0.31 mg/kg/day (Zhang et al. 2009), or in female F0 mice fed cornflakes dosed with 1 mg/kg/day of tetraBDE for 4 weeks prior to mating through PND 21 (Koenig et al. 2012; Ta et al. 2011)

DecaBDE: One study reported significant decreases in growth in rats exposed to decaBDE via gavage for 90 days (Wang et al. 2011b). "Whole-body growth rates," defined by the study authors as the average growth rate after 90 days, were reported as 1.57, 0.59, and 0.76% in the 0, 10, and 50 mg/kg/day groups, respectively (Wang et al. 2011b). No changes in body weight or body weight gain were observed in other studies of rats exposed to decaBDE doses up to 1,000 mg/kg/day for 21–90 days via gavage (Bruchajzer et al. 2010; Lee et al. 2010; Van der ven et al. 2008a; Wang et al. 2010; Zhang et al. 2013a), in mice exposed to decaBDE at doses up to 1,500 mg/kg/day for 15–60 days via gavage (Heredia et al. 2012; Liang et al. 2010; Sarkar et al. 2015; Tseng et al. 2006), or in rats and mice exposed to dietary decaBDE at doses of ≤8,000 and ≤9,500 mg/kg/day, respectively, for 28–90 days (NTP 1986; Sakamoto et al. 2013; Watanabe et al. 2010a). Dietary ingestion of 77.4% decaBDE mixture (containing 21.8% nonaBDE and

0.8% octaBDE) similarly caused no body weight changes in rats exposed to ≤800 mg/kg/day for 30 days or ≤1.0 mg/kg/day for 2 years (Kociba et al. 1975; Norris et al. 1973, 1975a).

Mean maternal weight was suppressed by ~10% in mice exposed to dietary decaBDE doses of 290 or 2,900 mg/kg/day from GD 10 to PND 21; however, the study authors did not report whether or not this finding was statistically significant (raw data not available for statistical analysis) (Watanabe et al. 2010b). A significant 12% decrease in body weight was also reported in rat dams exposed to decaBDE at 300 mg/kg/day via gavage for 3 weeks prior to mating until PND 21 (Liu et al. 2012). In other studies, decaBDE did not alter maternal body weight in rats exposed to doses up to 1,000 mg/kg/day from GD 10 to PND 21 via gavage (Biesemeir et al. 2011; Fujimoto et al. 2011) or in mice exposed to dietary decaBDE at doses up to 260 mg/kg/day from GD 10 to PND 21 (Watanabe et al. 2008).

Chronic-Duration Animal Studies

Lower-brominated PBDEs: No chronic-duration studies analyzing body weight effects were located for lower-brominated PBDEs.

DecaBDE: Body weight effects were not observed in rats and mice that were exposed to dietary decaBDE doses of ≤2,550 and ≤7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986).

Summary. No studies are available on body weight effects of PBDEs in humans. Although some acute- and intermediate-duration animal studies reported decreased body weight, several others reported no exposure-related changes in body weight. No changes in body weight were observed in chronic studies. Based on the body of evidence from animal studies, body weight effects are unlikely to occur following oral exposure to PBDEs at environmentally-relevant doses.

Metabolic Effects. No studies were located regarding metabolic effects in humans after oral exposure to PBDEs.

Animal Studies

Lower-brominated PBDEs: Exposure to pentaBDE via gavage for 28 days at doses of 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7, or 200 mg/kg/day caused significant, dose-related decreases in blood glucose levels in male rats (Van der ven et al. 2008b). Results, reported in terms of BMD analysis, indicated a BMD_{10} of

179.55 mg/kg/day and a $BMDL_{10}$ of 66.7 mg/kg/day (raw data and statistics for individual dose groups were not reported). No significant changes in blood glucose levels were found in female rats exposed to doses up to 200 mg/kg/day (Van der ven et al. 2008b). Reduced serum glucose levels were also reported in male rats exposed to 20 mg/kg/day of a dietary PBDE mixture containing 52.1% penta-decaBDE (DE-71), 44.2% decaBDE (BDE 209), and 0.4% octaBDE (DE-79) for 70 days; no changes were observed at ≤2 mg/kg/day (Ernest et al. 2012).

In wild-type mice exposed to tetraBDE at 0 or 1 mg/kg/day via gavage for 6 weeks, no exposure-related changes in circulating insulin levels, glucose tolerance, insulin resistance, or lipogenesis in the liver were observed (McIntyre et al. 2015). However, insulin sensitivity was significantly decreased in similarly-exposed *Pten -/-* mice (mice hypersensitive to insulin), compared to control *Pten -/-* mice. No exposure-related effects were observed in *Tsc1 -/-* (mice with mild insulin resistance).

Exposure to pentaBDE via gavage at 250 mg/kg/day for 28 days caused hypercalcemia, hypermagnesemia, and hyperphosphatemia in male rats and hyperatremia and hypokalemia in female rats (Oberg et al. 2010). No changes in blood calcium, magnesmium, potassium, phosphorus, or sodium levels were observed at doses ≤25 mg/kg/day (Oberg et al. 2010). No significant changes in blood calcium, magnesium, or phosphorus levels were observed in male rats exposed to ≤20 mg/kg/day of a dietary penta-decaBDE mixture containing 52.1% pentaBDE (DE-71), 44.2% decaBDE (BDE 209), and 0.4% octaBDE (DE-79) for 70 days (Ernest et al. 2012).

A single study evaluated fat pad weight and adipocyte number, size, viability, lipolysis, and glucose oxidation in male rats following exposure to pentaBDE at 14 mg/kg/day via gavage for 2 or 4 weeks (Hoppee and Carey 2007). No exposure-related effects were noted at 2 weeks. At 4 weeks, significant observations included increased adipocyte lipolysis and decreased adipocyte glucose oxidation; however, no changes in fat pad weight or the number, size, or viability of adipocytes were observed.

DecaBDE: DecaBDE exposure via gavage for 8 weeks caused elevated blood glucose levels at ≥0.05 mg/kg/day in male rats (Zhang et al. 2013a). Elevated glucose levels were increased 12–21% compared with controls, and were accompanied by significantly decreased serum insulin levels at ≥1 mg/kg/day; see Section 3.2.2.2, Endocrine System Effects for more details.

Summary. Data regarding metabolic effects of PBDE are too limited to adequately characterize if PBDE exposure could alter metabolism in humans.

3.2.2.3 Immunological and Lymphoreticular Effects

Human Studies. A significant negative statistical association (not further described) was found between serum concentrations of PBDE and number of circulating lymphocytes in a subset of 18 of a cohort of 33 children (18 girls and 15 boys) born in the Amsterdam/Zaandam area of the Netherlands and aged 14–19 years at the time of this study (Leijs et al. 2009). Serum PBDE concentrations (determined as the sum of congeners 28, 47, 85, 99, 100, 153, 154, and 183) ranged from 5 to 74 ng/g lipid with a mean of 13.9 ng/g lipid. Congener-specific analysis showed the main contributors to be BDE 183, BDE 154, and BDE 85. There were no other effects on leukocyte count or differential. Although dioxins and PCBs were also assessed, no association with lymphocytes was found for these chemicals. No effects on pokeweed mitogen-stimulated DNA proliferation or IgG immunoglobulin synthesis were found in human lymphocytes exposed to BDE 47 or BDE 85 *in vitro* (Fernlof et al. 1997). A cross-sectional study of 992 subjects from Sweden aged 70 years reported that serum levels of BDE 47 (median 12.6 ng/g lipid) were significantly ($p=0.03$) negatively associated with blood levels of protein complement 3 (C3), but not with serum levels of C3a or C4 (Kumar et al. 2014a); no other BDE congener was assessed. PCB levels were also negatively associated with protein complements. In a similar study of the same cohort, Kumar et al. 2014b) found no association between serum levels of BDE 47 and multiple inflammatory markers.

Acute-Duration Animal Studies

Lower-brominated PBDEs: Limited information is available on effects of acute-duration exposure to lower-brominated PBDEs on immunologic function in animals. A single gavage dose of 0.8–500 mg/kg pentaBDE did not affect the plaque-forming splenic cell antibody response to injected sheep red blood cells in mice (Fowles et al. 1994). Mice that were given 18, 36, or 72 mg/kg/day doses of pentaBDE via gavage for 14 days had significantly reduced antibody response to sheep red blood cells (63% of control value, $p<0.02$) and decreased thymus weight at 72 mg/kg/day (Fowles et al. 1994). There were no exposure-related effects of the 14-day exposure to ≤72 mg/kg/day on NKC activity to murine YAC-1 target cells; NKC activity was not evaluated in the single-dose study. Another 14-day study was conducted in which mice and rats were administered pentaBDE at 0, 18, or 36 mg/kg/day via gavage and were evaluated for spleen and thymus weights, numbers of splenic and thymic lymphocyte subsets (CD4+, CD8+, and CD45R+ cells), and *in vitro* IgG immunoglobulin production in pokeweed mitogen-stimulated splenocytes (Darnerud and Thuvander 1998). The only exposure-related effect in either species was significantly reduced *in vitro* production of IgG in pokeweed-stimulated splenocyte cultures

from the mice exposed to 36 mg/kg/day. Mice that were similarly exposed to tetraBDE at 18 mg/kg/day via gavage for 14 days had significantly reduced numbers of total splenocytes as well as CD4+, CD8+, and CD45R+ cells in spleen (Darnerud and Thuvander 1998), but no changes in spleen or thymic weight (Hallgren et al. 2001).

DecaBDE: No acute-duration studies analyzing immune effects were located for lower-brominated PBDEs.

Intermediate-Duration Animal Studies

Lower-brominated PBDEs: Immune function was evaluated in male mink exposed to dietary pentaBDE for 9 weeks (Martin et al. 2007). Initial exposure concentrations were 0, 1, 10, or 100 ppm; however, after the first week of exposure, the 100 ppm group was switched to 5 ppm for weeks 2–9 because of food avoidance and weight loss concerns. Dose levels in the 1, 5/100, and 10 ppm groups were calculated to be 0.08, 0.63, and 0.78 mg/kg/day, respectively, based on estimated daily intake ranges (Martin et al. 2007). Mink were assessed weekly during exposure for antibody-mediated immunity to KLH (a carrier protein from keyhole limpet that binds to haptens and is used to stimulate a response from the immune system in the form of antibody production) and at 9 weeks for PHA skin response. No dose-related effects were observed for antibody production to KLH; however, at each time-point tested, antibody production in the 0.63 mg/kg/day group (the 100 ppm/5 ppm group) was significantly increased compared with controls. No exposure-related changes were observed in the skin response to PHA challenge.

Martin et al. (2007) also reported a significant increase in the incidence of spleen hyperplasia in mink from the 0.63 and 0.78 mg/kg/day groups, with 25 and 40% incidence, respectively, compared with 0% incidence in the controls. In the 0.78 mg/kg/day group, the number of germinal centers in the spleen was also significantly increased. In mice, histological and morphometric changes were observed in the spleen and thymus of females exposed to dietary tetraBDE at 0.45 mg/kg/day for 28 days (only dose tested; males not evaluated) (Maranghi et al. 2013). In the spleen, follicular hyperplasia with germinal center development was observed in 9/10 exposed mice, compared with 4/10 controls, and lymphocytic infiltration involving the red pulp was observed in 5/10 exposed mice, compared with 0/10 controls. In the thymus, 7/9 exposed mice showed Hassal's bodies, compared with 2/10 controls, and 5/9 showed lymphocytic apoptosis, compared with 0/10 controls. The ratio between area of cortex and medulla in the thymus was significantly increased by 43% in exposed mice, compared with controls. In the thyroid, cellular debris was observed in the follicular lumen of 5/7 exposed mice, compared with 0/10 controls

(Maranghi et al. 2013). In other studies, no exposure-related changes were observed in spleen, thymus, lymph node, and/or bone marrow tissue histology in rats exposed to pentaBDE at gavage doses up to 250 mg/kg/day for 28 days (Oberg et al. 2010; Van der ven et al. 2008b), pentaBDE at dietary doses up to 100 mg/kg/day for 90 days (WIL Research Laboratories 1984), or octaBDE at dietary doses up to 750 mg/kg/day for 13 weeks (IRDC 1977). Additionally, no exposure-related changes were observed in the histology of the spleen, thymus, Peyer's patches, or mesenteric lymph nodes in rat dams exposed to pentaBDE at doses up to 25 mg/kg/day via gavage for 70 days prior to mating through PND 21 (~21 weeks) (Bondy et al. 2013). In offspring, a significant dose-related trend was observed in the incidence of apoptotic lymphocytes and tingible macrophages in the thymus of PND 43 males, but not females (Bondy et al. 2013); see Section 3.2.2.6 (Developmental Effects) for more details.

Martin et al. (2007) reported a significant 29% increase in relative, but not absolute, spleen weight in mink exposed to dietary pentaBDE at 0.78 mg/kg/day for 9 weeks. In a 28-day study, rats were exposed to pentaBDE at 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7, or 200 mg/kg/day via gavage (Van der ven et al. 2008b). Study authors reported a dose-related decrease in thymus weight in male rats (maximal decrease of 19.4%); however, the lowest dose at which the effect was observed was not reported. Instead, results were reported in terms of BMD analysis ($BMD/BMDL_{RD20\%}$=194.2/110 mg/kg/day). No dose-related changes were reported for female thymus weight, male or female spleen weight, or T-cell, B-cell, or macrophage population distribution in the spleen in either sex (Van der ven et al. 2008b). In other studies, no exposure-related changes were observed in spleen or thymus weights in rats exposed to penta- or diBDE at gavage doses up to 250 mg/kg/day for 28 days (Oberg et al. 2010; Zhang et al. 2014), in rats exposed to pentaBDE at gavage doses up to 0.015 mg/kg/day for 90 days (Daubie et al. 2011), in rats exposed to dietary penta- or octaBDE at doses up to 100 mg/kg/day for 28 or 90 days (IRDC 1976; WIL Research Laboratories 1984), in rats exposed to a dietary penta-decaBDE mixture (52.1% pentaBDE [DE-71], 44.2% decaBDE [BDE 209], 0.4% octaBDE [DE-79]) at doses up to 20 mg/kg/day for 70 days (Ernest et al. 2012), in rats exposed to dietary octaBDE at doses up to 750 mg/kg/day for 13 weeks (IRDC 1977), or in mice exposed to dietary tetraBDE at 0.45 mg/kg/day for 28 days (Maranghi et al. 2013). Additionally, no exposure-related changes in spleen or thymus weights were observed in rat dams or offspring exposed to pentaBDE at 18 mg/kg/day via gavage from GD 6 to PND 21 (Ellis-Hutchings et al. 2006), in rat dams exposed to pentaBDE at doses up to 25 mg/kg/day via gavage for 70 days prior to mating through PND 21 (~21 weeks) (Bondy et al. 2013), or in mink sows exposed to dietary pentaBDE at doses up to 0.31 mg/kg/day for 4 weeks prior to mating through PNW 6 (Zhang et al. 2009). Although immune function was not altered, increased thymus weights, as well as altered serum immunoglobin levels and lymphocyte proliferation, were observed in the offspring of rat dams exposed to pentaBDE at

doses ≥5 mg/kg/day via gavage for 70 days prior to mating through PND 21 (~21 weeks) (Bondy et al. 2013); see Section 3.2.2.6 (Developmental Effects) for more details.

In a poorly-reported study, immune end points were evaluated in mouse dams and offspring following exposure to pentaBDE at 0, 50, 100, or 200 mg/kg/day via gavage from GD 6 to PND 21 (Hong et al. 2010). The absolute and relative spleen weights were significantly decreased in the 100 and 200 mg/kg/day groups and the thymi of exposed dams were "weighed decreasingly" (quantitative data were not reported). No histopathological alterations were observed in the spleen or thymus. The study abstract reports decreased cellularity of the spleen and thymus in dams; however, cellularity data for the dams are not reported in the results section of the paper. No statistically significant increases in T- or B-cell lymphocyte proliferation were observed, and no exposure-related changes in T-cell, B-cell, or macrophage population distribution were observed in the spleen. The study authors reported decreases in serum IgM levels, but statistical significance was not reported. No changes in serum IgG1 were observed. The study authors also noted some immune effects in offspring, including decreased spleen weight and cellularity and altered serum immunoglobin levels on PND 21 in groups exposed to ≥100 mg/kg/day and increased T-cell proliferation following *in vitro* exposure to concanavalin A (ConA) at 200 mg/kg/day on PND 63; see Section 3.2.2.6 (Developmental Effects) for more details.

DecaBDE: One week following exposure to dietary decaBDE at 0 or 1,800 mg/kg/day for 28 days, mice were intranasally infected with the RSV (Watanabe et al. 2010a). No exposure-related differences in pulmonary viral titers were observed 5 days post-infection (Watanabe et al. 2010a). In contrast, pulmonary viral loads were increased post-infection in PND 28 offspring of mouse dams exposed to dietary decaBDE at doses ≥260 mg/kg/day from GD 10 to PND 21 (Watanabe et al. 2008, 2010b); see Section 3.2.2.6 (Developmental Effects) for more details. In another high-dose study, female mice exposed to decaBDE at 800 mg/kg every other day showed impaired CD4 T-cell function from 4 to 10 months of exposure, compared with controls (Feng et al. 2016b). Significant alterations in peripheral CD4 T-cells from exposed mice included significant decreases in *in vitro* cytokine production of TNF-α, IFN-γ, and IL-2, decreased percentage of multi-functional CD4 T-cells (cells capable of producing multiple cytokines following mitogen production), increased percentage of T regulatory cells (CD4+CD25+Foxp3+), decreased percentage of proliferating CD4 T-cells, and decreased percentage of antigen-specific CD4 T cells following *in vivo Listeria monocytogenes* infection.

In rats exposed to decaBDE at 0 or 300 mg/kg/day via gavage for 21 days prior to mating through PND 21, the distribution of T-lymphocytes in the thymus was significantly altered in exposed rat dams,

with significantly decreased CD3+, CD4+, CD4+/CD8+, CC3+/CD8+, and CD3+/CD4+ T-cells and significantly increased CD4-/CD8- T-cells compared with control dams (Liu et al. 2012). CD161+ NKCs were also significantly decreased, compared with controls. Additionally, the lymphocyte stimulation index in response to *in vitro* PHA exposure was significantly reduced by ~2-fold in lymphocytes harvested from exposed rat dams, compared with control dams (Liu et al. 2012). In another study, no dose-related changes were reported for T-cell, B-cell, or macrophage population distribution in the spleen of rats exposed to decaBDE at doses up to 60 mg/kg/day via gavage for 28 days (Van der ven et al. 2008a).

Following exposure to decaBDE at 300 mg/kg/day via gavage for 21 days prior to mating through PND 21, serum IgM and IgG were significantly decreased by 28 and 4%, respectively, compared with controls (Liu et al. 2012). No exposure-related changes were observed in IFN- γ or IL-4 (Liu et al. 2012).

Histopathological examinations of spleen, thymus, lymph node, and/or bone marrow tissues showed no effects in rats or mice exposed to dietary decaBDE at doses up 8,000 or 9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986). In rat dams exposed to decaBDE at 0 or 300 mg/kg/day via gavage for 21 days prior to mating through PND 21 (~11 weeks), the exposed group showed significantly increased "histological scores" in the spleen and thymus compared with controls (methods of histological scoring and incidences of lesions were not reported) (Liu et al. 2012). Lesions observed in the thymus of exposed rats included thickened thymus capsule, decreased lymphoid tissue in the cortex with adipose tissue replacement, increased medulla size, and obscured corticomedullary junction. The spleen showed decreased size and number of lymphoid nodules, thinner lymphatic sheath around arteries, and fibrotic tissue with macrophages in the medulla.

In a 28-day study, rats were exposed to decaBDE at 0, 1.87, 3.45, 7.5, 15, 30, or 60 mg/kg/day via gavage (Van der ven et al. 2008a). The study authors reported a dose-related decreased in thymus weight in female rats (maximal decrease of 16.6%); however, the lowest dose at which the effect was observed was not reported. Instead, results were reported in terms of BMD analysis ($BMD/BMDL_{RD20\%}$= 75/43 mg/kg/day). No dose-related changes were reported for male thymus weight or male or female spleen weight (Van der ven et al. 2008a). Absolute and relative spleen weights were significantly decreased by 12 and 27%, respectively, in rat dams exposed to decaBDE at 300 mg/kg/day via gavage for 21 days prior to mating through PND 21 (~11 weeks); no exposure-related changes were observed in thymus weight (Liu et al. 2012). In other studies, no exposure-related changes in spleen weight were observed in rats exposed to decaBDE at doses up to 20 mg/kg/day via gavage for 8 weeks (Zhang et al.

2013a) or in mouse dams or PND 71 male offspring exposed to decaBDE at doses up to 1,500 mg/kg/day from GD 0 to 17 via gavage (Tseng et al. 2008).

In a poorly-reported study, immune end points were evaluated in mouse dams and offspring following exposure to decaBDE at 0, 500, 2,500, or 12,500 mg/kg/day via gavage on GD 6 to PND 21 (Hong et al. 2010). No exposure-related changes were reported for maternal spleen or thymus weight or histology. T-cell lymphocyte proliferation (in response to ConA) was "slightly" increased. No statistically significant increases in B-cell lymphocyte proliferation were observed and no exposure-related changes in T-cell, B-cell, or macrophage population distribution were observed in the spleen. The study authors reported increased serum IgG1 and IgM levels; however, statistics were not reported and changes do not appear dose-related in graphically-presented data. In offspring, altered spleen cell populations were noted on PND 21; see Section 3.2.2.6 (Developmental Effects) for more details.

Chronic-Duration Animal Studies

Lower-brominated PBDEs: No chronic-duration studies analyzing immune effects were located for lower-brominated PBDEs.

DecaBDE: Following chronic ingestion of decaBDE for 103 weeks, an increased incidence of splenic hematopoiesis was observed in female rats at ≥1,200 mg/kg/day (12/49, 24/48, and 17/50 at 0, 1,200, and 2,550 mg/kg/day, respectively); only the incidence in the mid-dose group was statistically significant. In male rats, the incidence of splenic fibrosis was significantly increased at 2,240 mg/kg/day (5/49, 8/50, and 13/49 at 0, 1,120, and 2,240 mg/kg/day, respectively) (NTP 1983). The incidence of lymphoid hyperplasia in the mandibular lymph node was also significantly increased in male rats at 2,240 mg/kg/day (4/50, 6/50, and 13/49 at 0, 1,120, and 2,240 mg/kg/day, respectively) (NTP 1983). No exposure-related histopathological lesions were observed in male or female mice following chronic ingestion of decaBDE for 103 weeks at doses up to 6,650 and 7,780 mg/kg/day, respectively (NTP 1983).

Summary. Evidence from animals suggest that PBDE exposure may cause immune suppression, particularly in infants or children (see Section 3.2.2.6, Developmental Effects for more details), but data are limited and inconsistent. Additionally, comprehensive immunological evaluations have not been performed and human data are limited. Therefore, currently available information is insufficient to adequately characterize the human immunotoxic potential of PBDEs. The highest NOAEL values and all

LOAEL values from each reliable study for immunological effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

Human Studies. No association was found between serum PBDE concentrations (BDE 28, BDE 47, BDE 66, BDE 85, BDE 99, BDE 100, BDE 138, BDE 153, BDE 154, and their sum) and neuropsychological function assessed by 34 tests of cognitive and motor function, affective state, and olfactory function in a study population of 144 volunteers (67 males and 77 females) between the ages of 55 and 74 who lived for at least 25 years in the upper Hudson valley of New York State (Fitzgerald et al. 2012). Median total serum PBDE concentration in the study population was 23.9 ppb (lipid weight), with the detection limit exceeded in 89% of the population. Neurobehavioral function in adolescents was studied by Kicinski et al. (2012). The study population included 515 secondary school students (271 boys) from Flanders, Belgium, with a mean age of 14.9 years. The students were given a computerized battery of neurological tests. PBDE concentrations (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183, and BDE 209) were measured in the serum. The median concentration of total PBDE in the serum was 7 ng/L. Serum PBDE concentrations were not associated with most aspects of neurological performance investigated, but there was a significant deterioration in performance in the finger tapping test with increasing PBDE level, suggesting an effect of PBDE on motor activity. Studies of neurological function in relation to PBDE concentrations in young children are discussed in Section 3.2.2.6 on Developmental Effects.

Acute-Duration Animal Studies

Lower-brominated PBDEs: In a neurotoxicity screen, adult male rats were exposed once to pentaBDE at 0, 0.6, or 1.2 mg/kg via gavage (Belles et al. 2010). Rats were assessed using a functional observation battery 3, 21, and 44 days after pentaBDE administration. After 45 days, rats were assessed in a variety of behavioral tests over 9 days, including open-field activity, passive avoidance test, and the Morris water maze. Following completion of behavioral tests, rats were sacrificed and brains were removed for biochemical analysis of oxidative stress markers (right hemisphere) and histopathological (left hemisphere) examinations of the cortex, hippocampus, and cerebellum. No exposure-related neurobehavioral, histological, or biochemical effects were observed.

In developing animals, a series of studies evaluated neurobehavior at 2–8 months of age following single-day exposure to lower-brominated PBDEs on PND 3, 10, or 19 in rats and mice. Collectively, these studies showed altered open-field activity, impaired habituation and altered learning and memory in rats and mice exposed to penta-, hexa-, tetra-, or octaBDE at doses as low as 0.4, 0.45, 1, or 16.8 mg/kg, respectively (Eriksson et al. 2001, 2002b, 2006; Fischer et al. 2008; Hallgren et al. 2015; He et al. 2009, 2011; Gee and Moser 2008; Sand et al. 2004; Viberg et al. 2002, 2003a, 2004a, 2004b, 2005, 2006). No changes in open-field behavior were observed in mice exposed to heptaBDE at 15.2 mg/kg or nonaBDE at 18.5 on PND 10 (Viberg et al. 2006). Besides behavioral changes, other significant neuro-developmental effects observed in these studies included ultrastructural changes, altered nicotinic receptor density, and altered gene and protein expression levels in the hippocampus. Altered open-field behavior was also observed in PND 36 offspring following acute gestational exposure to pentaBDE at doses as low as 0.06 mg/kg via gavage on GD 6 (Kuriyama et al. 2004). See Section 3.2.2.6 (Developmental Effects) for more details regarding neurodevelopmental effects of acute exposure to lower-brominated PBDEs.

DecaBDE: No studies evaluating neurobehavior or neuropathology in adult rats or mice following acute exposure to decaBDE were identified; however, there were no overt signs of neurotoxicity in rats and mice exposed to decaBDE in estimated dietary doses of ≤16,000 and ≤19,000 mg/kg/day, respectively, for 14 days (NTP 1986).

In developing animals, a series of studies reported decreased spontaneous activity and impaired habituation in open-field testing in 2–6-month-old mice that were exposed to decaBDE on PND 3 at doses as low as 2.22 mg/kg (Buratovic et al. 2014; Johansson et al. 2008; Viberg et al. 2003b, 2007). These changes in open-field behaviors were not observed in animals exposed on PND 10 or 19. In contrast, significantly increased locomotor activity during the first 1.5 hours of a 2-hour observation period was observed in PND 70 males following exposure to decaBDE at 20 mg/kg/day via micropipette from PND 2 to 15 (Rice et al. 2007). No changes in locomotor activity were observed at PND 70 in females or at 1 year in either sex at doses up to 20 mg/kg/day (Rice et al. 2007). In operant training and visual discrimination tasks, learning impairments and impulsivity were observed in aging mice (16 months old), but not young adult mice (3 months old), that had been exposed to decaBDE at 20 mg/kg/day from PND 2 to 15 (Rice et al. 2009). See Section 3.2.2.6 (Developmental Effects) for more details regarding neurodevelopmental effects of acute exposure to decaBDE.

Intermediate-Duration Animal Studies

Lower-brominated PBDEs: No exposure-related changes were observed in open-field behavior, anxiety-like behavior in the elevated plus-maze performance, or learning and memory in the Morris water maze in male rats following exposure to pentaBDE at doses up to 0.015 mg/kg/day (highest dose tested) via gavage for 90 days (Daubie et al. 2011). In a higher dose study, impaired attention and inhibitory control was observed in a series of 5-choice serial reaction time tasks assessed from PND 40 to 125 in male mice exposed to dietary pentaBDE at 26.2 mg/kg/day from PND 1 to 125; no exposure-related changes were observed at 17.5 mg/kg/day (Driscoll et al. 2009). In adult male rats exposed to tetraBDE at 0, 0.1, 0.5, or 1 mg/kg/day via gavage for 30 days, rats in all exposure groups showed impaired learning and memory in the Morris water maze (Yan et al. 2012). Exposed rats required significantly more time to find the hidden platform in the Morris water maze compared with control group, without showing any differences in swim speed. Additionally, significant decreases in the time spent in the target quadrant and the number of crossings over the original platform location were observed during the retention trial on day 5 (Yan et al. 2012).

No exposure-related changes in brain weight and/or histology were observed in rats exposed to pentaBDE at gavage doses up to 250 mg/kg/day for 28 days (Oberg et al. 2010; Van der ven et al. 2008b), in rats exposed to pentaBDE at gavage doses up to 0.015 mg/kg/day for 90 days (Daubie et al. 2011), in rats exposed to penta- or octaBDE at dietary doses up to 750 mg/kg/day for 28 or 90 days (IRDC 1976, 1977; WIL Research Laboratories 1984), in rat dams or pups exposed to pentaBDE at a gavage dose of 18 mg/kg/day from GD 6 to PND 18 (Ellis-Hutchings et al. 2006), or in mink sows or kits exposed to pentaBDE at dietary doses up to 0.31 mg/kg/day for 4 weeks prior to mating through PNW 6 or 33 (Zhang et al. 2009).

The density of NMDA receptor subunits, NR1 and NR2B, and the glutamate receptor, Glu, was determined in the CA1, CA3, and dentate gyrus of the hippocampus in adult male rats exposed to tetraBDE at 0, 0.1, 0.5, or 1 mg/kg/day via gavage for 30 days (Yan et al. 2012). Immunohistochemical staining showed significant decreases in the density of NR_1 and Glu in the hippocampus of all exposed rats and NR_2B in the hippocampus of rats exposed to 0.5 and 1 mg/kg/day. Additionally, significant decreases in hippocampal mRNA levels were observed for NR1 and NR2C in all dose groups and NR_2D in the 0.5 and 1 mg/kg/day groups. No exposure-related changes were observed in NR_2A or NR_2B mRNA levels (Yan et al. 2012).

Cholinergic effects were evaluated in mink sows and kits exposed to dietary pentaBDE at 0, 0.01, 0.05, or 0.25 mg/kg/day from pre-mating day 28 to PNW 6 (Bull et al. 2007). No exposure-related effects were observed in acetylcholinesterase concentration (ACh), acetylcholinesterase activity (ChE), or muscarinic receptor (mAChR) or nicotinic receptor (nAChR) binding in the cerebral cortices of sows or kits. Plasma ChE activity in sows from the 0.25 mg/kg/day group was significantly increased by 3-fold, compared with controls. Plasma ChE activity was not significantly correlated with cortical ChE activity; however, it was significantly correlated with absolute and relative liver weights. Therefore, altered ChE activity in plasma in the high-dose sows may reflect exposure-related effects in the liver, rather than the central nervous system. No changes were observed in plasma ChE activity in kits (Bull et al. 2007).

Several studies have reported delayed ontogeny of reflexes, neurobehavioral impairments, and ultrastructural and biochemical changes in the hippocampus of offspring after gestational and lactational exposure to penta- or tetraBDE in rats and mice at doses as low as 2 and 0.03 mg/kg/day, respectively (Blanco et al. 2013; Bowers et al. 2015; Branchi et al. 2001, 2002, 2005; Cheng et al. 2009; Koenig et al. 2012; Ta et al. 2011; Woods et al. 2012). See Section 3.2.2.6 (Developmental Effects) for more details regarding neurodevelopmental effects of intermediate-duration exposure to lower-brominated PBDEs.

DecaBDE: Following a 15-day exposure to decaBDE at 20 mg/kg/day via gavage, male mice showed decreased anxiety behavior in the elevated zero maze, including decreased latency to first entry into the open region, increased time spent and number of entries into the open region, and increased number of head dips, compared with controls (Heredia et al. 2012). No exposure-related changes were observed in anxiety behaviors in the light/dark test, in learning or memory in the Morris water maze, or in general neurological behaviors assessed using a functional observation battery (Heredia et al. 2012). In another study, no changes were observed in open-field behavior of male rats exposed to decaBDE at doses up to 50 mg/kg/day via gavage for 90 days (Wang et al. 2011b). No overt signs of neurotoxicity were observed in rats and mice exposed to decaBDE in estimated dietary doses of ≤8,000 and ≤9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986).

Rats exposed to decaBDE at dietary doses up to 90 mg/kg/day for 28 days showed no change in brain weight (IRDC 1976). No exposure-related changes in brain weight or AchE activity were observed in mice exposed to decaBDE at doses up to 160 mg/kg/day via gavage for 15, 30, or 60 days (Liang et al. 2010).

There is limited evidence for neurodevelopmental effects following gestational and lactational exposure to decaBDE. No neurobehavioral changes were observed in rat offspring exposed to decaBDE at doses up to 1,000 mg/kg/day from GD 6 to PND 21 (Biesemeier et al. 2011). However, impaired spatial learning was observed in offspring of rat dams exposed to decaBDE at doses ≥30 mg/kg/day from GD 1 to 14 (Chen et al. 2014). Additionally, altered hippocampal electrophysiology was observed in mice offspring exposed to 20.1 mg/kg/day from GD 1 to PND 41 (Xing et al. 2009) and altered hippocampal immunohistochemistry was observed in mice offspring exposed to ≥15 mg/kg/day from GD 10 to PND 21 (Fujimoto et al. 2011). See Section 3.2.2.6 (Developmental Effects) for more details regarding neurodevelopmental effects of intermediate-duration exposure to decaBDE.

Chronic-Duration Animal Studies

Lower-brominated PBDEs: No chronic-duration studies analyzing neurological effects were located for lower-brominated PBDEs.

DecaBDE: There were no indications of neurotoxicity in rats and mice in lifetime feeding studies of decaBDE at doses as high as 2,550 and 7,780 mg/kg/day, respectively, as assessed by overt clinical signs and nervous system histopathology (NTP 1986). Although the high doses and extended exposure durations in the NTP (1986) studies provided opportunities for the induction and/or development of effects, neurotoxicity is incompletely evaluated due to the lack of testing for subtle behavioral and other sensitive neurological end points.

Summary. While the nervous system is a target of concern during early development (see Section 3.2.2.6, Development Effects for more details), it is unclear if the developed nervous system is a target of oral PBDE toxicity. Animal data suggest the oral PBDE exposure may lead to neurobehavioral changes; however, available information is insufficient to adequately characterize the neurotoxic potential of PBDEs in adults and adolescents. The highest NOAEL values and all LOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

Human Studies. Two studies have demonstrated reproductive effects in men associated with exposure to PBDEs. In a small study limited to 10 young adult Japanese males, strong, statistically significant inverse

correlations were found between serum levels of BDE 153 and sperm concentration and testis size (Akutsu et al. 2008). Both blood and sperm were collected monthly and pooled for each participant over 1 year. BDE 153 concentrations for the 10 participants ranged from 0.37 to 1.1 ng/g lipid. There was no relationship to sperm concentration or testes size for other PBDE congeners or for the sum of the most prevalent congeners, which included BDE 153 (BDE 47, BDE 99, BDE 100, and BDE 153) and ranged from 1.1 to 8.6 ng/g lipid for the 10 participants. Among a group of 52 Canadian men recruited at a fertility clinic, sperm mobility was significantly reduced in association with increased serum PBDE concentrations (BDE 47, BDE 100, and total) (Abdelouahab et al. 2011). Results for BDE 153 were similar, but not statistically significant. Relationships between PBDE and sperm concentration were consistently negative as well, but also not significant. The median ∑PBDE concentration in this population was 0.302 ng/mL and the maximum was 2.250 ng/mL. A study of 468 male partners of couples discontinuing contraception for purposes of becoming pregnant from Michigan and Texas found that, in general, serum concentrations PBDEs were negatively associated with parameters of semen quality, specifically increased percentages of abnormal morphology (Mumford et al. 2015). The study evaluated 10 BDEs: BDE 17, BDE 28, BDE 47, BDE 66, BDE 85, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 183. BDE 153, however, was associated with increased sperm concentration. The 95th percentile serum concentrations (unadjusted for lipid content) for the BDEs analyzed ranged from 0.007 ng/g serum for BDE 183 to 0.761 ng/g serum for BDE 47. Significant associations at the α=0.01 level were found only for BDE 17 (increased sperm with coiled tail) and for BDE 28 (increased bicephalic sperm). A cross-sectional study that included 99 men from Greenland, 100 from Poland, and 100 from Ukraine found no association between serum levels of BDE 47 and BDE 153 with parameters of semen quality including markers of DNA damage and apoptosis (Toft et al. 2014). Among the three groups, median concentrations of BDE 47 ranged from 0.2 to 2.0 ng/g lipid and median concentrations of BDE 153 ranged from 0.3 to 2.7 ng/g lipid.

Studies of hormone levels in men in relation to PBDE concentrations have not produced consistent results. Concentrations of PBDE (BDE 47, BDE 99, and BDE 100) in house dust samples were significantly associated with changes in some sex hormone levels (decreased FSH, LH, and free androgen index, and increased Inhibin B and sex hormone binding globulin [SHBG]), although not testosterone or estradiol, in 24 adult men recruited through a Massachusetts infertility clinic (Meeker et al. 2009). Median and maximum concentrations of PBDE in dust collected from houses of study subjects were 500 and 7,620 ng/g dust for BDE 47, 838 and 9,220 ng/g dust for BDE 99, and 180 and 2,830 ng/g dust for BDE 100. A larger subsequent study by the same researchers with 38 additional subjects using the same design found a significant negative correlation with FSH and significant positive correlations with

estradiol and SHBG for pentaBDE (sum of BDE 47, BDE 99, and BDE 100; median and maximum concentrations of 1,049 and 22,300 ng/g in dust), significant positive correlations with testosterone and LH for octaBDE (sum of BDE 183 and BDE 201; median and maximum concentrations of 30.5 and 1,181 ng/g in dust), and a significant negative correlation with testosterone for decaBDE (sum of BDE 206, BDE 207, BDE 208, and BDE 209; median and maximum concentrations of 1,800 and 38,483 ng/g in dust) (Johnson et al. 2013). No relationships were found between serum concentrations of BDE 47 and testosterone, FSH, LH, or prolactin in adult male Baltic Sea area residents of Sweden and Latvia with a wide range of fish consumption behavior and BDE 47 concentrations ranging from 0.10 ng/g lipid at the 10^{th} percentile to 5.16 ng/g lipid at the 90^{th} percentile (median=1.04 ng/g lipid) (Hagmar et al. 2001). In a study of serum PBDE and hormone levels in adult male sport fish consumers from the Great Lakes, a significant positive relationship was found for BDE 47 (but not other congeners) and serum testosterone levels (Turyk et al. 2008). BDE 47 concentrations in this population ranged from 0.01 to 5.90 ng/g (median=0.11 ng/g), while ∑PBDE concentrations ranged from 0.13 to 10.15 ng/g (median=0.26 ng/g). Serum concentrations of FSH, LH, or estradiol were not associated with serum levels of BDE 47 or BDE 153 in a study of 299 spouses of pregnant women from Greenland, Poland, and Ukraine (Toft et al. 2014). Median concentrations of BDE 47 ranged from 0.2 to 2.0 ng/g lipid and median concentrations of BDE 153 ranged from 0.3 to 2.7 ng/g lipid.

In women, Chao et al. (2007) found no significant relationship between concentrations of PBDE in breast milk and pre-pregnancy menstrual cycle length (by questionnaire) in an analysis of 20 pregnant women from Taiwan with median PBDE concentrations in breast milk of 3.65 ng/g lipid, predominantly BDE 47 and BDE 153. However, in a larger study of 46 pregnant Taiwanese women recruited several years later, Chao et al. (2010) found significant increases in average length of pre-pregnancy menstrual cycle with increased breast milk concentrations of PBDE (total and multiple individual congeners). Median breast milk total PBDE concentration in this study group was 2.84 ng/g lipid, with predominant congeners being BDE 47, BDE 153, and BDE 209. A study of 42 Cree women of James Bay, Canada, reported that concentrations of BDE 47 and BDE 153 in plasma were associated with increased menstrual cycle length (Wainman et al. 2016). Geometric mean plasma concentrations of BDE 47 and BDE 153 were 14.1 and 4.5 ng/g lipid, respectively, which according to the investigators, were comparable to those reported for women 20–39 years old from the general Canadian population; serum cadmium and selenium were also associated with increased cycle length. In the study by Chao et al. (2010), age at menarche was not related to breast milk PBDE concentrations, but an analysis of data from a sample of 271 adolescent U.S. girls age 12–19 years with serum total PBDE concentrations ranging from 6.4 to 636.5 ng/g lipid (median=44.7 ng/g lipid) from NHANES (2003–2004) found that higher serum PBDE concentrations

were associated with younger age of menarche in this population (Chen et al. 2011). There was no relationship between mid-pregnancy serum PBDE concentrations and pre-pregnancy menstrual cycling in a cohort of 223 pregnant low-income women from the Salinas Valley of California (part of the CHAMACOS study) (Harley et al. 2010), but there were significant decreases in fecundability (i.e., increases in time to pregnancy between stopping contraception and becoming pregnant) associated with increased serum concentrations of BDE 47 (geometric mean=14.9 ng/g lipid), BDE 99 (geometic mean=4.4 ng/g lipid), BDE 100 (geometic mean=2.8 ng/g lipid), BDE 153 (geometic mean=2.5 ng/g lipid), and their sum in this population and/or a subset comprising 107 women actively trying to become pregnant. However, fecundability was not significantly related to serum PBDE concentrations (concentrations not reported) in a cohort of 501 Michigan and Texas couples followed prospectively for 1 year after discontinuing contraception for the purpose of becoming pregnant (Buck Louis et al. 2013). Blood was collected from both male and female partners in this study and fecundity of the couple was assessed in relation to each partner's exposure. A study of 65 women undergoing *in vitro* fertilization found no association between serum PBDE concentrations (median=12.6 ng/g lipid, maximum=113 ng/g lipid) and risk of implantation failure, but did find a significantly increased risk of failure associated with increased (i.e., detectable) concentrations of BDE 153 (but not other congeners or total PBDE) in follicular fluid (Johnson et al. 2012). No significant relationship was found between concentrations of the PBDE metabolite 6-OH-BDE-47, which ranged from <4 to 127 pg/g wet weight, with a median of 26 pg/g wet weight) and 17β-estradiol in umbilical cord serum collected from 26 births in South Korea (Wan et al. 2010). Serum PBDE concentrations had no effect on expression of genes for the sex steroid enzymes aromatase (CYP19A1) and 17-α-hydroxylase or estrogen receptors α and β (ESR1 and ESR2) in leukocytes collected from 139 adult daughters of Michigan fisheaters with serum total PBDE concentrations ranging from 4.3 ng/g lipid at the 5^{th} percentile to 209.5 ng/g lipid at the 95^{th} percentile (median=33.8 ng/g lipid) (Karmaus et al. 2011).

Vagi et al. (2014 examined the association between blood levels of multiple environmental pollutants and Polycystic Ovary Syndrome (PCOS). Fifty-two PCOs patients and 50 controls were included in the study. PCOS is an endocrine- metabolic disorder characterized by ovulatory dysfunction, hirsutism, or elevated levels of androgens in blood. Eleven PBDEs were analyzed in blood, but only six had detectable serum concentrations (however, only five are listed in the report: BDE 28, BDE 47, BDE 99, BDE 100, and BDE 153). Cases and controls had comparable serum concentrations of these BDEs, and in both groups, BDE 47 had the highest concentration (~25.5 ng/g lipid). After controlling for age, BMI, and ethnicity, none of the measured BDEs was associated with increased risk of PCOS.

Concentrations of PBDEs in omental fat or serum were not associated with risk of uterine fibroids in women undergoing laparoscopy or laparotomy in a study conducted in Salt Lake City, Utah, and San Francisco, California (Trabert et al. 2015). Ninety-nine women had fibroids and 374 had none. Seven PBDEs were measured: BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183, and BDE 209. Whether in omental fat or serum, geometric mean concentrations of PBDEs were comparable between women who had fibroids and those who did not. Odds of a fibroid diagnosis (and corresponding 95% CIs) showed no association with PBDEs.

Acute-Duration Animal Studies

Lower-brominated PBDEs: There are limited data regarding the reproductive effects of acute exposure to lower-brominated PBDEs in females. In rat dams exposed once to tetraBDE at 0, 0.14, or 0.7 mg/kg on GD 6 via gavage, a significant 17% increase in paired ovary weight was observed at 0.14 mg/kg, but not 0.7 mg/kg (Talsness et al. 2008). No treatment-related changes in gravid uterine weight or number of implantation or resorptions were observed in rat dams exposed to pentaBDE at doses up to 2 mg/kg/day from GD 6 to 19 (Blanco et al. 2012). In ovariectomized female mice, no exposure-related changes were observed in uterine wet weight, uterine epithelial height, or vaginal epithelium thickness following exposure to pentaBDE at doses up to 300 mg/kg/day for 3 days via gavage, with or without co-exposure to β-estradiol-3-benzoate (Mercado-Feliciano and Bigsby 2008a).

The effects of acute pentaBDE exposure on androgen-dependent tissue weights was assessed in castrated male rats supplemented with subcutaneous injections of testosterone (to ensure equal levels of circulating testosterone in the exposed and control groups) in a Herschberger assay (Stoker et al. 2005). Male rats that were castrated on PND 42 were exposed to pentaBDE at 0, 30, 60, 120, or 240 mg/kg/day from PND 53 to 61 via gavage, and daily gavage exposures were followed by daily subcutaneous injections of 0.4 mg/kg testosterone. On PND 62, significant decreases in androgen-dependent tissue weights included a ~20–55% decrease in ventral prostate weight at ≥30 mg/kg/day, a ~20–60% decrease in seminal vesicle weight at ≥60 mg/kg/day, a ~28–41% decrease in Cowper's gland weight at ≥120 mg/kg/day, and a 22–29% decrease in the gland penis and levator ani bulbo cavernosus weight at 240 mg/kg/day (Stoker et al. 2005).

There is limited evidence for exposure-related effects on serum reproductive hormones levels in male rats following acute exposure to pentaBDE. Serum testosterone levels were significantly decreased by ~40–45% in male rats 45 days after a single gavage exposure to pentaBDE at doses ≥0.6 mg/kg; serum

progesterone was significantly decreased by ~40% at 1.2 mg/kg (data presented graphically) (Alonso et al. 2010). Significantly increased serum levels of LH were reported in male rats (~65%, data reported graphically) following exposure to pentaBDE at 60 mg/kg/day for 3 days (Stoker et al. 2005). No exposure-related changes were observed in serum testosterone, androsteridione, or estrone levels at doses up to 60 mg/kg/day (Stoker et al. 2005). Following a single gavage administration of pentaBDE at 0, 0.06, or 0.3 mg/kg on GD 6, no exposure-related changes in serum testosterone or LH levels were observed in F1 male rats on PND 140 (Kuriyama et al. 2005).

As discussed in Section 3.2.2.6 (Developmental Effects), F1 reproductive success was assessed following a single gavage administration of pentaBDE at 0, 0.06 or 0.3 mg/kg or tetraBDE at 0, 0.14, or 0.7 mg/kg on GD 6 in rat dams (Kuriyama et al. 2005; Talsness et al. 2005, 2008). Following pentaBDE exposure, no significant exposure-related effects were observed in F1 male fertility when exposed males were mated with unexposed females, and the only mating behavior significantly altered was a 32% decrease in the percent of males with two or more ejaculations in F1 males from the 0.3 mg/kg group (Kuriyama et al. 2005). Similarly, no changes in female pregnancy rate was observed in F1 females mated to unexposed males following exposure to pentaBDE or tetraBDE (Talsness et al. 2005, 2008). F1 male reproductive performance was not assessed following tetraBDE exposure. Despite a lack of exposure-related impairment in reproductive success following acute developmental exposure to penta- or tetraBDE, reductions in testicular weight, sperm/spermatid number, and daily sperm production were observed in F1 males exposed to pentaBDE at ≥0.06 mg/kg/day, and a decreased number of secondary follicles and ultrastructural changes in the ovaries were observed in F1 females exposed to pentaBDE at ≥0.06 mg/kg/day or tetraBDE at ≥0.14 mg/kg/day (Kuriyama et al. 2005; Talsness et al. 2005, 2008).

In a one-generation study in mink, females exposed to pentaBDE at doses ≥0.25 mg/kg/day from pre-mating day 28 through PNW 6 did not whelp (Bull et al. 2007; Zhang et al. 2009). It is not clear in the Bull et al. (2007) study whether mink exposed to 0.25 mg/kg/day never became pregnant or had complete litter loss. However, Zhang et al. (2009) reported that females exposed to 0.31 mg/kg/day had no exposure-related changes in mating success; rather, sows showed complete litter loss with 70% showing clear postimplantation loss.

DecaBDE: In mouse dams exposed to decaBDE at 0, 150, 750, 1,500, or 2,000 mg/kg/day via gavage from GD 7 to 9, the percentage of postimplantation loss per litter was significantly increased by 3, 2.7, and 9.8% at 750, 1,500, and 2,000 mg/kg/day, respectively, compared with control (Chi et al. 2011). At 1,500 and 2,000 mg/kg/day, the percentage of resorptions per litter was also significantly increased by

2.7 and 8.6%, respectively. Additionally, the percentage of live fetuses per litter was significantly decreased by 10% in the high-dose group (Chi et al. 2011). These effects could reflect reproductive toxicity in the dams or developmental toxicity in the fetuses (see Section 3.2.2.6, Developmental Effects). No other acute-duration studies analyzing reproductive effects were located for decaBDE.

Intermediate-Duration Animal Studies

Lower-brominated PBDEs: No exposure-related changes were observed in reproductive end points (number of pregnancies, gestation length, number, size, or sex ratio of litters) in rats exposed to pentaBDE at doses up to 25 mg/kg/day for 70 days prior to mating until PND 42 via gavage (Bondy et al. 2013), in rats fed vanilla wafers containing pentaBDE at doses up to 11.4 mg/kg/day from pre-mating day 28 through PND 21 (Poon et al. 2011), in mice exposed to tetraBDE at doses up to 0.1 mg/kg/day via gavage from pre-mating day 28 through PND 21 (Woods et al. 2012), or in mice fed cornflakes containing tetraBDE at doses up to 1 mg/kg/day from pre-mating day 28 through PND 21 (Koenig et al. 2012; Ta et al. 2011). Similarly, no exposure-related effects on litter parameters (successful delivery of litters, gestation length, litter size, sex ratio, number of live pups) were observed in rats or mice exposed to pentaBDE at doses up to 2 or 10 mg/kg/day, respectively, from GD 1 or 6 to PND 21 via gavage (Branchi et al. 2005; Cheng et al. 2009; Zhao et al. 2014), in rats exposed to pentaBDE at doses up to 30 mg/kg/day from GD 1 to PND 21 via dosed cookies (Bowers et al. 2015), or rats exposed to dietary tetraBDE at doses up to 32 mg/kg/day from GD 1 to PND 14 (Wang et al. 2011a). The number of litters surviving until PND 8 was significantly decreased following exposure to tetraBDE at 0.1 mg/kg/day from pre-mating day 28 through PND 21 in one study (Woods et al. 2012); however, reduced pup survival was not reported in other studies (Bondy et al. 2013; Koenig et al. 2012; Poon et al. 2011; Ta et al. 2011; Wang et al. 2011a).

Male rats exposed to tetraBDE at 1 mg/kg/day via gavage for 8 weeks showed a significant 24% decrease in daily sperm production; no exposure-related effects were observed at doses ≤0.03 mg/kg/day (Zhang et al. 2013b). In another study, sperm morphology, motility, and capacitation were evaluated in mice following gavage exposure to tetraBDE at 0, 0.0015, 0.045, 1.5, or 30 mg/kg/day for 30 days (Wang et al. 2013). No exposure-related changes in sperm morphology or sperm motility were observed. A significantly decreased rate of sperm capacitation (% B-type [mature] sperm) was observed in the 0.0015, 0.045, and 30 mg/kg/day groups, but not in the 1.5 mg/kg/day group (Wang et al. 2013). No exposure-related changes in sperm counts, motility, or DNA damage were observed in rats exposed to a penta-

decaBDE mixture (52.1% pentaBDE, 44.2% decaBDE, 0.4% octaBDE) at dietary doses up to 20 mg/kg/day for 70 days (Ernest et al. 2012).

Microscopic changes in the testes of rats were observed following exposure to tetraBDE at ≥0.03 mg/kg/day via gavage for 8 weeks, including increased epithelial thickness, vacuolar spaces in the seminiferous epithelium, and increased number of multinucleated giant cells (arising from spermatocytes that aborted meiosis); no histopathological changes were observed at 0.001 mg/kg/day (Huang et al. 2015; Zhang et al. 2013b). Additionally, the number of apoptotic cells was significantly increased by 2- and 3-fold in the testes of rats from the 0.03 and 1 mg/kg/day groups, respectively (Huang et al. 2015; Zhang et al. 2013b). In similarly-exposed rats, co-treatment with dexamethasone phosphate (DEX; a CYP3A1 inducer) "aggravated" the observed histopathological effects (Zhang et al. 2013b). Mice exposed to tetraBDE at 0, 0.0015, 0.045, 1.5, or 30 mg/kg/day via gavage for 30 days also showed testicular effects at doses ≥0.045 mg/kg/day, including "some" seminiferous tubules with complete germ cell loss and a Sertoli cell-only phenotype (incidence not reported) and a dose-related increase in the number of apoptotic cells (Wang et al. 2013). No exposure-related changes were observed in testes weight at doses up to 30 mg/kg/day (Wang et al. 2013). In other studies, exposure-related changes were not observed in organ weight or histology in male or female reproductive tissues from mice exposed to tetraBDE at dietary doses of 0.45 mg/kg/day for 28 days (Maranghi et al. 2013), rats exposed to penta- or octaBDE at dietary doses up to 750 mg/kg/day for 28–90 days (IRDC 1976, 1977; WIL Research Laboratories), rats exposed to a penta-decaBDE mixture (52.1% pentaBDE, 44.2% decaBDE, 0.4% octaBDE) at dietary doses up to 20 mg/kg/day for 70 days (Ernest et al. 2012), rats exposed to pentaBDE at gavage doses up to 250 mg/kg/day for 15–28 days (Becker et al. 2012; Oberg et al. 2010), or ovariectomized mice exposed to pentaBDE at 50 mg/kg/day for 34 days via gavage (with or without β-estradiol-3-benzoate co-exposure) (Mercado-Feliciano and Bigsby 2008a). Additionally, organ weight changes were not observed in F0 or F1 rats exposed to pentaBDE at doses up to 25 mg/kg/day via gavage from pre-mating day 70 to PND 42 (Bondy et al. 2013) or F0 or F1 mink exposed to dietary pentaBDE at doses up to 0.31 mg/kg/day for 4 weeks prior to mating through PNW 6 or 33 (Zhang et al. 2009).

Following exposure to pentaBDE at 0, 3, 30, or 60 mg/kg/day for 15 days via gavage, serum prolactin was significantly decreased by 67% at 60 mg/kg/day in male rats (Becker et al. 2012). While testosterone and FSH levels were not significantly altered at any specific dose (based on pair-wise analysis), trend tests showed significant dose-dependent increases in testosterone and FSH levels; LH and E2 were below the detection limit in all groups (Becker et al. 2012). In contrast, a repeat of the same study in a different laboratory showed no exposure-related changes in serum testosterone, LH, FSH, E2, or prolactin in male

rats exposed to pentaBDE at doses up to 60 mg/kg/day for 15 days (Becker et al. 2012). Similarly, male rats exposed to a penta-decaBDE mixture (52.1% pentaBDE, 44.2% decaBDE, 0.4% octaBDE) at dietary doses up to 20 mg/kg/day for 70 days did not show altered serum testosterone levels (Ernest et al. 2012). After exposure to tetraBDE for 8 weeks via gavage (6 days/week), male rats showed significant reductions in serum testosterone of ~35, 54, and 63% at 0.001, 0.03, or 1 mg/kg/day, respectively (data reported graphically) (Zhang et al. 2013b). No exposure-related changes were observed in serum E2, FSH, or LH levels at doses up to 1 mg/kg/day (Zhang et al. 2013b). In female mice exposed to dietary tetraBDE for 28 days, serum testosterone and E2 were significantly increased by 57 and 18% at 0.45 mg/kg/day (only tested dose) (Maranghi et al. 2013).

Biochemical analysis of rat testes showed significant dose-related elevations of mRNA levels of several apoptosis genes following exposure to tetraBDE at doses of 0.001–1 mg/kg/day via gavage for 8 weeks (6 days/week) (Zhang et al. 2013b). Additionally, elevated levels of ROS were observed at 1 mg/kg/day (Zhang et al. 2013b). Co-exposure to DEX (a CYP3A1 inducer) enhanced ROS-induction in the testes, with significant elevations observed at ≥0.001 mg/kg/day (Zhang et al. 2013b). No exposure-related changes were observed in testicular mRNA expression levels for genes involved in steroidogenesis (Star, Cyp17a1, Ar, Srd5a1, Srd 5a2, Cyp19a1, Esr1, Esr2) in rats following exposure to a penta-decaBDE mixture (52.1% pentaBDE, 44.2% decaBDE, 0.4% octaBDE) at dietary doses up to 20 mg/kg/day for 70 days (Ernest et al. 2012).

Exposure to lower-brominated PBDEs has been reported to cause reproductive effects in developing rats. Two companion studies evaluated reproductive system development following exposure to pentaBDE at 0, 3, 30, or 60 mg/kg/day via gavage during pubertal development (PNDs 23–53 in males or PNDs 22–41 in females) (Stoker et al. 2004) or 0, 60 or 120 mg/kg/day via gavage during pubertal development in males (PNDs 23–53) (Stoker et al. 2005). In males, preputial separation (PPS) was significantly delayed by 1.7–5 days at ≥30 mg/kg/day, absolute ventral and lateral prostate weights and seminal vesicle weights were significantly decreased 16–29% at ≥60 mg/kg/day (in the absence of body weight effects), and serum prolactin was increased 2-fold at 60 mg/kg/day (not evaluated in the 120 mg/kg/day group). There were no exposure-related changes in testicular weight or histology, epididymides histology, serum testosterone or LH, or pituitary LH or prolactin levels at doses up to 60–120 mg/kg/day (Stoker et al. 2004, 2005). In females, vaginal opening was significantly delayed by 1.8 days in the 60 mg/kg/day group. No changes were observed in estrous cycling or ovarian or uterine weight or histology at doses up to 60 mg/kg/day (Stoker et al. 2004). As summarized in Section 3.2.2.6 Developmental Effects, observed effects in F1 rats exposed to pentaBDE at 30.1 mg/kg/day from GD 6 to PND 21 included decreased

anogenital distance, delayed PPS, and decreased serum testosterone in male offspring and decreased mammary gland development in female offspring (Kodavanti et al. 2010).

DecaBDE: Information on effects of intermediate-duration exposure to decaBDE on reproductive function is limited to negative findings in a one-generation study in rats using a low-purity (77.4%) decaBDE mixture (Dow Chemical Co. 1975; Norris et al. 1975a). Male and female rats were exposed to 0, 3, 30, or 100 mg/kg/day doses in the diet for 60 days prior to mating through PND 21. Parameters monitored included length of time between first day of cohabitation and parturition, numbers of live and dead newborn, number of live pups (PNDs 1, 7, 14, and 21), litter weight (PNDs 1, 7, and 14), and weanling weight (PND 21). Comprehensive histological examinations (adults and weanlings), skeletal examinations (weanlings), and cytogenetic evaluation of bone marrow (adults and weanlings) were also performed on PND 21. There were no exposure-related effects on reproductive parameters or any indications of maternal or neonatal toxicity. Additionally, no exposure-related changes were observed in litter parameters in mice exposed to decaBDE at gavage doses up to 1,500 mg/kg/day from GD 0 to 17 (Tseng et al. 2008), in rats exposed to decaBDE at gavage doses up to 1,000 mg/kg/day from GD 6 to PND 21 (Biesemeier et al. 2011), or in rats or mice exposed to decaBDE at dietary doses up to 146 or 3,100 mg/kg/day, respectively, from GD 10 to PND 21 (Fujimoto et al. 2011; Watanabe et al. 2008, 2010b).

Sperm parameters have been assessed in rats and mice following intermediate-duration exposure to decaBDE. In the rat study, no exposure-related changes were observed in male epididymal sperm counts or morphology following exposure to decaBDE at gavage doses up to 60 mg/kg/day for 28 days (Van der ven et al. 2008a). In one mouse study, sperm count and viability were significantly reduced by about 40% in males exposed to 950 mg/kg/day via gavage for 35 days, compared with controls; no exposure-related changes in sperm count and viability were observed at 750 mg/kg/day (Sarkar et al. 2015). In contrast, no exposure-related changes were observed in sperm count, sperm motility, or the percent of abnormal sperm heads in mice exposed to decaBDE at doses up to 1,500 mg/kg/day via gavage for 50 days (Tseng et al. 2006). For sperm velocity, the lateral head amplitude was significantly decreased in the 500 and 1,500 mg/kg/day groups, compared with control; no exposure-related changes were observed in curvilinear, average path, or straight line velocity or beat-cross frequency. No exposure-related changes were observed in sperm damage parameters; however, sperm H_2O_2 production was significantly increased at 500 and 1,500 mg/kg/day (no change in O_{2^-} production). The percentage of sperm with high mitochondrial membrane potential was significantly decreased in the 1,500 mg/kg/day group, compared with controls.

There is limited evidence of histopathological damage in reproductive organs following intermediate-duration exposure to decaBDE. Various degenerative changes were observed in the seminiferous tubules of mice exposed to decaBDE at gavage doses of 950 mg/kg/day for 35 days, including thinning of the germinal epithelium, decreased diameter of the seminiferous tubules, depletion of germ cells, exfoliation of germ cells, and intraepithelial vacuolation (Sarkar et al. 2015). Both the height of the germinal epithelium and the diameter of seminiferous tubules were significantly decreased at 950 mg/kg/day, compared with controls. No exposure-related testicular lesions were observed at 750 mg/kg/day. In female rats exposed to decaBDE at 0 or 300 mg/kg/day from 3 weeks of age, through mating to untreated males, gestation, and lactation (~11 weeks), the ovaries in the exposed rats had significantly increased "histological scores" than the ovaries from the control rats (methods of histological scoring and incidences of lesions were not reported) (Liu et al. 2012). Observations in the ovaries of exposed rats included atrophic changes, decreased number of follicles, and increased fibrotic tissue (Liu et al. 2012). F0 reproductive success and F1 developmental end points were not reported in this study. However, in other intermediate-duration studies, no histopathological changes were observed following exposure to decaBDE in male or female reproductive tissues from rats exposed for 28–50 days at gavage doses up to 1,500 mg/kg/day (Tseng et al. 2006; Van der ven et al. 2008a), rats exposed for 28–30 days at dietary doses up to 800 mg/kg/day (IRDC 1976; Norris et al. 1973, 1975a), or rats or mice exposed for 13 weeks at dietary doses up to 8,000 or 9,500 mg/kg/day, respectively (NTP 1986).

In a 28-day study, rats were exposed to decaBDE at 0, 1.7, 3.75, 7.5, 15, 30, or 60 mg/kg/day via gavage (Van der ven et al. 2008a). The study authors reported a dose-related decrease in epididymis weight in male rats (maximal decrease of 22.5%) and a dose-related increase in seminal vesicle/coagulation gland weight (maximal increase of 38.3%); however, the lowest doses at which the effects were observed were not reported. Instead, results were reported in terms of BMD analysis ($BMD_{RD10\%}$ for epididymis= 4.0 mg/kg/day, $BMDL_{RD10\%}$ was not determined; $BMD/BMDL_{RD10\%}$ for seminal vesicle= 1.5/0.2 mg/kg/day). No exposure-related changes in organ weight were reported for testes, ovaries, or uterus at doses up to 60 mg/kg/day (Van der ven et al. 2008a). In other rat studies, no exposure-related changes in testes, epididymides, or seminal vesicle weights were observed following exposure to dietary decaBDE doses up to 800 mg/kg/day for 28–30 days (IRDC 1976; Norris et al. 1973, 1975a). In mice, one study reports a significant 13–18% decrease in relative testis and epididymides weight following exposure to decaBDE at 950 mg/kg/day via gavage for 35 days, compared with controls; no exposure-related changes in male reproductive organ weights were observed at 750 mg/kg/day (Sarkar et al. 2015). In contrast, no exposure-related changes in testes, epididymides, or seminal vesicle weights were

observed in another mouse study that exposed animals to decaBDE at gavage doses up to 1,500 mg/kg/day for 50 days (Tseng et al. 2006).

In a 35-day study, serum testosterone levels were significantly decreased by 65% in mice following exposure to decaBDE at gavage doses of 950 mg/kg/day, compared with controls; testosterone levels at 750 mg/kg/day were comparable to control (Sarkar et al. 2015).

There is limited evidence that exposure to decaBDE in developing animals results in reproductive effects. In male rats exposed during pubertal development, exposure to decaBDE at doses up to 600 mg/kg/day did not cause exposure-related changes in testes, epididmides, or prostate organ weight, testicular histology, or testicular mRNA expression levels of steriodogensis-related genes (Lee et al. 2010). As summarized in Section 3.2.2.6 (Developmental Effects), only one intermediate-duration gestational exposure study reported reproductive effects in mouse offspring exposed to decaBDE doses of 10–1,500 mg/kg/day from GD 0 to 17 via gavage, including testicular lesions, decreased AGD, and altered sperm parameters (Tseng et al. 2013). No exposure-related changes in AGD, onset of puberty (PPS or vaginal opening), estrous parameters, or reproductive organ weight and histology were reported in offspring exposed to decaBDE at doses up to 1,000 mg/kg/day during gestation and lactation (Biesemeier et al. 2011; Fujimoto et al. 2011) or doses up to 20 mg/kg/day from PND 2 to 15 (Rice et al. 2007).

Chronic-Duration Animal Studies

Lower-brominated PBDEs: No chronic-duration studies evaluating reproductive system effects were located for lower-brominated PBDEs.

DecaBDE: No histopathological changes were observed in male or female reproductive tissues from rats or mice that were exposed to decaBDE at dietary doses up to 2,550 or 7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986). In the only other chronic-duration study, a low-purity mixture (77.4% purity) did not cause histopathological changes in male or female reproductive tissues from rats at doses up to 1.0 mg/kg/day for 2 years (Kociba et al. 1975; Norris et al. 1975a),

Summary. Based on the evidence in humans and animals, lower-brominated PBDEs are potentially toxic to the male reproductive system in humans, including the developing reproductive system in children (see Section 3.2.2.6, Developmental Effects for more details). Available data for decaBDE provide very limited evidence of male reproductive damage, and are insufficient to determine if oral decaBDE

exposure can damage the male reproductive system in humans. For female reproductive end points, data are inconsistent in humans and animals; therefore, it is unclear whether PBDEs affect the female reproductive system in adults or developing infants/children. The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

Human Studies. Numerous studies have investigated potential relationships between developmental PBDE exposure and birth outcomes (e.g., birth weight, length, head circumference, gestational age) and/or postnatal development of various systems (neurological, reproductive, endocrine, immune).

Physical Growth and Development and Related Birth Outcomes

Robledo et al. (2015a) studied potential relationships between pre-conception maternal and paternal serum levels of 10 PBDEs (BDE 17, BDE 28, BDE 47, BDE 66, BDE 85, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 183) and birth weight and length, head circumference, and ponderal index (100x[birth weight in g/birth length in cm^3]) in a prospective cohort study in Michigan and Texas (LIFE study). Geometric means of maternal and paternal serum PBDE levels (n=234 pairs) were 0.001–0.116 ng/g serum and 0.001–0.113 ng/g serum, respectively. The relative concentrations of PBDEs was similar in maternal and paternal serum, with BDE 47 having the highest level (Robledo et al. 2015b). Levels were not reported in terms of ng/g lipid. Maternal serum BDE 28 and BDE 183 levels and paternal serum BDE 183 were significantly associated with lower birth weight in female infants, with a decrease of 84.6–151.33 g per 1 standard deviation (SD) increase in ln- transformed chemical concentration. Maternal serum BDE 28 was also significantly associated with smaller birth length (-1.14 cm per 1-SD increase) and head circumference (-1.05 cm per 1-SD increase) in female infants. However, in male infants, maternal serum BDE 66 and BDE 99 were significantly associated with higher birth weight (+85.21–125.04 g per 1-SD increase) and larger head circumference (+0.6–0.91 cm per 1-SD increase. Maternal serum BDE 99 was also significantly associated with larger birth length in boy infants (+0.76 cm per 1-SD increase). No other significant associations were observed.

Maternal serum concentrations were studied in a population of 286 low-income women living in the Salinas Valley of California (a subset of the CHAMACOS study) (Harley et al. 2011). The main PBDEs detected in maternal blood, collected during the second trimester of pregnancy, were BDE 47, BDE 99, BDE 100, and BDE 153 (median concentrations of 14.57, 3.85, 2.45 and 2.03 ng/g lipid, respectively).

Serum concentrations of each of these congeners, and their sum, were significantly inversely related to infant birth weight in crude analysis. After adjustment for covariates, regression analysis showed that each 10-fold increase in BDE 47, BDE 99, or BDE 100 was associated with roughly a 115-g decrease in birth weight. These associations were no longer statistically significant when maternal weight gain was included in the models. There were no significant relationships between maternal serum PBDE and infant birth length or head circumference, or length of gestation. In a follow-up study of the CHAMACOS cohort, the potential association between obesity indices (BMI, waist circumference, obesity/overweight status) at 2–7 years of age and maternal serum PBDE levels was evaluated (BDE 47, BDE 99, BDE 100, BDE 153, and their sum); child serum PBDE levels were also determined at age 7. The geometric means of the total maternal (n=224) and child (n=216) serum PBDE concentration were 25.35 and 83.03 ng/g lipid, respectively (Erkin-Cakmak et al. 2015). No significant associations were observed between obesity measures and maternal serum concentrations of individual or summed PBDEs at any age when both sexes were examined together; however, statistical analysis revealed a significant effect modification by sex. When evaluated separately, a significant negative relationship was observed between BMI *z*-score in 3.5-year-old females and a 10-fold increase in summed maternal serum PBDE levels (adjusted β -0.64, 95% CI -1.23, -0.06), and a significant positive relationship was observed between BMI *z*-score in 3.5-year-old males and a 10-fold increase in summed maternal serum PBDE levels (adjusted β 0.99, 95% CI 0.32, 1.66). This sex difference was also observed at 7 years of age, with a significant negative association in females (adjusted β -0.41, 95% CI -0.87, -0.05) and a near-significant positive association in males (adjusted β 0.26, 95% CI: -0.19, 0.72). Similar trends were observed for waist circumference and obesity status (data not reported). When evaluating child serum PBDE levels, significant associations after adjustment for potential cofounders included a negative association between BDE 153 and ∑PBDEs and BMI and waist circumference and a reduced risk for being overweight. There was no significant effect by sex modification in the analysis of child serum levels. This follow-up study suggests possible obesogenic effects of *in utero* PBDE exposure in boys. In contrast, Vuong et al. (2016b) found no significant association between PBDEs (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, and their sum) measured in maternal serum at 16 weeks of gestation (geometric mean 39.1 ng/g lipid) and height or weight of boys and girls at 1–8 years of age. However, BDE 153 was associated with lower BMI at 2–8 years, smaller waist circumference at 4–8 years, and lower percent body fat at 8 years. The Vuong et al. (2016b) study comprised 318 mother-child pairs in the Health Outcomes and Measures of the Environment (HOME) Study in Cincinnati, Ohio. Agay-Shay et al. (2015) also evaluated the potential association between maternal PBDE exposure and childhood obesity. This study did not find significant associations between maternal PBDE colostrum levels (BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 209, and their sum) and BMI *z*-scores or risk of being overweight (BMI >85th percentile) in

470 7-year-old boys and girls from a Spanish birth cohort study; however, sex-specific analyses were not conducted. Geometric mean concentrations of BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 209 in colostrum were 0.5, 0.3, 0.2, 0.7, 0.7,and 0.8 ng/g lipid, respectively.

Results from other studies evaluating potential associations between maternal serum PBDE levels during pregnancy and/or at delivery and birth outcomes are inconsistent between studies and congeners. The Healthy Pregnancy, Healthy Baby (HPHB) birth cohort in North Carolina also evaluated potential associations between third trimester maternal serum PBDE concentrations and birth weight, length, head circumference, and birth weight percentile for gestational age (Miranda et al. 2015). Serum was evaluated for 27 PBDE congeners and 6 OH-PBDEs; however, only congeners and metabolites detected in >50% of the subjects (n=137) were included in the analysis, including BDE 47, BDE 99, BDE 100, BDE 153, 4'-OH-BDE-49, and 6'-OH-BDE-47 (median serum concentrations of 18.87, 5.5, 4.61, 5.65, 0.12, and 0.19 ng/g lipid, respectively). The only significant finding was a negative association between maternal BDE 153 and head circumference (0.32 cm decrease per 2-fold increase in BDE 153); however, this association was no longer significant after adjustment for maternal risk factors. Similarly, Serme-Gbedo et al. (2016) did not find a statistically significant relationship between birth weight and BDE 47, BDE 99, BDE 100, BDE 153, or their sum in maternal serum collected during early pregnancy (~12 weeks; median concentration of 32.99 ng/g lipid for total PBDE). This study evaluated 349 Canadian women (GESTE birth cohort), and utilized both unadjusted models and multivariate regression models adjusted for a full range of clinical risk factors known to affect fetal growth as well as other environmental pollutants that are likely to impact fetal growth (PCBs, mercury, lead, cadmium, manganese). In contrast, Chen et al. (2015) reported negative associations between lower levels of maternal serum PBDEs and birth weight and length in the prospective Laizhou Wan birth cohort (LWBC) in 215 Chinese women. In this study, median maternal serum levels of PBDEs, collected at delivery, were 2.27, 2.26, 3.53, 2.13, and 4.87 ng/g lipid for BDE 28, BDE 47, BDE 99, BDE 100, and BDE 153, respectively (median concentration of total PBDEs was 21.68 ng/g lipid). After adjustment for potential covariants, a near-significant negative association was observed between birth weight and maternal serum BDE 28; when stratified by sex, this decrease was significant for males (253.76 g decreased per 10-fold increase in BDE 28), but not females. A negative association was observed between birth length and maternal serum levels of all congeners; however, the association was only statistically significant for BDE 28 and BDE 100 when males and females were combined (0.92–0.97 cm decrease per 10-fold BDE increase). When stratified by sex, BDE 99 was significantly negatively associated with male infant length and BDE 100 was significantly negatively associated with female infant length (1.47–1.50 cm decrease per 10-fold BDE increase). There were no significant relationships between maternal serum PBDE and

infant head circumference or length of gestation. In a case-control study of 197 full-term births and 82 pre-term births, identified at the Centennial Women's Hospital in Nashville, serum BDE 47 levels were divided into the following ordinal scale: 25–135, 136–200, 200–321, 322–1,000, and >1,000 pg/mL (Peltier et al. 2015). The distribution for controls into the five bins was 23, 22, 21, 22, and 12%, respectively, while the distribution for cases was 10, 6, 20, 35, and 29%, respectively; these distributions are statistically significantly different. When cases and controls were combined for analysis, women from the two higher exposure bins (>323 pg/mL BDE 47) showed a significantly increased risk for preterm birth.

Several investigators have also evaluated associations between serum maternal and umbilical cord PBDE levels, and their potential associations with birth indices. As observed in maternal serum studies, data are inconsistent between studies and congeners. In a study of 97 Canadian women (a subset of the FAMILY study), Foster et al. (2011) found no significant relationship between infant birth weight and maternal serum PBDE (BDE 17, BDE 28, BDE 47, BDE 66, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183, and their sum), whether the serum was collected during the second trimester of pregnancy or at delivery. PBDE concentrations in this study were relatively high (median concentrations of 52.1 and 50.1 ng/g lipid for total PBDE at mid-pregnancy and delivery). Foster et al. (2011) also evaluated the potential relationship between umbilical cord serum PBDE (BDE 17, BDE 28, BDE 47, BDE 66, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183, and their sum) and birth weight. They found a significant negative association between birth weight and concentrations of one PBDE congener (BDE 99) in umbilical cord serum. PBDE concentrations in umbilical cord serum were 1.7–3.4 times higher than in maternal serum at delivery (median concentration of 100.0 ng/g lipid for total PBDE). These results are in contrast to Mazdai et al. (2003), who found umbilical cord concentrations of PBDE (BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183, and their sum) to be similar to maternal serum concentrations at delivery (median concentrations of 39 and 37 ng/g lipid for total PBDE, respectively) in a small study of 12 women recruited upon presenting in labor to Indianapolis hospitals. In this study, no relationship of maternal or cord blood serum to infant birth weight was observed. In contrast to these findings, a low exposure study in 686 Spanish women (INMA cohort; median total serum PBDE concentrations in maternal blood collected at gestational week 12 and umbilical cords of 10.74 and 7.51 ng/g lipid, respectively) reported a significant relationships between PBDE levels and some birth indices (Lopez-Espinosa et al. 2015). While concentrations were similar between maternal and umbilical cords serum, Pearson's correlations between maternal and cord $\log_2$(PBDEs) were low (0.18, 0.06, 0.04, 0.09, and 0.07 for BDE 47, BDE 99, BDE 153, BDE 159 and BDE 209, respectively). Potential associations were examined between maternal or cord serum and birth weight, length, and head circumference for each

congener and their sum. A significant inverse association was observed between birth weight and maternal serum BDE 99 levels (Lopez-Espinosa et al. 2015). After adjustment for covariates, regression analysis showed that each 2-fold increase in BDE 99 was associated a 1.4% decrease (~46.8 g) decrease in birth weight. Maternal serum BDE 99, as well as the sum of all PBDEs, was also significantly associated with a 2.1–2.9% decrease in head circumference per 2-fold increase in PBDE concentration (~0.7–1.0 cm). No significant associations were observed between birth weight or head circumference for other maternal serum congeners or any cord serum congeners, and no significant associations were observed for birth length. Lopez-Espinosa et al. (2015) also evaluated fetal growth indices during pregnancy using ultrasound measurements in mid-pregnancy (gestational weeks 12–20) and late pregnancy (gestational weeks 20–34), including abdominal circumference, fetal length, biparietal distance, and estimated fetal weight. Significant associations, observed only during gestational weeks 20–34, included inverse relationships between maternal BDE 99 and biparietal distance, abdominal circumference, and estimated fetal weight; cord BDE 99 and ∑PBDEs and fetal abdominal circumference and estimated fetal weight; and maternal ∑PBDEs and biparietal distance (1.3–3.5% decrease per 2-fold increase in PBDE concentration). In another low-exposure study, Tan et al. (2009) also found no relationship between PBDE in umbilical cord blood and infant birth weight (or length, head circumference, or sex) in babies from 41 native mothers admitted to the Singapore National Hospital for Cesarean section. Median PBDE concentrations in this study population 3.3 ng/g lipid for total PBDE in cord blood. There was reported to be a small positive association between cord blood concentrations of the PBDE congeners BDE 47 and BDE 99 and Apgar score at 1 minute in this study.

Wu et al. (2010) also studied cord blood, but did not assess effect on birth weight alone, using instead a composite of adverse birth outcomes, including low birth weight but also premature delivery and stillbirth. These researchers reported significant associations between umbilical cord PBDE (BDE 28, BDE 47, BDE 99, BDE 153, BDE 183, and total) and adverse birth outcomes in a comparison of 128 normal births and 25 cases of adverse birth outcomes from two towns (Guiyu and Chaonan) in China. The two towns represent an e-waste recycling area (Guiyu, with a median total PBDE concentration of 13.8 ng/g lipid [n=102]) and a control area with no e-waste recycling workshops (Chaonan, with a median total PBDE concentration of 5.2 ng/g lipid [n=51]). In a companion study, neonatal physiological indices were compared between 69 births in Guiyu (e-waste recycling) and 86 births in Haojiang (no e-waste re cycling), using placental PBDE concentrations as an exposure metric (Xu et al. 2015b). Median total placental PBDE concentrations were 32.25 ng/g lipid in Guiyu and 5.13 ng/g lipid in Haojiang; measured congeners included BDE 29, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183, and BDE 209 (mean placental concentrations of 11.66, 1.61, 0.32, 0.14, 3.17, 0.34, 2.20, and 3.30 ng/g lipid,

respectively). Head circumference, BMI, and Apgar1 score were significantly decreased by 4–8% and body length significantly increased by 4% in the Guiyu cohort, compared with the Haojiang cohort. No differences were observed in birth weight between the two cohorts. When data from both cohorts were combined for multiple linear regression analysis, significant negative associations were observed between ∑PBDEs and BDE 47 and head circumference; ∑PBDEs, BDE 47, and BDE 99 and neonatal BMI, and ∑PBDEs, BDE 29, BDE 47, BDE 153, and BDE 183 and Apgar1 score. A significant positive association was observed between BDE 47 and body length.

Concentrations of PBDE in breast milk have also been studied in relation to adverse birth outcomes. A study of births from 20 healthy pregnant women in Taiwan found that increased PBDE (BDE 47, BDE 99, BDE 100, and BDE 209) in breast milk was associated with significantly reduced infant birth weight, length, chest circumference, and Quetelet's index (BMI) (Chao et al. 2007). The median total PBDE concentration in breast milk was 3.65 ng/g lipid in the study population. A significant negative association between breast milk PBDE concentrations (BDE 47, BDE 99, BDE 100, BDE 153, and their sum) and birth weight was also identified in a Swedish cohort of 254 women with a median total PBDE concentration of 2.4 ng/g lipid (Lignell et al. 2013). In contrast, a significant positive association was observed between colostrum (breast milk collected in the first week after delivery) PBDE concentrations (BDE 47, BDE 99, BDE 100, BDE 153, and their sum) and birth weight and birth length in a Tanzanian cohort of 95 women with a median total PBDE concentration of 19.8 ng/g lipid (Müller et al. 2016). When stratified by sex, the only significant finding was a positive association between birth weight and PBDE concentration in female infants. The high PBDE levels were attributed to the consumption of Pemba (clay soil), which is sold in Tanzanian markers to pregnant women as a mineral supplement and nausea-relieving aid. Women who ate Pemba (64%) had significantly higher PBDE concentrations in colostrum, compared to non-Pemba eating women.

Reproductive System Development

Main et al. (2007) found a significant positive relationship between concentrations of PBDE in breast milk and congenital cryptorchidism (undescended testes) in male offspring. The study compared concentrations of 14 PBDE congeners in breast milk of mothers of 62 Danish and Finnish boys with cryptorchidism to mothers of 68 controls from the same population. Significant increases were seen for the sum of the 7 most prevalent congeners (found in all mothers), for several of the individual congeners, and for the sum of all 14 congeners in cases versus controls. No such relationship was found, however, when placental blood PBDE concentrations, rather than maternal breast milk PBDE concentrations, were

used to represent exposure. In a comparison of 86 paired samples from this study, PBDE concentrations in placenta and breast milk were correlated, but absolute PBDE concentrations were 3–4 times higher in breast milk (median of 3.23 ng/g fat for total PBDE) than in placenta (median of 1.19 ng/g fat for total PBDE). The researchers hypothesized that while breast milk PBDE concentrations reflect the accumulated body burden of the mother, placental PBDE concentrations may resemble measurements in single blood samples, reflecting the situation at delivery, but not the long-term exposure. In another case-control study in Danish and Finnish boys, no significant difference was observed in the concentrations of 14 PBDE congeners in subcutaneous tissue samples from the inguinal region during orichidopexy surgery (44 cases) or hernia surgery (38 controls) (Koskenniemi et al. 2015). Mean age at surgery was 2.3 and 2.9 years for cases and controls, respectively. Median concentrations of total PBDEs in adipose tissue were 4.90 ng/g in cases and 5.54 ng/g in controls.

Other studies of reproductive development found no relationship between PBDE concentrations in mid-pregnancy serum samples from California mothers (median ∑PBDE=33.7 ng/g lipid for 20 cases and 38.6 ng/g lipid for 28 controls) and hypospadias in their male offspring (Carmichael et al. 2010) and no relationship between current serum concentrations of PBDE (range of 4.9–73.6 ng/g lipid, with median of 9.9 ng/g lipid) and various measures of sexual maturation (e.g., initiation of breast development, current breast development, age at menarche), in a small cohort of 18 (9 boys and 9 girls) teen-aged Dutch children (Leijs et al. 2008). A study of 55 Dutch boys found significant positive associations between maternal serum concentrations of BDE 154 (median=0.5 ng/g lipid) collected on week 35 of pregnancy (but not other congeners measured, including BDE 47 and BDE 153 that occurred at higher concentrations [medians of 0.9 and 1.6 ng/g lipid, respectively]) and serum concentrations of the sex hormones, E2, free E2, and inhibin B (but not testosterone, LH, FSH, or sex hormone binding globulin), in the baby boys at 3 months of age and testes volume in the boys at 18 months of age, but no effect on penile length at either age (Meijer et al. 2012). In a French birth cohort (n=262 mother-child pairs; 141 male infants, 141 female infants), cord blood total testosterone was significantly decreased in male infants with detectable BDE 209 levels (>0.05 ng/g lipid) compared with male infants without detectable BDE 209 levels (Warembourg et al. 2016). No associations were observed for female total testosterone or other sex hormones measured in male and/or female infants (sex hormone-binding globulin, E2, free testosterone, aromatase index, or Anti-Müllerian hormone).

Delayed onset of puberty was significantly associated with higher PBDE concentrations in serum in a cohort of 645 ethnically diverse girls (recruited at 6–8 years of age and followed annually) from California and Ohio (Breast Cancer and the Environment Research program [BCERP] cohort) (Windham

et al. 2015a). A cross-sectional analysis, conducted at enrollment, showed a significant decrease in the percentage of females showing Tanner stage 2 breast development per quartile increase in serum ∑PBDE levels; no association was observed with pubic hair development. Median serum ∑PBDE levels at enrollment were 78.3 ng/g lipid (Windham et al. 2015b). In follow-up evaluations, the longitudinal analysis showed a significant increase in age at pubertal transition (both breast and pubic hair Tanner stages) with per quartile increase in serum ∑PBDE levels. When analyzed by individual congener, significant delays in pubic hair development were observed for all congers evaluated (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, and BDE 154) and significant delays in breast development were observed for BDE 47, BDE 99, BDE 100, and BDE 153 (associations were marginal for BDE 28 and BDE 154).

Neurodevelopment

Numerous studies have reported results suggestive of an effect of PBDE on neurodevelopment in children. Associations are more consistent when blood concentrations were used as a biomarker of exposure, compared with studies utilizing breast milk concentrations as a biomarker of exposure.

A series of studies evaluated neurodevelopmental outcomes in a longitudinal study of the HOME study cohort from Cincinnati, Ohio (full sample 349 mother-child pairs) (Braun et al. 2014; Chen et al. 2014; Donauer et al. 2015; Vuong et al. 2016a). Several environmental chemicals, including PBDEs, were quantified in maternal blood collected at approximately 16 weeks of gestation. In 5-week-old infants (n=326), no associations were observed between neonatal neurobehavior and maternal serum PBDEs (Donauer et al. 2015). The geometric mean of the sum of the most frequently detected PBDEs (sum_4BDE=BDE 47, BDE 99, BDE 100, and BDE 153) in maternal serum from mother-child pairs in the neonatal study was 37.12 ng/g lipid (Donauer et al. 2015). The majority of children from the HOME cohort (n=309) were examined for cognitive and motor abilities at 1, 2, and 3 years of age, intelligence at 5 years of age, and general behavior at 2, 3, 4, and 5 years of age (Behavioral Assessment System for Children 2 [BASC-2]) (Chen et al. 2014). This analysis focused primarily on maternal BDE 47 exposure (geometric mean=20.1 ng/g lipid; median=18.9 ng/g lipid); however, some analysis were conducted for sum_4BDE (geometric mean=37.7 ng/g lipid; median=34.6 ng/g lipid). Maternal serum levels of BDE 47 or sum_4BDE were not significantly associated with Bayley mental or psychomotor development indices at 1–3 years of age. At 5 years, however, adjusted regression analysis showed a significant 4.5-point decrease in full-scale IQ per 10-fold increase in maternal serum BDE 47 (95% CI -8.8, -0.1). For the sum_4BDE analysis, a marginal decrease in full-scale IQ was observed (β [95% CI] -4.38 [-8.9, 0.14]). In the BASC-2 behavioral assessments at 2–5 years of age, significant associations observed included a

positive association between maternal BDE 47 serum levels and the Externalizing Problems score at 2 and 3 years of age (~2–3-point increase per 10-fold increase in serum BDE 47) and a positive association between maternal BDE 47 serum levels and the Hyperactivity subscore at 2, 3, and 5 years of age (~2–3-point increase per 10-fold increase in serum BDE 47); other ages showed marginal positive associations in these domains. Similar trends were observed in BASC-2 analysis of sum_4BDE analysis. Further analysis of neurodevelopment at 4 and 5 years of age in a subset of the HOME cohort (n=175) was reported by Braun et al. (2014), with a specific focus on autistic behaviors (social, repetitive, and stereotypic behaviors). Specific analysis were conducted for BDE 28, BDE 47, BDE 85, BDE 99, BDE 100, BDE 153, and BDE 154 (maternal serum geometric mean of 0.8, 20.1, 3.1, 4.7, 3.8, 5.1, and 2.6 ng/g lipid, respectively). A marginal positive association was observed between maternal serum BDE 28 levels and autistic behaviors (β [95% CI] 2.5 [-0.6, 5.6]). In contrast, a significant negative association was observed between maternal serum BDE 85 levels and autistic behaviors (β [95% CI] -3.2 [-5.9, -0.5]); however, BDE 85 was detected in <50% of the maternal serum samples. In the most recent report, Vuong et al. (2016a) evaluated potential associations between maternal PBDE levels and executive function at 5 and/or 8 years in 256 children from the HOME cohort. Specific analysis were conducted for BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, and their sum (maternal serum geometric mean of 1.2, 21.5, 5.0, 4.2, 5.5, and 41.3 ng/g lipid, respectively). After adjusted regression analysis, a significant positive association was observed between impaired behavior regulation and maternal serum BDE 153. Increased BDE 153 was also significantly associated with higher odds of having a behavior regulation (OR, 3.92 95% CI 1.76, 8.73) or a global executive function score (OR 2.34, 95% CI 1.05, 5.23) greater than 1 standard deviation above the mean, indicating increased risk of impaired behavior regulation and/or executive function deficits. No significant associations were observed for other congeners or their sums.

In another longitudinal birth cohort of predominantly Mexican-American families in the Salinas Valley of California (CHAMACOS), PBDE levels were analyzed in maternal serum (collected at mid-pregnancy or at delivery) and child serum at 7 years of age (Eskenazi et al. 2013). Significant negative associations were found for both maternal serum PBDE and current child serum PBDE and measures of attention, fine motor coordination, and cognitive function (particularly verbal comprehension) in children evaluated at 5 (n=249) and/or 7 (n=270) years of age. PBDE concentrations for this subset of the CHAMACOS cohort are not available. A follow-up study in the CHAMACOS birth cohort (n=622) evaluated potential associations between PBDE levels in maternal serum (collected at mid-pregnancy or delivery) and current child serum (collected at age 9) and numerous measures of attention and executive function at 9, 10.5, and 12 years of age (Sagiv et al. 2015). Significant negative associations were observed between several

measures of attention and executive function at 9–12 years of age and maternal serum PBDE (geometric mean of BDE 47, BDE 99, BDE 100, and BDE 153 of 26.3 ng/g lipid), but not current child serum PBDE (geometric mean of BDE 47, BDE 99, BDE 100, and BDE 153 of 63.2 ng/g lipid).

Several studies in infants and toddlers report associations between neurodevelopmental deficits and umbilical cord serum PBDE levels. Taiwanese infants (n=36) evaluated for neurological development at age 8–12 months by Bayley Scales of Infant and Toddler Development and parental questionnaire showed significant deficits in cognitive score and adaptive behavior associated with umbilical cord blood concentrations of total PBDE (range of 2.24–49.1 ng/g lipid, with median of 4.63 ng/g lipid) and concentrations of several individual congeners (Shy et al. 2011). Children from a New York City cohort (n=152) who had higher cord blood concentrations of PBDE congeners BDE 47, BDE 99, and BDE 100 scored significantly lower on tests of mental and physical development at ages 12, 24, and 36 months (Bayley Scales of Infant Development) and ages 48 and 72 months (Wechsler Preschool and Primary Scale of Intelligence) than children with lower cord blood concentrations (Herbstman et al. 2010). Median and maximum concentrations of BDE congeners in cord blood were 11.2 and 613.1 ng/g lipid for BDE 47, 3.2 and 202.8 ng/g lipid for BDE 99, and 1.4 and 71.9 ng/g lipid for BDE 100. Ding et al. (2015) evaluated the potential association between motor, adaptive, language, and social developmental quotients in Chinese children at 12 months (n=192) and 24 months (n=149) and cord blood concentrations of BDE 28, BDE 47, BDE 85, BDE 99, BDE 100, and BDE 153 (median concentrations of 2.05, 3.71, 1.47, 6.70, 2.63, and 2.19 ng/g lipid, respectively) and the sum of BDE 47, BDE 99, BDE 100, and BDE 153 (the four congeners detected in >80% of samples). No significant associations were observed at 12 months. At 24 months, a 10-fold increase in cord blood BDE 99 was significantly associated with a 2.16-fold decrease in the language domain developmental quotient and a 10-fold increase in cord blood BDE 47 was significantly associated with a 1.89-fold decrease in the social domain developmental quotient. No significant associations were observed for the other congeners or the sum of BDE 47, BDE 99, BDE 100, and BDE 153.

Studies in preschool and school-aged children (≥4 years of age) also report associations between neurodevelopmental impairments and maternal, child, or umbilical cord serum/plasma PBDEs. Both cord and current blood concentrations of BDE 47 (cord blood median and maximum of 2.10 and 16.8 ng/g lipid [n=88], current blood median and maximum of 0.12 and 130.2 ng/g lipid [n=244]) were also negatively associated with cognitive and motor functions in 4-year-old children from a Spanish cohort (McCarthy Scales of Children's Abilities), although in this study, the results were not statistically significant, except for associations with symptoms of poor social competence and ADHD (Gascon et al.

2011). Positive associations between attention problems (Child Behavior Checklist) and cord plasma levels of BDE 47, BDE 99, BDE 100, and BDE 153 were also observed in 4-year-old children (n=109) from a New York City cohort of mothers pregnant during the September 11, 2001 World Trade Center terrorist attacks; however, associations were only significant for BDE 47 and BDE 153 (Cowell et al. 2015). No significant associations were observed in 6-year-old children. Median BDE 47, BDE 99, BDE 100, and BDE 153 cord plasma levels were 11.2, 3.2, 1.4, and 07 ng/g lipid, respectively. Significant negative correlations were observed for maternal serum PBDE (median ∑PBDE=3.4 ng/g lipid, n=62) in the 35th week of pregnancy and fine manipulative abilities, verbal memory, and sustained attention in a cohort of Dutch children tested at age 5–6 years (Roze et al. 2009), although some aspects of neurodevelopment appeared to be improved with higher PBDE concentrations in this study, including coordination, visual perception, and behavioral outcome reported by parents and teachers. A case-control study of 100 California children, including 51 with autism/autism spectrum, 26 developmentally delayed but not autistic, and 23 with typical development, found no association of autism or developmental delay with concentrations of PBDE in serum collected from the children after assessment of developmental status at 36 months (Hertz-Picciotto et al. 2011). PBDE concentrations for this study were not available numerically.

Studies attempting to discern relationships between PBDE concentrations in breast milk, rather than blood, and neurodevelopmental end points produced more uncertain results. A study of 70 infants in Taiwan found no correlation between total PBDE concentrations in breast milk collected within 1 month of delivery (range=1.44–118 ng/g lipid, median=2.92 ng/g lipid) and neurodevelopment as assessed in infants at 8–12 months of age using the Bayley Scales (Chao et al. 2011). There was, however, a significant inverse association between BDE 209 concentration in breast milk and cognitive score, suggesting delayed cognitive development associated with that particular congener. Gascon et al. (2012) reported similar findings in a Spanish cohort of 290 infants. In this study, total PBDE concentrations in colostrum collected at the hospital within 4 days of delivery (range=0.31–32.66 ng/g lipid, median= 4.05 ng/g lipid) were negatively, but not significantly, associated with the Bayley score for cognitive development in children assessed at age 12–18 months, but again, a significant negative relationship was found for BDE 209, the congener found at the highest concentrations in this population (median of 1.02 ng/g lipid). Studies of a North Carolina cohort found modest and imprecisely estimated associations between PBDEs (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, and their sum) in breast milk collected 3 months postpartum (median ∑PBDE=47.3 ng/g lipid, n=222) and (1) increased extermalizing behaviors, primarily driven by activity/impulsivity behaviors, in children assessed at 24–36 months for social and emotional development; (2) higher anxiety and withdrawal in children assessed at 36 months

for behavioral development using a parental rating scale; and (3) improved cognitive skills in children assessed at 36 months for cognitive development using the Mullen Scales of Early Learning (Adgent et al. 2014; Hoffman et al. 2012).

Potential associations between household BDE 99 and BDE 209 dust and cognitive ability of 6-year-old children (verbal comprehension, working memory) were evaluated in French birth cohort (n=246); potential associations with cord blood BDE 209 were also evaluated (Chevrier et al. 2016). After adjustment for potential covariates, children from homes with BDE 99 dust levels above the median value (54 ng/g) or BDE 209 dust levels in the 2nd or 3rd exposure tertile (≥257 ng/g) had significantly increased risk of poor performance on verbal comprehension assessments. No association was observed between dust BDE 99 or BDE 209 levels and working memory, and no association was observed between cord BDE 209 and cognitive function.

A case-control study of a Chinese population found no relationship between risk of neural tube defects and placental PBDE concentrations (median ∑PBDE=0.55 ng/g lipid for 80 cases and 0.54 ng/g lipid for 50 controls) (Ma et al. 2012b; Ren et al. 2011).

Endocrine System Development

A number of studies evaluated effects on thyroid hormones in neonatal serum or cord blood associated with developmental exposure to PBDEs; however, findings are inconsistent between studies and congeners.

Abdelouahab et al. (2013) evaluated the potential associations between thyroid hormone levels in the umbilical cord blood and maternal serum concentrations of PBDEs collected at <20 weeks of pregnancy (for ∑PBDE, median=30.92 ng/g lipid, maximum=726.09 ng/g lipid, n=380). Significant negative associations were observed between maternal PBDE levels and both free and total T_4 in cord blood, but not free or total T_3 or TSH in cord blood. Similarly, neonatal TSH assessed in blood samples collected 24 hours after birth (on average) was not related to PBDE concentrations (median ∑PBDE=25.4 ng/g lipid) in maternal serum collected at the start of the third trimester or at delivery from 289 expectant mothers living in the Salinas Valley of California (Chevrier et al. 2011). In a low-exposure Korean birth cohort (n=104), no significant associations were observed between neonatal free or total T_3, free or total T_4, or TSH and maternal PBDE concentrations in maternal serum collected at delivery (median ∑PBDE=2.2 ng/g lipid) (Kim et al. 2015)

Other studies of thyroid hormone changes in infants looked at hormone levels in umbilical cord or neonatal blood in relation to PBDE concentrations in maternal breast milk. Kim et al. (2011a) reported a significant negative correlation between breast milk PBDE (BDE 28 only, mean ≈0.1 ng/g lipid) and free T_4 in cord blood. Other studies that measured PBDEs in breast milk to assess exposure found no association with TSH in neonatal blood collected 3 days after delivery in a Norwegian population of 239 mother-baby pairs (median ∑PBDE in breast milk=1.91 ng/g lipid) (Eggesbo et al. 2011), no association with T_3, T_4, free T_4, or TSH in cord blood in a population of 149 Taiwanese mothers (median ∑PBDE in breast milk=3.38 ng/g lipid from 42 mothers in Central Taiwan and 3.13 ng/g lipid from 107 mothers in Southern Taiwan) (Shy et al. 2012), and no association with free T_4, total T_3, or TSH in infant serum at 3 weeks or 3 months in 186 Swedish mother-child pairs (median breast milk concentrations of tetra-pentaBDE [BDE 47, BDE 99, BDE 100] of 2.3 ng/g lipid and BDE 153 of 0.48 ng/g lipid) (Lignell et al. 2016).

Inconsistent findings were also observed when thyroid hormones and PBDEs levels were evaluated in infant serum and/or cord blood. For example, Mazdai et al. (2003) found no correlations between PBDE concentrations (for ∑PBDE, range=14–460 ng/g lipid, median=39 ng/g lipid, n=12) and thyroid hormone levels (free and total T_4 and free and total T_3) in umbilical cord blood. Similarly, there was no correlation between PBDEs and thyroid hormone levels in umbilical cord blood in another study of 21 South Korean mothers undergoing Cesarean section (Kim et al. 2012a). In this study, ∑PBDE in cord blood ranged from 2.28 to 30.94 ng/g lipid, with a median of 12.04 ng/g lipid. Both T_3 and free T_3 in cord blood were significantly inversely related to PBDE in cord blood (median ∑PBDE=3.49 ng/g lipid) in another study of 54 Taiwanese births (Lin et al. 2011). T_4, free T_4, and TSH were unaffected in this study. Wan et al. (2010) found no significant relationship between the PBDE metabolite, 6-OH-BDE-47 (which ranged from <4 to 127 pg/g wet weight, with a median of 26 pg/g wet weight), and T_4 in cord serum in 26 pregnant South Korean women. In an analysis of 289 births at Johns Hopkins Hospital in Maryland, PBDE in cord blood (median ∑PBDE=18.7 ng/g lipid) was compared to T_4, free T_4, and TSH in cord blood and T_4 from blood spots collected from newborns at 2 and 18 days of age (on average) (Herbstman et al. 2008). There were consistent negative associations between PBDEs and free and total T_4 in cord blood and/or spot samples, although these were primarily nonsignificant. Kim et al. (2011d, 2012b) analyzed blood samples collected from infants in neonatal screening tests. They found a positive relationship between PBDEs with TSH (BDE 197 and BDE 196 only), a negative association with T_3 (BDE 154 only) for control babies (for ∑PBDE, range=1.61–252.9 ng/g lipid, mean=56.70 ng/g lipid, n=12), and no significant relationships between PBDEs and thyroid hormones in babies with congenital

hypothyroidism (for ∑PBDE, range=2.22–861.0 ng/g lipid, mean=59.84 ng/g lipid, n=26). In another Korean birth cohort (n=104), significant positive associations were observed between neonatal serum TSH and cord BDE 99 levels and cord TSH and cord BDE 47 levels; no significant associations were observed for free or total T_3 or T_4 and ∑PBDE, BDE 47, or BDE 99 levels in cord blood (median ∑PBDE in cord=8.8 ng/g lipid) (Kim et al. 2015).

Immune System Development

A study of developmental immunity found reduced risk of atopic dermatitis in Japanese infants (diagnosed at 7 months of age by questionnaire) with higher concentrations of PBDE in umbilical cord blood (median ∑PBDE=41 pg/g wet weight for 27 cases and 54.5 pg/g wet weight for 54 controls) (Ochiai et al. 2014).

Animal Studies

Teratology, Fetotoxicity, and Physical Growth and Development

Lower-brominated PBDEs: No exposure-related developmental changes were observed following gestational exposure to pentaBDE in GD 12 rat embryos from dams exposed to doses up to 120 mg/kg/day via gavage from GD 6.5 to 11.5 (Ellis-Hutchings et al. 2009), in GD 20 rat fetuses from dams exposed to doses up to 120 mg/kg/day via gavage from GD 6.5 to 19.5 (Ellis-Hutchings et al. 2009), or in GD 20 rat fetuses from dams exposed to doses up to 200 mg/kg/day via gavage from GD 6 to 15 (Argus Research Laboratories 1985a). End points evaluated included fetal/embryo survival, resorptions, fetal weight and length, gross abnormalities, and skeletal and soft tissue abnormalities. In these studies, maternal toxicity (significantly decreased maternal weight gain) was observed at doses ≥100 mg/kg/day (Argus Research Laboratories 1985a; Ellis-Hutchings et al. 2009). Similarly, rat dams exposed to pentaBDE at doses up to 30.6 mg/kg/day from GD 6 to 21 did not show any exposure-related changes in pregnancy or birth indices (Branchi et al. 2001, 2002, 2005; Kodavanti et al. 2010; Zhou et al. 2002). Zhou et al. (2002) also reported no change in offspring viability and growth, as assessed by numbers of pups at birth and on PNDs 4–21, body weight of pups on PNDs 4–90, and eye opening status on PNDs 11–18; however, Kodavanti et al. (2010) observed significantly decreased body weight from PND 29 to 58 in female offspring at ≥10.2 mg/kg/day (8–10% reduction at PND 60). No exposure-related changes were observed in litter size, live births per litter, sex ratio, implantation sites, timing of eye opening, or body or organ weights (brain, liver, thymus) on PND 21 in offspring from rat dams fed

vanilla wafers dosed with commercial pentaBDE (DE-71) at doses up to 11.4 mg/kg/day from 28 days pre-mating through PND 21 (Poon et al. 2011). In a similar study, no exposure-related changes were observed in litter size, live pups per litter, sex ratio, offspring survival, or timing of eye opening or pinna detachment in offspring from rat dams fed cookies dosed with DE-71 at doses up to 30 mg/kg/day from GD 1 to PND 21; however, a significant decrease in offspring body weight was observed during early development (up through PND 30) at 30 mg/kg/day, but not at doses ≤3 mg/kg/day (Bowers et al. 2015).

Several studies examined developmental effects of very low doses of pentaBDE. Following maternal exposure to pentaBDE at doses of 0, 0.5, 1, or 2 mg/kg/day via gavage from GD 6 to 19, GD 20 rat fetuses showed delayed ossification and an increased incidence of internal variations at 2 mg/kg/day (Blanco et al. 2012). Significantly increased skeletal variations included delayed ossification of parietal and occipital bones, caudal vertebrae, and floating ribs. Soft tissue variations included significant increase in the size of the ventricles of the heart and liver enlargement. No significant changes were observed in the number of live fetuses, the sex ratio, the average fetal body weight/litter, or external malformations (Blanco et al. 2012). In another study, incisor eruption was delayed in offspring from rat dams exposed on GD 6 to a single gavage dose of pentaBDE at 0.3 mg/kg, but not 0.06 mg/kg (average age at eruption was not reported) (Kuriyama et al. 2005). No exposure-related delays were reported for fur development or eye opening (Kuriyama et al. 2005). Following single gavage doses of pentaBDE up to 0.3 mg/kg or tetraBDE up to 0.7 mg/kg/day in F0 rats on GD 6, no significant, exposure-related changes were observed in F1 body weights through PND 100 or F2 fetal end points (implantation sites, implantation sites/dam, number of live fetuses, fetuses/dam, mean fetal weight, resorption rate, or incidence of skeletal anomalies) on GD 21 (Talsness et al. 2005, 2008).

In several other studies assessing offspring body weight following developmental exposure to pentaBDE (without assessing any teratogenic or fetotoxic end points), no consistent body weight effects were found. No dose-related body weight changes were observed in offspring of male and female rats exposed to pentaBDE at doses up to 25 mg/kg/day via gavage for 70 days pre-mating through PND 42 (Bondy et al. 2011, 2013). No exposure-related body weight effects were observed in rat pups from dams exposed to pentaBDE at doses up to 30 mg/kg/day via gavage or pentaBDE-dosed cookies from GD 6 to PND 18 or 21 (Bansal et al. 2014; Cheng et al. 2009; Ellis-Hutchings et al. 2006; Miller et al. 2012; Zhao et al. 2014), or in mouse pups from dams exposed to pentaBDE at 452 mg/kg/day every third day from GD 4 to PND 17 via gavage (Skarman et al. 2005). A poorly-reported study indicated that pup weight was significantly decreased at PND 21 by 8, 16, and 15% following maternal exposure to pentaBDE at 50, 100, at 200 mg/kg/day, respectively, from GD 6 to PND 21 via gavage; by PND 63, no body weight

effects were observed (Hong et al. 2010). Another study reported significantly elevated pup body weight on PND 21 in rat offspring from dams exposed to pentaBDE at 2 mg/kg/day via gavage from GD 6 to PND 21; no changes were observed at 1 mg/kg/day (Blanco et al. 2014).

Three studies exposed rat dams to different commercial octaBDE mixtures at 0, 2.5, 10, or 25 mg/kg/day via gavage on GDs 6–15, including FR-1208 (Life Science Research Israel Ltd. 1987), Saytex 111 (Argus Research Laboratories 1985b), and DE-79 (WIL Research Laboratories 1986). Following exposure to FR-1208, postimplantation loss was significantly elevated at 10 and 25 mg/kg/day when Freeman-Tukey arcsine transformed values were tested as normally distributing data (Student's t-test); however, no statistical differences were observed with untransformed data and values were within the range of laboratory historical control values. There were no indications of skeletal malformation or variations or delayed or retarded ossification in any dose group (Life Science Research Israel Ltd. 1987). Following exposure to Saytex 111, the number of resorptions per litter was significantly increased by 8-fold and the number of live fetuses per litter was decreased 6.6% in the 25 mg/kg/day group (Argus Research Laboratories 1985b). Average fetal body weights were also significantly reduced at 25 mg/kg/day (Argus Research Laboratories 1985b). Following exposure to DE-79, effects observed at 50 mg/kg/day included significantly reduced mean maternal body weight gain during the post-treatment period (GDs 16–20) and fetotoxicity as indicated by increased postimplantation loss due to late resorptions (not significantly increased compared to control group but exceeded historical control range), 39% reduced mean fetal weight ($p<0.01$), skeletal variations (e.g., reduced ossification of the skull and various unossified bones) that were associated with the reduced fetal weights in this group, and single instances of malformations (fetal anasarca, bent limb bones, unilateral absence of 13^{th} rib) commonly associated with maternal toxicity (WIL Research Laboratories 1986).

In rabbit does exposed to octaBDE (Saytex 111) at 0, 2, 5, or 15 mg/kg/day on GDs 7–19 via gavage, the 15 mg/kg/day group showed slight fetotoxicity, as indicated by a significantly ($p\leq0.05$) increased incidence of delayed ossification of the sternebrae (Breslin et al. 1989). This finding was accompanied by evidence of slight maternal toxicity as indicated by decreased body weight gain during GDs 7–20 and 7–28 (not statistically identified), reduced body weight on GD 28 (7% less than controls, $p\leq0.05$), and significantly increased absolute and relative liver weights on GD 28 (Breslin et al. 1989).

Developmental effects were assessed in mice from dams fed cornflakes containing tetraBDE at doses of 0, 0.03, 0.1, or 1 mg/kg/day from 28 days premating to PND 21 (Ta et al. 2011). At PND 21, a significant decrease in crown-rump length of pups was observed in the 0.1 mg/kg/day group, compared

with controls; however, no other dose-group showed this effect and the ponderal index (weight/crown-rump length) was not statistically different among treatment groups. Similarly, body weight was significantly decreased by ~13–17% from PND 14 to 18 in the 0.1 mg/kg/day group, compared with controls, but not in the 0.03 or 1 mg/kg/day groups. No changes were observed in gestation length, litter size, or sex ratio (Ta et al. 2011). In another study using the same protocol, no changes were observed in the number of pups per litter, the sex ratio, or pup body weight (Koenig et al. 2012). Pre-weaning weights were significantly reduced by ~10% in female offspring of female mice exposed to tetraBDE at 0.03 mg/kg/day via gavage for 28 days premating through PND 21, but only in one of two experimental replicates (Woods et al. 2012). In a dietary study, no exposure-related changes were observed in pregnancy length, litter sizes, pup mortality, sex ratio, or pup weight in rats exposed to tetraBDE at doses up to 32 mg/kg/day from GD 1 to PND 14 (Wang et al. 2011a).

DecaBDE: Developmental effects were assessed in mice following maternal exposure to decaBDE at gavage doses of 0, 150, 750, 1,500, or 2,000 mg/kg/day from GD 7 to 9 (Chi et al. 2011). No gross external malformations were observed; however, significant exposure-related changes were observed in postimplantation loss, resorptions, number of live litters, and fetal body weight on GD 16. The percentage of postimplantation loss per litter was significantly increased by 3, 2.7, and 9.8% at 750, 1,500, and 2,000 mg/kg/day, respectively, compared with control. At 1,500 and 2,000 mg/kg/day, the percentage of resorptions per litter was also significantly increased by 2.7 and 8.6%, respectively. Additionally, the percentage of live fetuses per litter was significantly decreased by 10% in the high-dose group. Fetal body weight was significantly decreased by 10, 10, and 22% at 750, 1,500, and 2,000 mg/kg/day, respectively, compared with controls (Chi et al. 2011). A significant 15% reduction in pup body weight was observed in offspring of mouse dams fed dietary decaBDE at 260 mg/kg/day from GD 10 through PND 21 (Watanabe et al. 2008); however, another study using the same protocol did not observe body weight effects in PND 21 pups at doses up to 2,900 mg/kg/day (Watanabe et al. 2010b). No exposure-related changes were observed in the number of liters or survival rate of pups in either study at doses up to 3,100 mg/kg/day (Watanabe et al. 2008, 2010b). In other studies, no exposure-related teratogenic, fetotoxic, or body weight effects, and/or delays in attainment of developmental landmarks, were observed following decaBDE exposure in rat offspring from dams exposed to gavage doses up to 146 mg/kg/day from GD 10 to PND 20 or 21 (Fujimoto et al. 2011; Saegusa et al. 2012), in rat offspring from dams exposed to gavage doses up to 1,000 mg/kg/day from GD 0 to 19 or from GD 6 to PND 21 (Hardy et al. 2001, 2002; Biesemeier et al. 2011), in mouse offspring from dams exposed to gavage doses up to 1,500 mg/kg/day from GD 0 to 17 (Tseng et al. 2008, 2013), or in mouse offspring from dams exposed to doses up to 20 mg/kg/day via micropipette from PND 2 to 15 (Rice et al. 2007). In a poorly-

reported study, the study authors reported that pup weight was significantly decreased in mouse offspring at PND 21 following maternal exposure to decaBDE at 500, 2,500, and 12,500 mg/kg/day via gavage from GD 6 to PND 21; however, the included graph and table do not support that statement (Hong et al. 2010).

A lower purity commercial decaBDE product (77% decaBDE, 22% nonaBDE, 0.8% octaBDE) used in the 1970s was fetotoxic in rats at high, albeit not maternally toxic, dose levels. Developmental effects were investigated in GD 21 rat fetuses from dams exposed to decaBDE at doses of 10, 100, or 1,000 mg/kg/day by gavage on GDs 6–15 (Dow Chemical Co. 1985; Norris et al. 1975a). The numbers of fetuses with subcutaneous edema and delayed ossification of normally developed skull bones were significantly increased at 1,000 mg/kg/day. Resorptions were significantly increased at ≥10 mg/kg/day, but the increases were not dose-related and rates in the high dose group were comparable to historical control values. As discussed in Section 3.2.2.5 (Reproductive Effects), a one-generation study of the 77% commercial decaBDE mixture at ≤100 mg/kg/day in rats found no effects on numbers of live pups at birth or during lactation, body weights of pups at birth or weaning, or skeletal development or soft-tissue histology of pups at weaning (Dow Chemical Co. 1975; Norris et al. 1975a).

An additional study evaluated developmental effects in neonatal mice engineered to express neonatal human apolipoprotein E (three genotypes: apoE2, apoE3, and apoE4) exposed once to decaBDE at 0, 10, or 30 mg/kg via micropipette on PND 10 (Reverte et al. 2014). Investigators were assessing the potential effect of different human apoE genotypes on susceptibility to decaBDE exposure during development, as apolipoprotein is a genetic factor that is associated with varied vulnerability for the development of neurodegenerative disease. No exposure-related changes were observed in survival, body weight gain, ontogeny of reflexes, pinna detachment, or incisor eruption. However, eye opening was significantly delayed in apoE2 mice exposed to 30 mg/kg, compared with apoE2 controls.

Neurodevelopment

Lower-brominated PBDEs

Neurobehavior: In a series of one-day exposure neonatal gavage studies using similar experimental designs, mice exposed to pentaBDE at doses ≥0.8 mg/kg on PND 3 or PND 10 consistently showed alterations in open-field behavior at 2–8 months of age, characterized by decreased activity during the first 20-minute period followed by increased activity during the third 20-minute period; exposure-related

effects were not observed in any study at ≤0.4 mg/kg/day or following exposure to pentaBDE at 8 mg/kg on PND 19 (Eriksson et al. 2002b, 2006; Fischer et al. 2008; Hallgren et al. 2015; Sand et al. 2004; Viberg et al. 2002, 2004a, 2004b). These findings indicate an initial decrease in activity, but also a lack of habituation to new surroundings. The study authors noted that this nonhabituating behavior profile (i.e., decreased activity early in the test period and increased activity late in the test period) has also been reported in adult mice neonatally exposed to certain *ortho*-PCB congeners (Eriksson and Fredriksson 1996a, 1996b). Several other 1-day exposure studies report similar findings in rats and mice following exposure to various lower-brominated PBDEs. Decreased spontaneous activity and/or impaired habituation was observed in rats exposed to pentaBDE at 8 mg/kg on PND 10, mice exposed to hexaBDE at ≥0.45 mg/kg on PND 10, mice exposed to tetraBDE at 10.5 mg/kg on PND 10, mice exposed to heptaBDE at 15.2 mg/kg on PND 3, and mice exposed octaBDE at 16.8 mg/kg on PND 3 or 10 (Eriksson et al. 2001; Viberg et al. 2003a, 2005, 2006). Increased vertical activity was significantly increased at 4 months, but not 2 months, in mice exposed to tetraBDE at ≥1 mg/kg on PND 10; no changes were observed in horizontal activity or habituation (Gee and Moser 2008). No changes in open-field behavior were observed in mice exposed to heptaBDE at 15.2 mg/kg or nonaBDE at 18.5 on PND 10 (Viberg et al. 2006). The observed effects may be modulated by the cholinergic system, as mice exposed to pentaBDE at 8 mg/kg on PND 10 showed significantly altered responses in a nicotine-induced behavior task (decreased instead of increased activity) (Viberg et al. 2002).

Evidence for exposure-related changes in open-field behavior is less consistent in pre- and perinatal studies. Male offspring from mouse dams exposed to pentaBDE at 18 mg/kg/day via gavage or "self-administration" from a modified syringe from GD 6 to PND 21 showed significantly increased motor activity during the 3rd 10-minute block of an open field test on PND 34, indicating decreased habituation (female offspring not evaluated) (Branchi et al. 2005). This effect was transient, as it was no longer observed in male offspring at PND 60 to 120. No exposure related changes were observed in the amount of time spent in the center versus the middle of the open field (Branchi et al. 2005). However, male and female offspring from mouse dams exposed to pentaBDE at 0, 0.6, 6, or 30 mg/kg/day via gavage from GD 6 to PND 21 showed changes suggestive of an age-dependent alteration in activity at ≥6 mg/kg/day; effects included hyperactivity (increased locomotion and rearing) and impaired habituation at PNDs 34 and 60, altered thigmotaxis (reduced time near walls) at PND 60, and a tendency to hypoactivity (reduced locomotion) at PND 120 (Branchi et al. 2001, 2002). In 24-hour observations of open-field behavior, total activity, time spent active, duration of activity per active phase, and total activity per active phase were all significantly increased in PND 36 male rat offspring following a single maternal exposure to 0.3 mg/kg on GD 6 via gavage (Kuriyama et al. 2005). At PND 71, the increases in total activity and

time spent active persisted in the 0.3 mg/kg group, and were also significantly increased at 0.06 mg/kg (Kuriyama et al. 2005). Bowers et al. (2015) reported a significant increase in rearing behavior in rats at PND 110 following maternal exposure to pentaBDE at doses up to 30 mg/kg/day via dosed cookie intake from GD 1 to PND 21; however, rearing behavior did not differ from control on PND 16, 55, or 230, and no other changes in motor activity or habituation behavior were observed in open-field testing at any time-point. Kodavanti et al. (2010) also reported a lack of exposure-related changes in open-field behavior in rats at PND 100, 114, or 273 following maternal exposure to pentaBDE at doses up to 30.6 mg/kg/day via gavage from GD 6 to PND 21. Similarly, no exposure-related changes were observed in general motor activity in an open field in PND 22 male and female offspring of rat dams exposed to pentaBDE at doses up to 2 mg/kg/day via gavage from GD 6 to PND 21; however, the offspring exposed to 2 mg/kg/day spent a significantly greater percentage of time in the center of the open field, indicating decreased anxiety (anxiolytic effect) (Blanco et al. 2013). However, no effect on anxiety behavior was observed in the emergence latency assay in PND 35 or 80 rat offspring of dams fed pentaBDE-dosed cookies at intake levels up to 30 mg/kg/day from GD 1 to PND 21 (Bowers et al. 2015). In offspring of mouse dams fed cornflakes containing tetraBDE at doses up to 1 mg/kg/day from pre-mating day 28 through PND 21, no changes in locomotor activity were observed at PNW 8 or 17 (Koenig et al. 2012). In contrast, a second study using the same protocol reported significantly decreased locomotion and distance travelled in open-field testing of PND 60 females exposed to ≥0.1 mg/kg/day; at 0.03 mg/kg/day, PND 60 female mice showed decreased activity in the center of the open field (Ta et al. 2011). No exposure-related changes in open-field behavior were observed at PND 60 in males or PND 42 in either sex (Ta et al. 2011). Open field behavior was not significantly altered in PND 21 male offspring of mouse dams administered tetraBDE at 0.2 mg/kg/day from GD 8 to PND 21, compared with control (Kim et al. 2015).

In 1-day exposure neonatal gavage studies, significant impairments were also observed in learning and memory in the Morris water maze test in mice exposed to pentaBDE at 0.8 mg/kg on PND 10, mice exposed to hexaBDE at ≥0.9 mg/kg on PND 10, and mice exposed to octaBDE at 16.8 mg/kg on PND 10, or in rats exposed to tetraBDE at ≥1 mg/kg on PND 10 (Fischer et al. 2008; He et al. 2009, 2011; Viberg et al. 2003a, 2006). Observed changes included increased latencies to find a hidden platform during a 4-day training period and decreased distance travelled in the quadrant containing the hidden platform compared with controls; no changes were observed during a reversal phase on the 5th day (when the platform was moved to a new quadrant). Similarly, impairments in learning and memory were observed in the radial arm maze in mice exposed to pentaBDE at 0.8 mg/kg on PND 10, as evidence by the increased number of re-entries into maze arms from which the food pellet had already been eaten (Fischer

et al. 2008). No exposure-related effects on learning or memory were observed in the Morris water maze in mice exposed to octaBDE at 16.8 mg/kg on PND 3 or nonaBDE at 18.5 mg/kg on PND 10 (Viberg et al. 2006). Based on the observed deficits, some of the studies evaluated the density of nicotinic cholinergic receptors in the hippocampus. Significant decreases in receptor density of 7–31% were observed in mice exposed to penta- or hexaBDE at ≥9 mg/kg on PND 10 (Viberg et al. 2003a, 2004b, 2005). Another study reported a significant 23% decrease in cortical density of nicotinic cholinergic receptors in mice exposed to pentaBDE at 0.8 mg/kg on PND 10; hippocampal density was not altered at this dose (Fischer et al. 2008). The observed effects may be modulated by the cholinergic system, as mice exposed to pentaBDE at 8 mg/kg on PND 10 showed significantly altered responses in a nicotine-induced behavior task (decreased instead of increased activity) (Viberg et al. 2002).

Learning and memory impairments have also been reported following pre- and perinatal exposures. Following maternal exposure to pentaBDE at 0, 1, or 2 mg/kg/day via gavage from GD 6 to PND 21, a nonsignificant trend toward increased time to locate the hidden platform in the Morris water maze was observed on days 2–4 in PND 22 rat offspring from both dose groups; however, the increase was only significant on day 4 in the 2 mg/kg/day group (Blanco et al. 2013). In another study, significantly increased latency to find the escape platform was observed in the Morris water maze on training days 2–3 in PND 34 offspring of rat dams exposed to pentaBDE at 2 mg/kg/day from GD 6 to PND 21 (Cheng et al. 2009). In a low-dose study, maternal exposure to 0.2 mg/kg/day from GD 1 to PND 21 via gavage did not impair spatial learning in PND 34–36 offspring (Zhao et al. 2014). Effects observed at higher doses may be transient, as no exposure-related changes were observed in the Morris water maze in PND 235 rat offspring following maternal exposure to pentaBDE at doses up to 30 mg/kg/day via dosed cookie intake from GD 1 to PND 21 (Bowers et al. 2015). No exposure-related deficits in the Morris water maze were observed during PNW 7 or 11 in offspring of mouse dams exposed to tetraBDE at 0.03 mg/kg/day via gavage from pre-mating day 28 to PND 21 (Woods et al. 2012). Similarly, offspring of mouse dams fed cornflakes containing tetraBDE at 0, 0.03, 0.1, or 1 mg/kg/day from pre-mating day 28 through PND 21 did not show exposure-related impairments in the Morris water maze at PNW 8 (Ta et al. 2011). However, another study using the same protocol reported a significant increase in the latency to find a hidden escape hole in the Barnes maze on the first day of training at ≥0.03 mg/kg/day in PNW 8 offspring (Koenig et al. 2012). No exposure-related effects were observed during trial days 2–4 or during reversal learning on day 5 (escape hole placed in a different location) at doses up to 1 mg/kg/day (Koenig et al. 2012). Other studies assessed learning and attention using 5-choice visual learning and attention tasks conducted from PND 30 to 95 in offspring of rat dams exposed to pentaBDE via gavage from PND 6 to 12 (Driscoll et al. 2012; Dufault et al. 2005). Offspring exposed to 30 mg/kg/day required significantly

more trials to reach "passing" criterion in the visual discrimination task, and committed significantly more errors than controls prior to reaching the criterion (Dufault et al. 2005). The number of omission errors (no choice made) was also significantly increased in rats exposed to 30 mg/kg/day. No exposure-related impairments were observed in the visual discrimination task at doses ≤15 mg/kg/day, and no exposure-related impairments were observed in the sustained attention task at doses up to 30 mg/kg/day (Driscoll et al. 2012; Dufault et al. 2005).

In the three-chambered sociability task, general sociability (time spent in the chamber with a social target) was decreased in female offspring of mouse dams exposed to tetraBDE at 0.03 mg/kg/day via gavage from pre-mating day 28 to PND 21 (only dose evaluated); no exposure-related changes were observed in male offspring (Woods et al. 2012). No changes were observed in social novelty (time spent in the chamber with a new social target vs. a familiar social target) or barrier social interaction tests (time spent interacting with social target) in either sex (Woods et al. 2012). No exposure-related changes in social interaction were observed in offspring of mouse dams fed cornflakes containing tetraBDE at 0, 0.03, 0.1, or 1 mg/kg/day from pre-mating day 28 through PND 21 (Ta et al. 2011). No performance deficits were observed in additional neurobehavioral tests in these studies, including the elevated plus maze, acoustic startle and prepulse inhibition, and fear conditioning (Ta et al. 2011; Woods et al. 2012). Similarly, no significant, exposure-related changes were observed in social interaction or social novelty indices in PND 70 offspring of dams administered tetraBDE at 0 or 0.2 mg/kg/day via gavage from GD 8 to PND 21 (Kim et al. 2015). Additionally, no exposure-related effects were observed in social dominance testing (tube test, urine marking test).

Sensory and motor development. Sensory and motor development (righting reflex, forelimb stick grasping reflex, forelimb placing reflexes, negative geotaxis, screen grasping and climbing, pole grasping, ultrasonic vocalizations, homing test) were assessed in male and female offspring of mouse dams exposed to pentaBDE at doses of 0, 0.6, 6, or 30 mg/kg/day via gavage from GD 6 to PND 21 (Branchi et al. 2001, 2002). The screen climbing response was delayed by approximately 2 days in the 30 mg/kg/day group, compared with controls; no other exposure-related changes were observed in reflex development (Branchi et al. 2001, 2002). However, in rats, development of the cliff drop and negative geotaxis reflexes was significantly delayed in male pups following maternal exposure to pentaBDE at 2 mg/kg/day via gavage from GD 6 to PND 21; no delays were observed in the development of the righting reflex (Cheng et al. 2009). Similarly, development of the cliff drop reflex was significantly delayed in male rat pups following a single maternal exposure to pentaBDE at 0.3 mg/kg on GD 6, but not at 0.06 mg/kg (average age at which the reflex developed was not reported) (Kuriyama et al. 2005). However, no exposure-

related changes were observed in the ability of male offspring to stay on a rotating rod for 3 minutes at doses up to 0.3 mg/kg (Kuriyama et al. 2005). In a low-dose study, no exposure-related changes in reflex maturation or motor coordination were observed in PND 3–30 offspring of dams exposed to pentaBDE at 0.2 mg/kg/day from GD 1 to PND 21 via gavage (Zhao et al. 2014). Similarly, no changes in grip strength or motor coordination (beam test) were observed in PND 12–60 rat offspring of dams fed pentaBDE-dosed cookies at intake levels up to 30 mg/kg/day from GD 1 to PND 21 (Bowers et al. 2015). However, the acoustic startle response was significantly increased at the highest intensity levels in rats on PND 9, but not PND 20 (Bowers et al. 2015).

Sensory and motor development was also tested in offspring of mouse dams fed cornflakes containing tetraBDE at doses of 0, 0.03, 0.1, or 1 mg/kg/day from pre-mating day 28 to PND 21 (Ta et al. 2011). No significant exposure-related effects were found in the Wahlsten battery for sensory and motor development (righting reflex, cliff aversion, needle grasp, visual placing, vibrissa placing, eye opening, ear opening, ear twitch response, screen pull, screen cling/climb, startle reflex) on PNDs 8–18 or rotarod performance on PNDs 35–36; however, significant differences in ultrasonic pup vocalization (UPV) measures were observed between the 1 mg/kg/day group and control, including increased duration of UPVs on PND 13 and increased UPV bout duration on PND 9 and 13. A nonsignificant trend was also observed in the 1 mg/kg/day group in the number of UPVs on PNDs 13 and 17 (Ta et al. 2011). Similarly, female offspring of mouse dams exposed to tetraBDE at 0 or 0.03 mg/kg/day via gavage from pre-mating day 28 to PND 21 showed significantly decreased UPV between PND 8 and 16; no exposure-related deficits were observed in male or female offspring in sensory or motor test batteries from PND 8 to 21 or rotarod performance at PNDs 29–30 or 66–68 (Woods et al. 2012). Koenig et al. (2012) also reported a lack of exposure-related changes motor tests (grip strength, ladder walk, gait analysis) in PND 14–42 offspring of mouse dams fed cornflakes containing tetraBDE at doses up to 1 mg/kg/day from pre-mating day 28 through PND 21 (Koenig et al. 2012).

In other studies, no exposure-related changes were observed in functional observation batteries in male offspring of rat dams exposed to pentaBDE at doses up to 30.6 mg/kg/day via gavage from GD 6 to PND 21 (assessed at PND 24 or 60) or male mice exposed to single tetraBDE doses up to 30 mg/kg via gavage on PND 10 (assessed at PNDs 12–18 or at 1 or 3 months of age) (Gee and Moser 2008; Kodavanti et al. 2010).

Electrophysiology. Following a single exposure to tetraBDE at 0, 6.8, or 68 mg/kg via gavage on PND 10, hippocampal slices from PND 17 to 19 male mice were prepared for field-excitatory

postsynaptic potential (fEPSP) recordings (Dingemans et al. 2007). No exposure-related differences in the stimulus-response relation were seen in fEPSPs. However, post-tetanus potential (PTP) and long-term potential (LTP) were significantly decreased in the hippocampus of exposed mice, compared with controls. No exposure-related effects on paired-pulse inhibition were observed.

To assess auditory function, distortion product otoacoustic emissions (DPOAEs) were measured in adult offspring of rat dams fed vanilla wafers containing pentaBDE at doses of 0, 5.7, or 11.4 mg/kg/day from pre-mating day 28 to PND 21 (Poon et al. 2011). No exposure-related changes were observed in DPOAE amplitudes, frequencies, or thresholds.

Histology and organ weight. Ultrastructural changes were observed in the hippocampus of 2-month-old rats exposed once to tetraBDE on PND 10 at ≥5 mg/kg, but not at 1 mg/kg (He et al. 2009). At 5 mg/kg, the endoplasmic reticulum appeared increasing swollen and degranulated, and at 10 mg/kg, the neurons were acutely affected, with puffed periplast, dissolved cell organelles, and vacuolized mitochondria (no incidence data reported) (He et al. 2009). No exposure-related changes in hippocampal histology were observed in PND 70 offspring of mouse dams fed cornflakes containing tetraBDE at 0, 0.03, 0.1, or 1 mg/kg/day from pre-mating day 28 through PND 21 (Ta et al. 2011).

No exposure-related changes in brain weight were observed in neonatal, weanling, or adult offspring following maternal exposure to pentaBDE at 18 mg/kg/day via gavage on GD 6 to PND 21 (Ellis-Hutchings et al. 2006), maternal exposure to pentaBDE-dosed cookies at doses up to 30 mg/kg/day from GD 1 to PND 21 (Bowers et al. 2015), or maternal exposure to pentaBDE-dosed vanilla wafers at doses up to 11.4 mg/kg/day from pre-mating day 28 to PND 21 (Poon et al. 2011). Similarly, cerebrum weight was unaffected in 2-month-old rats exposed once to tetraBDE at doses up to 10 mg/kg via gavage on PND 10 (He et al. 2011).

Biochemical, proteomic, genomic, and epigenetic changes. Hippocampal and cortical tissues of rat pups were evaluated for mRNA and protein expression levels of several T_3-mediate proteins (BDNF, NCAM1, and GAP-43) following maternal exposure to tetraBDE at 0, 3.2, or 32 mg/kg/day via gavage from GD 1 to PND 14 (Wang et al. 2011a). At PNDs 1, 7, and 14, multiple alterations in mRNA and protein levels were observed; however, magnitude, direction, and significance of the changes were time- and region-dependent, with no clear exposure-related pattern (Wang et al. 2011a). In another study, hippocampal and cortical tissues were evaluated for gene expression levels of TRα1, TRα2, TRβ1, and BDNF in PND 21 offspring of rat dams exposed to pentaBDE at 0, 1, or 2 mg/kg/day via gavage from GD 6 to

PND 21 (Blanco et al. 2013). The only exposure-related change observed was a significant decrease in BDNF expression in the hippocampus at 2 mg/kg/day (Blanco et al. 2013).

In 2-month-old rats exposed once to tetraBDE at 0, 1, 5, or 10 mg/kg on PND 10, various dose-related changes were observed in the mRNA and protein expression levels of apoptotic proteins in the hippocampus, including induction of caspase3, caspase12, and cytochrome C (He et al. 2009). Costa et al. (2015) reported similar induction of caspase3 in mice sacrificed 24 hours following exposure to 20 mg/kg of tetraBDE on PND 10, compared with control. Markers of oxidative stress were also elevated in exposed mice, including malondialdehyde (MDA), 8-isoprostane, and reactive protein carbonyls, and tetraBDE-mediated induction of caspase3 was significantly greater in glutathione-deficient knockout mice (*Gclm* -/-) than in wild-type mice (Costa et al. 2015). In another 1-day neonatal exposure study, brain levels of calcium/calmodulin-dependent protein kinase II (CaMKII), NMDA interacting proteins PSD-95 and SAP97, AMPA receptor subunit GluR1, and NMDA receptor subunits NR1, NR2A, and NR2B were measured in PNDs 17–19 mice exposed to tetraBDE at 0, 6.8, or 68 mg/kg on PND 10 (Dingemans et al. 2007). Protein expression levels of NR2B, GluR1, and the autophosphorylated-active form of CaMKII were significantly decreased in exposed mice compared with control. No exposure-related changes were observed for total CaMKII, NR1, NR2A, PSD-95, or SAP97 protein expression levels. Additionally, K+-evoked catecholamine release was evaluated from chromaffin cells isolated from mice in the 0 and 68 mg/kg groups; no significant exposure-related effects were observed (Dingemans et al. 2007).

Hippocampal, cortical, and cerebellar tissues were evaluated for markers of oxidative stress in PND 37 male offspring of rat dams exposed to pentaBDE at 0 or 2 mg/kg/day via gavage from GD 6 to PND 21 (Cheng et al. 2009). In the hippocampus of offspring of exposed dams, the specific activities of SOD and GSH-Px were significantly decreased, lipid peroxidase, H_2O_2, and NO generation were significantly increased, and the number of free radicals was significantly increased, compared with controls. No significant changes were observed for these measures in the cerebral cortex or cerebellum, with the exception of significantly increased H_2O_2 generation in the cerebellum. No changes in brain GSH concentrations were observed (Cheng et al. 2009). Increased markers of apoptosis and oxidative stress were also observed in the hippocampus of offspring of rat dams exposed to the commercial pentaBDE mixture DE-71 at 30.6 mg/kg/day via gavage from GD 6 to PND 14 (Kodavanti et al. 2015). In a low-dose study, no exposure-related changes were observed in cortical, cerebellar, or hippocampal levels of SOD, GSH-Px, GSH, H_2O_2, NO, or lipid peroxidation in PND 37 male offspring of dams exposed to 0.2 mg/kg/day via gavage from GD 1 to PND 21, compared with controls (Zhao et al. 2014).

DNA methylation and mRNA levels of DNA methyltransferase (*Dmnt1*) were measured in adult offspring of mouse dams exposed to tetraBDE at 0 or 0.03 mg/kg/day via gavage from pre-mating day 28 to PND 21 (Woods et al. 2012). Global DNA methylation was significantly decreased in female, but not male, offspring; no changes were observed in *Dmct1* transcript levels.

Cortical and hippocampal cholinergic gene transcription was evaluated in 2-month-old mice exposed to pentaBDE at 12 mg/kg via gavage on PND 10 (Hallgren et al. 2015). Significant transcriptional findings included increased cortical nAchR-β2, AChR5, and nAChR-α4 in exposed mice, compared with controls; a near-significant increase in cortical AchE transcription was also observed. No exposure-related cholinergic gene transcription changes were observed in the hippocampus of 2-month-old mice. Additionally, no exposure-related cholinergic gene transcription changes were observed in the cortex or hippocampus of mice sacrificed 24 hours postexposure (i.e., PND 11). Cholinergic effects were also evaluated in offspring from a one-generation study in mink (Bull et al. 2007). F0 females were exposed to dietary pentaBDE at 0, 0.01, 0.05, or 0.25 mg/kg/day from pre-mating day 28 to PNW 6. No kits were born in the 0.25 mg/kg/day group; for the other groups, 6 kits/group were sacrificed at PNW 6 and 10 kits/group continued dietary exposure until PNW 27 and were sacrificed at PNW 45. In both PNW 6 and 45 kits, ChE was determined in blood plasma and cerebral cortex. In the cerebral cortex, ACh, mAChR, and nAChR binding were also measured. No exposure-related effects were observed in any cholinergic measures in 6-week-old kits or 27-week-old juveniles.

DecaBDE

Neurobehavior. As seen in the lower-brominated PBDE neurobehavioral section, decreased spontaneous activity and impaired habituation in open-field testing were observed at 2 and 4 months in mice exposed once to decaBDE on PND 3 at doses ≥2.22 mg/kg (Buratovic et al. 2014; Johansson et al. 2008; Viberg et al. 2003b, 2007). Also as observed with pentaBDE, decaBDE-exposed mice showed significantly altered responses in a cholinergic-induced behavior task (decreased instead of increased activity) at ≥5.76 mg/kg on PND 3 (Buratovic et al. 2014; Johansson et al. 2008). Following exposure on PND 10 or 19, however, no exposure-related changes in open-field behavior were observed in mice exposed to decaBDE at doses up to 20.1 mg/kg (Viberg et al. 2003b). In contrast, significantly increased locomotor activity during the first 1.5 hours of a 2-hour observation period was observed in PND 70 males following exposure to decaBDE at 20 mg/kg/day via micropipette from PND 2 to 15 (Rice et al. 2007). No changes in locomotor activity were observed at PND 70 in females or 1 year in either sex at doses up to 20 mg/kg/day (Rice et al. 2007). In a perinatal exposure study, no exposure-related changes in motor

activity were observed in neonatal, weanling, juvenile, or adult offspring of rat dams exposed to decaBDE at doses up to 1,000 mg/kg/day via gavage from GD 6 to PND 21 (Biesemeier et al. 2011).

Operant training and visual discrimination were assessed in young adult (3-month-old) and aging (16-month-old) mice following exposure to decaBDE at 0, 6, or 20 mg/kg/day via micropipette on PNDs 2–15 (Rice et al. 2009). Neurobehavioral tasks included lever-press training followed by a series of operant procedures, including a fixed-ratio (FR) schedule of reinforcement, a fixed-interval (FI) 2-minute schedule, and a light-dark visual discrimination. No significant, exposure-related effects were observed in the young adult cohort; however, learning impairment and impulsivity were observed in the aging cohort. In the FR task, exposure did not lead to impaired performance; however, there was a significant main effect of decaBDE exposure on the slope parameter for the number of earned food pellets. The control group earned the fewest reinforcers at the beginning of the task, and the high-dose group earned the most; by the end of the 10 sessions, all dose groups earned about the same number of reinforcers. In the FI task, there were significant main effects of decaBDE exposure on the fitted mean for the overall response rate, with the high-dose having a marginally higher response rate than the control group ($p=0.06$). This means that the exposed mice were emitting more responses for the same number of reinforcers. No significant exposure-related changes were observed for other parameters measured in the FI task (pause time, run rate, index of curvature, or number of responses during the feed cycle). A number of significantly altered parameters were observed during the visual discrimination task (41 trials). Significantly decreased number of first choice errors and shorter response latencies were observed in 6 and 20 mg/kg/day females and 20 mg/kg/day males; these findings were particularly pronounced in the earlier trials. However, mice in the 20 mg/kg/day group showed a significantly higher rate of error in the last 15 trials, compared with controls. Additionally, after an initial error, mice in the 20 mg/kg/day group made significantly more “perseverative” errors than controls.

Altered spatial learning and memory have been reported in the Morris water maze following prenatal or neonatal exposure to decaBDE. While initial spatial learning was not impaired in mice exposed to 5.76 or 13.4 mg/kg on PND 3, reversal learning (ability to find the escape platform in a new location after initial training) was significantly impaired at ≥5.76 mg/kg at 5 and 7 months (Buratovic et al. 2014). Following gestational exposure from GD 1 to 14, rat offspring showed significantly impaired spatial learning on PND 25 at ≥30 mg/kg/day, but not at 10 mg/kg/day, compared with controls; reversal learning was not assessed in this study (Chen et al. 2014). An additional study evaluated spatial learning and memory in neonatal mice engineered to express neonatal human apolipoprotein E (three genotypes: apoE2, apoE3, and apoE4) exposed once to decaBDE at 0, 10, or 30 mg/kg via micropipette on PND 10 (Reverte et al.

2013). Investigators were assessing the potential effect of different human apoE genotypes on susceptibility to decaBDE exposure during development, as apolipoprotein is a genetic factor that is associated with varied vulnerability for the development of neurodegenerative disease. Impaired learning and memory were observed at 4 months in apoE3 and apoE4 male mice at ≥10 mg/kg/day and at 12 months in apoE3 females exposed to 30 mg/kg (lack of preference for target quadrant). No wild-type mice were evaluated.

In other behavioral tests, no exposure-related changes were observed in auditory startle responses, or learning and memory in the water-filled T-maze in weanling, juvenile, or adult offspring from rat dams exposed to decaBDE at doses up to 1,000 mg/kg/day via gavage from GD 6 to PND 21 (Biesemeier et al. 2011) or in the elevated plus maze in 4-month-old mice exposed once to decaBDE at doses up to 20.1 mg/kg via gavage on PND 3 (Johansson et al. 2008). No changes in were observed in surface-righting or geotaxis reflexes or motor function assays (tail pull, cling and climb test) at PNDs 12–16 in mice engineered to express neonatal human apolipoprotein E (three genotypes: apoE2, apoE3, and apoE4) exposed once to decaBDE at 0, 10, or 30 mg/kg via micropipette on PND 10 (Reverte et al. 2014).

Sensory and motor development. A comprehensive functional observation battery was conducted every other day from PND 2 to 20 in mice exposed to decaBDE at 0, 6, or 20 mg/kg/day via micropipette from PND 2 to 15 (Rice et al. 2007). The only exposure-related changes observed were a significant reduction in the number of male and female pups performing the palpebral reflex on PND 14 and a significant reduction in the number of male pups performing an effective forelimb grip on PNDs 14 and 16 in the 20 mg/kg/day group.

Electrophysiology. Five groups of developing mice were exposed to decaBDE at 0 or 20.1 mg/kg/day via gavage during the following periods: gestational (GDs 1–21, via dam), lactational (PNDs 1–21, via dam), neonatal (PNDs 3–21, direct), post-weaning (PNDs 22–41), or gestational, lactational, and post-weaning (GD 1–PND 21, via dam, and PNDs 22–41, direct) (Xing et al. 2009). In the control group, dams were administered the vehicle only from GD 1 to PND 21 and offspring were administered the vehicle from PND 22 to 41. The dams and pups in all exposure groups were administered the vehicle during non-exposure periods (e.g., the group exposed to decaBDE during gestation only was administered the vehicle daily from PND 1 to 41). In all groups, *in vivo* extracellular recording of synaptic transmission in the hippocampus was measured on PND 60 by placing the anesthetized mice in a stereotaxic head holder. Significantly decreased synaptic potency, short-term plasticity, and long-term potentiation were observed in exposed mice from the neonatal, post-weaning, and gestation+lactation+post-weaning groups. In the

lactation-only group, long-term potentiation was significantly decreased, but no exposure-related changes were observed in synaptic potency or short-term plasticity. No exposure-related changes were observed in the gestation-only exposure group.

Histology and organ weight. No exposure-related changes were observed in brain weight, histology, or morphometry in weanling or juvenile offspring from rat dams exposed to decaBDE at doses up to 1,000 mg/kg/day via gavage from GD 6 to PND 21 or PND 10 to 21 (Biesemeier et al. 2011; Fujimoto et al. 2011; Saegusa et al. 2012) or juvenile offspring from rat dams exposed to decaBDE at doses up to 1,500 mg/kg/day via gavage from GD 0 to 17 (Tseng et al. 2008). Absolute brain weight was significantly decreased in GD 16 fetuses following maternal exposure to decaBDE at 2,000 mg/kg/day via gavage on GDs 7–9; however, the difference was no longer significant once brain weights were adjusted for body weight (Chi et al. 2011).

Biochemical, proteomic, and genomic changes. Seven months following exposure to decaBDE at 0 or 13.4 mg/kg on PND 3, the cortical and hippocampal expression of CaMKII, Gap-43, Tau, and synaptophysin were evaluated in male and female mice (Buratovic et al. 2014). Significant increases in protein levels of CaMKII, Gap-43, and Tau were observed in the cortex and hippocampus in exposed male mice and increased levels of Tau were observed in the cortex and hippocampus of exposed female mice, compared with control. No changes in synaptophysin were observed.

Neonatal mice engineered to express neonatal human apolipoprotein E (three genotypes: apoE2, apoE3, apoE4) were exposed once to decaBDE at 0, 10, or 30 mg/kg via micropipette on PND 10 and evaluated for BDNF levels in the hippocampus (Reverte et al. 2013). When all genotypes were combined for analysis, hippocampal BDNF levels were significantly elevated in males and females (combined) in the 30 mg/kg group. No wild-type mice were evaluated.

Following exposure to decaBDE at 0, 2, 15, or 146 mg/kg/day from GD 10 to PND 21, brains from offspring sacrificed on PND 20 and 77 were fixed for hippocampal immunohistochemistry (reelin, flutamic acid decarboxylase 67 [GAD67], EphA5, Tacr3, and neuron-specific nuclear protein [NeuN]) and cresyl violet staining of apoptotic bodies (Saegusa et al. 2012). Significant, exposure-related findings included an increase in the number of reelin-immunoreactive cells in the dentate hilus in groups exposed to ≥15 mg/kg/day on PND 20, an increase in the number of EphA5-postive cells in the CA1 layer on PND 20, and a minimal increase in the number NeuN-immunoreactive cells in the hilus in the 146 mg/kg/day group on PND 77.

Reproductive System Development

Lower-brominated PBDEs: Reproductive performance was assessed in F1 male and female offspring from rat dams exposed to single pentaBDE doses of 0, 0.06, or 0.3 mg/kg or tetraBDE doses of 0, 0.14, or 0.7 mg/kg on GD 6 via gavage (Kuriyama et al. 2005; Talsness et al. 2005, 2008). Following pentaBDE exposure, no significant exposure-related effects were observed in F1 male fertility or F2 litter parameters when exposed males were mated with unexposed females (Kuriyama et al. 2005). Additionally, ejaculatory and mounting latencies, intromission frequency and latency, and number of penetrations were not altered in F1 exposed mice, compared with controls; however, the percent of males with two or more ejaculations was significantly decreased by 32% in the F1 males from the 0.3 mg/kg group (Kuriyama et al. 2005). In pentaBDE-exposed F1 females mated to unexposed males, there were no exposure-related changes in female pregnancy rate, total implantation sites, implantation sites/dam, F2 fetuses/gravid dam, or total number of live F2 fetuses (Talsness et al. 2005). However, the resorption rate was 12 and 15% in the 0.06 and 0.03 mg/kg groups, respectively, compared with the control rate of 9%, and the percentage of litters with resorptions was 69 and 72% in the 0.06 and 0.3 mg/kg groups, respectively, compared with the control percentage of 47% (Talsness et al. 2005). Following tetraBDE exposure, reproductive performance of F1 females was unaltered by developmental tetraBDE exposure (Talsness et al. 2008). No exposure-related changes were observed for the following F2 litter parameters: total number of implantation sites, implantation sites/dam, number of live fetuses, fetuses/dam, mean fetal weight, or resorption rate. In the 0.7 mg/kg group, the sex ratio was significantly altered; however, comparison of the altered sex ratio with controls from two different historical experiments (n=24 and 43 litters) revealed no differences (Talsness et al. 2008). F1 male reproductive performance was not assessed following tetraBDE exposure.

Male offspring of rat dams exposed to pentaBDE at 0, 1.7, 10.2, or 30.6 mg/kg/day via gavage from GD 6 to PND 21 showed a significant 1.8-day delay in PPS (an external sign of pubertal development), compared with controls (Kodavanti et al. 2010). A 5.5% decrease in AGD was also observed at PND 7 in the 30.6 mg/kg/day group; although not statistically significant, the study authors argue that this finding may be biologically relevant, as the findings were not confounded by body weight effects and were accompanied by the delay in PPS and a 20% decrease in mean testosterone concentration on PND 60 (Kodavanti et al. 2010). After maternal exposure to a lower pentaBDE dose (2 mg/kg/day) from GD 6 to PND 21 via gavage, no exposure-related changes in AGD were observed in male offspring on PND 1 (Cheng et al. 2009). Female offspring of rat dams exposed to pentaBDE at 0, 1.7, 10.2, or

30.6 mg/kg/day via gavage from GD 6 to PND 21 showed a significant reduction in mammary gland development on PND 21 at 10.2 and 30.6 mg/kg/day, showing lack of outgrowth, fewer lateral branches and limited terminal end bud development, compared with controls (Kodavanti et al. 2010). Vaginal opening was not assessed.

As discussed in Section 3.2.2.5 (Reproductive Effects), delays in reproductive development also occurred in male and female rats that were exposed to pentaBDE at 0, 3, 30, or 60 mg/kg/day via gavage during pubertal development (PNDs 23–53 in males or PNDs 22–41 in females) (Stoker et al. 2004). PPS was significantly delayed by 1.7 and 2.1 days in the 30 and 60 mg/kg/day groups, respectively, and vaginal opening was significantly delayed by 1.8 days in the 60 mg/kg/day group. However, no changes were observed in estrous cycling at doses up to 60 mg/kg/day (Stoker et al. 2004).

Following a single gavage administration of pentaBDE at 0, 0.06 or 0.3 mg/kg on GD 6 to rat dams, various exposure-related sperm effects were observed in male offspring on PND 140 in both dose groups (Kuriyama et al. 2005). Spermatid number and daily sperm production were significantly decreased by 31 and 34%, and sperm number was significantly decreased by 29 and 18%, at 0.06 and 0.3 mg/kg, respectively (Kuriyama et al. 2005).

Following a single gavage administration of pentaBDE at 0, 0.06 or 0.3 mg/kg on GD 6 to rat dams, multiple ultrastructural changes were noted in the ovaries of female offspring at PND 90 in both exposure groups, including destruction of the surface of the serosal epithelial cells, necrosis, and numerous vesicular structures with dense granular material within the cytoplasm (Talsness et al. 2005). Additional changes observed in the 0.3 mg/kg group included degenerative changes and aggregates of small and large vesicles filled with homogeneously dense granular material in the cytoplasm and clumped chromatin within the condensed nucleus. No statistically significant, exposure-related histological changes were observed at the light microscopic level in the ovary, uterus, or vagina of female offspring, and no exposure-related effects were observed on the number of ovarian follicles (Talsness et al. 2005). Following a single gavage administration of tetraBDE at 0, 0.14, or 0.7 mg/kg on GD 6 to rat dams, the mean number of secondary follicles in the ovaries of female offspring was significantly decreased by 43% in both exposure groups at PND 38, and the mean number of tertiary follicles was significantly decreased by 38% in the 0.7 mg/kg group, compared with controls (Talsness et al. 2008). No exposure-related changes were observed in the number of primordial, primary, or atretic follicles. No histopathological lesions or organ weight changes were observed in the ovary, uterus, or vagina at PND 38 or 100 (Talsness et al. 2008). However, ultrastructural changes of the ovaries were observed on PND 200 in F1 females

from both exposure groups, including an accumulation of vesicular structures with homogeneously dense granular material in the cytoplasm of the stromal cells, which appeared to fuse together to form large vacuoles (Talsness et al. 2008). No exposure-related histopathological changes were observed in the uteri or ovaries from female rats exposed to pentaBDE at gavage doses up to 60 mg/kg/day for 20 days during pubertal development (PNDs 22–41) (Stoker et al. 2004).

In 2-month-old females exposed to tetraBDE on PND 10, relative uterine weights were significantly decreased by 23–36% at doses ≥1 mg/kg and relative ovary weights were significantly increased by 27–35% at doses ≥5 mg/kg (He et al. 2011). In other studies, no exposure-related changes were observed in ovary or uterus weights in PND 100 offspring of rat dams exposed to tetraBDE at doses up to 0.7 mg/kg via gavage on GD 6 (Talsness et al. 2008), in PND 31 offspring of rat dams exposed to pentaBDE at 18 mg/kg/day from GD 6 to PND 18 via gavage (Ellis-Hutchings et al. 2006), in PND 43 offspring of rat dams exposed to pentaBDE at doses up to 25 mg/kg/day from pre-mating day 70 to PND 42 (Bondy et al. 2013), or in PND 42 rats exposed to pentaBDE at doses up to 60 mg/kg/day for 20 days during pubertal development (PNDs 22–41) (Stoker et al. 2004).

No exposure-related histopathological changes were observed in the epididymides or left testis from male rats exposed to pentaBDE at gavage doses up to 60 mg/kg/day for 31 days during pubertal development (PNDs 23–53) (Stoker et al. 2004). No other developmental studies examined male reproductive histology following exposure to lower-brominated PBDEs.

Relative testes weights were significantly increased by 1.3-fold in PND 31 male offspring of rat dams exposed to pentaBDE at 18 mg/kg/day from GD 6 to PND 18 via gavage (absolute weights not reported); no exposure-related changes were observed on PND 12 and 18 (Ellis-Hutchings et al. 2006). In contrast, following a single maternal exposure to pentaBDE on GD 6, adult male rat offspring showed significant 10 and 11% decreases in relative testes weights at 0.06 and 0.3 mg/kg, respectively, as well as a significant 5% decrease in relative epididymis weight at 0.3 mg/kg (Kuriyama et al. 2005). Neither absolute organ weights nor body weight were significantly altered (Kuriyama et al. 2005). In other studies, no exposure-related weight effects were observed in male reproductive organs (seminal vesicles, prostate, epididymides, or testes) in PND 60 offspring of rat dams exposed to pentaBDE at doses up to 30.6 mg/kg/day from GD 5 to PND 21 (Kodavanti et al. 2010), in 2-month-old rats exposed to tetraBDE at doses up to 10 mg/kg on PND 10 (He et al. 2011), in PND 43 offspring of rat dams exposed to pentaBDE from pre-mating day 70 to PND 42 at doses up to 25 mg/kg/day (Bondy et al. 2013), or in

PND 53 rats exposed to pentaBDE at doses up to 60 mg/kg/day for 31 days during pubertal development (PNDs 23–53) (Stoker et al. 2004).

After a single maternal gavage exposure to tetraBDE on GD 6, serum E2 levels in female rat offspring were significantly decreased on PND 28 by ~38% at 0.7 mg/kg; no change was observed in the 0.14 mg/kg group (Talsness et al. 2008). No exposure-related changes in serum testosterone or LH were observed in adult F1 males after a single exposure to pentaBDE at doses up to 0.3 mg/kg via gavage on GD 6 (Kuriyama et al. 2005). Exposure-related effects on serum testosterone levels were also not observed in male offspring of rat dams on PND 60 following exposure to pentaBDE at doses up to 30.6 mg/kg/day from GD 6 to PND 21 (Kodavanti et al. 2010). In male rats exposed to pentaBDE at 0, 3, 30, or 60 mg/kg/day via gavage during pubertal development (PNDs 23–53), serum prolactin was increased 2-fold in the 60 mg/kg/day group approximately 2 hours after the final exposure on PND 53; however, no exposure-related changes were observed in serum testosterone, serum or pituitary luteinizing, or pituitary prolactin levels (Stoker et al. 2004).

DecaBDE: Male offspring of mouse dams exposed to decaBDE at 0, 10, 500, or 1,500 mg/kg/day via gavage from GD 0 to 17 were assessed for reproductive system effects on PND 71 (Tseng et al. 2013). The mean AGD and AGI (anogenital index; corrected for body weight) were significantly reduced in the 1,500 mg/kg/day group, compared with control. No exposure-related changes were observed in testicular index ([testicular length x testicular width]/body weight) or male reproductive organ weights (testis, epididymis, cauda epididymis, or seminal vesicles). However, increased incidences of testicular lesions were observed in male offspring, with increased incidence of slight/moderate vacuolization in interstitial cells in all treated groups (0/5, 4/5, 3/5, or 5/5 at 0, 10, 500, or 1,500 mg/kg/day, respectively) and increased incidence of slight-severe vacuolization in seminiferous tubules at 1500 mg/kg/day (4/5; control incidence 0/5). Additionally, in the 1,500 mg/kg/day group, seminiferous tubules had lost almost all spermatozoa and spermatids. Analysis of male offspring sperm parameters (from the seminiferous tubules) showed a significant increase in percentage of abnormal sperm heads in the 1,500 mg/kg/day group (18.2%) compared with controls (10.3%). No changes were observed in abnormal sperm heads at lower doses, and no changes were observed in sperm count, motility, or velocity at any dose. However, evidence of sperm damage was observed in all exposed groups. The DNA fragmentation index (DFI) and level of sperm with DNA damage (X αT) were significantly elevated in all exposed groups in a dose-related manner. Sperm H_2O_2 generation was significantly elevated in a dose-related manner; however, only the values in the 10 and 1,500 mg/kg/day groups reached statistical significance in pairwise

comparisons with controls. No changes were observed in sperm O_{2-} generation. No changes in serum testosterone levels were observed.

In other studies, no exposure-related changes were observed in AGD, onset of puberty (PPS or vaginal opening), estrous parameters, and/or reproductive organ weight and histology in offspring of rat dams exposed to dietary decaBDE at doses up to 146 mg/kg/day from GD 10 to PND 21 (Fujimoto et al. 2011) or gavage doses up to 1,000 mg/kg/day from GD 6 to PND 21 (Biesemeier et al. 2011). Similarly, no exposure-related changes were observed in AGD or the onset of puberty (vaginal opening or descent of the testes) in mice exposed to decaBDE at doses up to 20 mg/kg/day via micropipette from PND 2 to 15 (Rice et al. 2007). No changes in were observed in vaginal opening or testicle descent in mice engineered to express neonatal human apolipoprotein E (three genotypes: apoE2, apoE3, and apoE4) exposed once to decaBDE at 0, 10, or 30 mg/kg via micropipette on PND 10 (Reverte et al. 2014).

Endocrine System Development

Lower-brominated PBDEs: Histological, ultrastructural, and morphometric changes of the thyroid were observed at PND 100 in female offspring of dams exposed once to tetraBDE via gavage on GD 6 at 0, 0.14, or 0.7 mg/kg; however, a clear dose-response pattern cannot be determined from the available data (Talsness et al. 2008). The study authors reported "occasional" follicular cyst formation in the 0.14 mg/kg group with multiple areas of degenerated follicular epithelium. In the 0.7 mg/kg group, only mild cyst formation was observed. Ultrastructural changes observed in both exposure groups included irregular, non-typical follicular shape and detached and swollen follicular cells. Incidence data were not reported for any of these end points. Morphometric analysis showed that the colloid area of the thyroid was also significantly increased in offspring, but only in the 0.14 mg/kg group (Talsness et al. 2008). In mink, no exposure-related changes were observed in thyroid histology in PNW 6 offspring of sows exposed to pentaBDE at doses up to 0.06 mg/kg/day from pre-mating week 4 to PNW 6 (Zhang et al. 2009).

In rats exposed once to tetraBDE via gavage on PND 10 at doses of 0, 1, 5, or 10 mg/kg, relative thyroid weights were significantly decreased by 11% in the 10 mg/kg group at 2 months of age (He et al. 2011). In other studies, no exposure-related changes in thyroid weight were observed in rat offspring of dams exposed once to tetraBDE at gavage doses up to 0.7 mg/kg on GD 6 (Talsness et al. 2008) or in mink offspring of sows exposed to pentaBDE at doses up to 0.06 mg/kg/day from pre-mating week 4 to PNW 6 (Zhang et al. 2009).

Significant reductions in serum T_4 ranging from 12 to 90% were observed in male and female offspring of rat dams exposed once to pentaBDE on GD 6 at 0.3 mg/kg (Kuriyama et al. 2007), exposed to pentaBDE or commercial pentaBDE mixtures from GD 6 to PND 18 or 21 via gavage at doses as low as 2 mg/kg/day (Blanco et al. 2013; Ellis-Hutchings et al. 2006; Kodavanti et al. 2010; Miller et al. 2012; Shah et al. 2011; Szabo et al. 2009; Zhou et al. 2002), exposed to pentaBDE from GD 1 or 6 to PND 21 via dosed cookies at intake levels ≥3 mg/kg/day (Bansal et al. 2014; Bowers et al. 2015), or exposed to dietary tetraBDE from GD 1 to PND 14 at doses ≥3.2 mg/kg/day (Wang et al. 2011a). These reductions were observed in offspring between PND 7 and 22. In studies described above that also evaluated offspring at older ages, it was found that the effects were no longer observed at PNDs 31–60 (Bowers et al. 2015; Ellis-Hutchings et al. 2006; Kodavanti et al. 2010; Szabo et al. 2009; Zhou et al. 2002), indicating that serum T_4 changes in offspring may be transient. In one-generation studies, offspring from rat dams fed pentaBDE-dosed vanilla wafers from pre-mating day 28 to PND 21 showed significant 43–55% reductions in serum T_4 on PND 21 at ≥5.7 mg/kg/day (Poon et al. 2011) and male and female offspring from rat dams exposed to pentaBDE via gavage from pre-mating day 70 to PND 42 showed significant 65–70% reductions in serum T_4 on PND 21 at 25 mg/kg/day (but not ≤5 mg/kg/day) (Bondy et al. 2011, 2013). In rats and mice exposed once to tetraBDE via gavage on PND 10 at doses up to 10 or 20 mg/kg, respectively, no exposure-related changes were observed in serum T_4 levels (Costa et al. 2015; Gee et al. 2008; He et al. 2011). In other mouse studies, maternal exposure to the pure pentaBDE congener BDE 99 at doses up to 452 mg/kg/day via gavage from GD 6 to PND 21 or from GD 4 to PND 17 did not lead to altered serum T_4 levels in offspring at PNDs 11–37 (Branchi et al. 2005; Skarman et al. 2005). In contrast, maternal exposure to the commercial pentaBDE mixture Bromkal 70-5DE at 452 mg/kg/day via gavage from GD 4 to PND 17 caused a significant 29–32% reduction in serum T_4 in PND 11 mouse offspring (Skarman et al. 2005). This effect was transient, as it was no longer observed at PND 18 (Skarman et al. 2005). In mink, no exposure-related changes were observed in serum T_4 levels in PNW 6 offspring of sows exposed to dietary pentaBDE at doses up to 0.06 mg/kg/day from pre-mating week 4 to PNW 6 (Zhang et al. 2009). In mink offspring that continued exposure through PNW 33, serum T_4 levels were significantly increased by 31% compared with control; however, this response was entirely attributable to the females, which were significantly elevated by 71% compared to males at the same dose (change compared with female controls was not reported) (Zhang et al. 2009).

Evidence for exposure-related serum T_3 changes is less consistent. In a one-generation study, male and female offspring from rat dams exposed to pentaBDE via gavage from pre-mating day 70 to PND 42 showed significant 16–27% reductions in serum T_3 on PND 21 at 25 mg/kg/day (but not ≤5 mg/kg/day)

(Bondy et al. 2013). Similarly, offspring of rat dams exposed to pentaBDE at ≥2 mg/kg/day via gavage from GD 6 to PND 21 showed a significant 19–25% reduction in serum T_3 levels on PNDs 21–23 and offspring from dams fed cookies dosed with ≥3 mg/kg/day pentaBDE from GD 1 to PND 21 showed a significant 5–40% reduction in serum T_3 levels on PND 21; no exposure-related effect was observed at doses ≤1.7 mg/kg/day (Blanco et al. 2013; Bowers et al. 2015; Shah et al. 2011). This effect was transient, as it was no longer observed at PND 50 or later (Bowers et al. 2015; Shah et al. 2011). In contrast, exposure-related changes in serum T_3 levels were not observed in offspring of rat dams exposed to pentaBDE at doses up to 30.6 mg/kg/day via gavage or pentaBDE-dosed cookies from GD 6 to PND 18 or 21 (Bansal et al. 2014; Ellis-Hutchings et al. 2006; Kodavanti et al. 2010; Szabo et al. 2009) or offspring of rat dams exposed to a single pentaBDE dose up to 0.3 mg/kg on GD 6 via gavage (Kuriyama et al. 2007). In rats and mice exposed once to tetraBDE via gavage on PND 10 at doses up to 10 or 20 mg/kg, respectively, no exposure-related changes were observed in serum T_3 levels (Costa et al. 2015; Gee et al. 2008; He et al. 2011). In mink, no exposure-related changes were observed in serum T_3 levels in PNW 6 offspring of sows exposed to pentaBDE at doses up to 0.06 mg/kg/day from pre-mating week 4 to PNW 6; however, in offspring that continued exposure through PNW 33, serum T_3 levels were significantly reduced by ~31% at 0.06 mg/kg/day (Zhang et al. 2009).

No exposure-related changes in serum TSH were observed in offspring of rat dams exposed to a single pentaBDE dose up to 0.3 mg/kg on GD 6 via gavage (Kuriyama et al. 2005), offspring of rat dams exposed to pentaBDE at doses up to 30.6 mg/kg/day via gavage or pentaBDE-dosed cookies from GD 6 to PND 21 (Bansal et al. 2014; Kodavanti et al. 2010), or rats exposed once to tetraBDE via gavage on PND 10 at doses up to 10 mg/kg (He et al. 2011).

DecaBDE: Following maternal exposure to decaBDE at 0, 2, 15, or 146 mg/kg/day via gavage from GD 6 to PND 21, the incidences of diffuse follicular cell hypertrophy in the thyroid of rat offspring on PND 21 were 0/10, 1/10, 3/10, and 9/10 in males and 0/10, 3/10, 2/10, and 4/10 in females, respectively (Fujimoto et al. 2011). No change was observed in thyroid weight (Fujimoto et al. 2011). Following maternal exposure to decaBDE at 0, 10, 500, or 1,500 mg/kg/day via gavage on GDs 0–17, histological evaluation of the thyroid glands in male offspring at PND 71 showed that a few acini were slightly enlarged in the 1,500 mg/kg/day group, compared to the controls (female offspring were not evaluated) (Tseng et al. 2008). The normal cuboidal epithelium had dose-dependently transformed into squamous epithelium, with the most notable change found in the 1,500 mg/kg/day group. Incidence data for histological lesions were not reported (Tseng et al. 2008).

Serum T_3 levels were significantly reduced by 16% on PND 21 in male offspring of rat dams exposed to decaBDE at 146 mg/kg/day via gavage from GD 6 to PND 21; no change was observed in serum T_3 levels in female offspring or serum T_4 or TSH levels in either sex at doses ≤146 mg/kg/day (Fujimoto et al. 2011). Following maternal exposure to decaBDE at 0, 10, 500, or 1,500 mg/kg/day via gavage on GDs 0–17, serum T_3 levels were significantly decreased by 21% in the 10 and 1,500 mg/kg/day groups in male mouse offspring examined on PND 71 (female offspring not examined); serum T_3 levels were not significantly altered in the 500 mg/kg/day group and serum T_4 levels were not altered in any group (Tseng et al. 2008). In neonatal male mice exposed to 0, 6, or 20 mg/kg/day from PND 2 to 15, serum T_4 was reduced by ~8 and 22% at 6 and 20 mg/kg/day, respectively (Rice et al. 2007). This finding was reported as a dose-related trend; however, pair-wise statistics were not reported. No exposure-related changes in serum T_4 levels were observed in similarly exposed neonatal female mice (Rice et al. 2007). Serum T_3 levels were not examined by Rice et al. (2007).

Immune System Development

Lower-brominated PBDEs: In a one-generation study, F0 rats were exposed to pentaBDE at 0, 0.5, 5, or 25 mg/kg/day via gavage for 70 days prior to mating, through mating, gestation, and lactation (PND 21) (Bondy et al. 2013). F1 rats continued exposure to their respective doses from PND 22 to 42. Half of the F1 rats were sacrificed on PND 43 and assessed for serum immunoglobin levels, B and T lymphocyte quantification in the spleen, spleen cell proliferation *in vitro*, and immune organ weight and histology. The remaining F1 rats were assessed for immune function at PND 56 using the KLH antigen immune challenge. Serum IgE and IgG1 levels were significantly reduced by 77 and 53%, respectively, in females at 25 mg/kg/day; no changes were observed in serum IgE or IgG1 levels in males or serum IgM, IgA, IgG2a, IgG2b, or IgGc levels in either sex. All exposed groups showed a significant, dose-related reduction in the proportion of B cells and a significant concomitant increase in the proportion of T cells in the spleen (7–18%). *In vitro*, increased proliferation of unstimulated spleen cells was observed in cells harvested from F1 males and females from the 25 mg/kg/day group; however, the proliferative response to ConA or lipopolysaccharide (LPS) stimulation was not affected by pentaBDE exposure. In the thymic cortex from F1 rats, mild increases in apoptotic lymphocytes and tingible macrophages were observed in F1 males (0/14, 1/19, 3/13, and 3/13) and F1 females (0/13, 3/17, 0/13, and 4/14) in control, 0.5, 5, and 25 mg/kg/day groups, respectively. Trend test analysis indicated that the increase was significantly dose-related in males, but not females. No treatment-related histopathological changes were observed in the Peyer's patches, mesenteric lymph nodes, or spleen of F1 rats. Absolute thymus weights were significantly increased in the 5 mg/kg/day males and females and relative thymus weight was only

increased in the 5 mg/kg/day females; no changes were observed in thymus weights at 25 mg/kg/day. These organ weight changes reflect observed body weight changes (significantly increased in the 5 mg/kg/day group). In the immune challenged rats, no exposure-related changes were observed in KLH-specific IgG levels or changes in delayed-type hypersensitivity responses to KLH injections (Bondy et al. 2013). In another one-generation study (pre-mating day 28 to PND 21), maternal exposure to pentaBDE-dose vanilla wafers at doses up to 11.4 mg/kg/day had no effect on thymus weight in PND 21 offspring (Poon et al. 2011).

In a poorly-reported study, mouse dams were exposed to pentaBDE at 0, 50, 100, or 200 mg/kg/day via gavage from GD 6 to PND 21 (Hong et al. 2010). In PND 21 offspring, study authors report exposure-related decreases in serum IgM and IgG1, decreased absolute and relative spleen weights at ≥100 mg/kg/day, and reduced cellularity levels at ≥100 mg/kg/day. No statistically significant increases in T- or B-cell lymphocyte proliferation were observed *in vitro*. The only significant effect observed in PND 63 offspring was increased T-cell proliferation following *in vitro* exposure to ConA at 200 mg/kg/day.

In another study, absolute spleen weight was significantly increased by 9% in PND 140 male offspring of rat dams exposed to single pentaBDE doses of 0.06 and 0.3 mg/kg on GD 6; relative spleen weight was only significantly increased in the 0.06 mg/kg group (12%) (Kuriyama et al. 2005). No organ weight changes were observed in the thymus of PND 140 male rats (Kuriyama et al. 2005). Following maternal exposure to pentaBDE at 0 or 18 mg/kg/day via gavage from GD 6 to PND 18, no exposure-related changes were observed in spleen or thymus weights measured in rat offspring on PNDs 3, 12, 18, and 31 (Ellis-Hutchings et al. 2006).

DecaBDE: Immune function in PND 28 mice was assessed using the RSV intranasal infection test following maternal exposure to decaBDE from GD 10 to PND 21 at doses of 0, 3.3, 34, 260, or 3,100 mg/kg/day (Watanabe et al. 2008). Typical features of pneumonia due to RSV infection were observed in all RSV-infected mice; however, exacerbation of histopathological changes in the lung was observed in 50% of mice exposed to 3,100 mg/kg/day, including hypertrophy and/or hyperplasia. Five days after the RSV infection, pulmonary viral titers of RSV and bronchoalveolar lavage fluid (BALF) levels of IFN-γ were significantly increased in the 260 mg/kg/day group (titers and BALF fluid not assessed in the 3,100 mg/kg/day group). Additionally, mRNA expression of RANTES (a characteristic marker of severity of inflammation in the lungs due to an RSV infection) was significantly elevated at 34 and 260 mg/kg/day (Watanabe et al. 2008). In a second study, immune function in PND 28 mice was

also assessed using the RSV infection test following maternal exposure to decaBDE from GD 10 to PND 21 at doses of 0, 290, or 2,900 mg/kg/day (Watanabe et al. 2010b). In this study, pulmonary viral titers of RSV were significantly increased in the 2,900 mg/kg/day group 1 and 5 days post-infection. BALF levels of TNF-α, IL-6, and IL1β were significantly decreased one day post-infection (cytokine levels at 5 days postexposure were not reported). No exposure-related changes were observed in BALF cell TNF-α production *in vitro* in response to LPS.

In a poorly-reported study, mouse dams were exposed to decaBDE at 0, 500, 2,500, or 12,500 mg/kg/day via gavage from GD 6 to PND 21 (Hong et al. 2010). Absolute and relative thymus weights were significantly increased in offspring the 2,500 mg/kg/day group at PND 21, but not the 500 or 12,500 mg/kg/day groups. At PND 21, no exposure-related changes were reported for spleen weight, spleen, or thymus cellularity, serum IgM or IgG1 levels, or T- or B-cell lymphocyte proliferation *in vitro*. The study authors report an increase in the relative B cell population and a decrease in the relative distribution of macrophage cells in spleens from pups exposed to decaBDE (no statistics provided). No exposure-related effects were observed at PND 63. In another study, no exposure-related changes in spleen weight were observed in PND 71 male offspring of mouse dams exposed to decaBDE at doses up to 1,500 mg/kg/day from GD 0 to 17 (Tseng et al. 2008).

Summary

Teratology, fetotoxicity, and physical growth and development: No human studies have evaluated associations between embryotoxicity or fetotoxicity and PBDE exposure. Evidence for altered physical grown and development from human studies is inconsistent. Available data from animal studies do not indicate that PBDEs are embryotoxic or fetotoxic at PBDE doses below doses that elicited maternal toxicity, although occasional observations of reduced pup weight were reported. Taken together, it is unlikely that oral PBDE exposure will cause embryotoxicity or fetoxicity in humans; however, data indicate that PBDE exposure could potentially lead to low birth weight.

Neurodevelopment: Evidence from both human and animal studies indicates that oral PBDE exposure can lead to adverse effects in neurodevelopment, leading to altered neurobehavior later in life.

Reproductive system development: Based on limited human and adequate animal data, it is possible that that oral PBDE exposure during development may adversely affect the developing reproductive system,

particularly the male reproductive system. However, data are too limited to adequately determine whether or not PBDE exposure in infants and children will lead to altered reproductive performance.

Endocrine system development: While human data are inconsistent, they suggest that PBDEs can interact with thyroid hormone homeostasis in infants and children. These data, along with available animal studies, indicate that the thyroid is a target of concern for PBDE exposure, especially lower-brominated PBDEs.

Immune system development: Animal data suggest that oral PBDE exposure during development may lead to immunosuppression; however, data are too limited to adequately assess the immunotoxic potential of PBDE exposure in infants and children.

The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

Human Studies. There was no clear association between risk of non-Hodgkin's lymphoma (NHL) and exposure to 2,2'4,4'-tetraBDE in a case-control study of 77 Swedish men and women who were recruited in 1995–1997 and ranged in age from 28 to 85 years (Hardell et al. 1998; Lindstrom et al. 1998). Adipose tissue concentrations of 2,2',4,4'-tetraBDE (BDE 47) (used as a marker for total PBDE exposure) were compared in 19 patients with NHL, 23 patients with malignant melanoma, 8 patients with other cancers or *in situ* changes, and 27 persons with no cancer diagnosis. The highest concentrations were seen in the patients with NHL. The mean concentration of BDE 47 was 13.0 ng/g (ppb) lipid (range 1.0–98.2 ppb) in the 19 NHL patients and 5.1 ppb (range 0.6–27.5 ppb) in the 27 persons without known malignancies. Logistic regression, adjusted for age, gender, sum of PCBs, and sum of chlordanes, was performed on cases and controls in three concentration groups (<2.05, 2.05–<5.43, and ≥5.43 ppb). A nonsignificantly elevated risk with a suggestive dose-response was found for NHL in the two highest concentration groups compared with the lowest group; the ORs and 95% CIs were 1.9 (0.3–14) and 3.8 (0.7–26) in the middle and high groups, respectively. Although the risk was highest in the group with the highest concentration of 2,2'4,4'-tetraBDE (p=0.09 for trend), there was no significant difference between cases and controls (p=0.14). The results for patients with malignant melanoma did not differ from controls.

Hardell et al. (2006) found no association between testicular cancer risk and serum PBDE (sum of congeners 47, 99, and 153, but concentrations not reported) in a study of 58 cases recruited from hospitals in Sweden and age-matched controls. Blood was also collected from the mothers of the cases and controls (44 of the case mothers and 45 of the control mothers agreed to participate). There was a marginally significant relationship between maternal PBDE and the risk of testicular cancer in sons (OR 2.5, 95% CI 1.02–6.0 using median concentration in mothers of controls as a cut-off) and some evidence of a dose-response (OR 3.2, 95% CI 1.1–11 for those with high concentrations of PBDE in blood [>75th percentile] and OR 1.8, 95% CI 0.6–7.9 for those with low concentrations of PBDE in blood [median–75th percentile]). The relevance of this finding is uncertain, as it is unclear the extent to which the case mothers' body burden of PBDE at the time of the study might relate to body burden when giving birth to the cases (approximately 30 years previously, as median age of the cases was 30 years). A case-control study of Singaporean males found no significant association between serum levels of BDE 47 and risk of prostate cancer (Pi et al. 2016). The study included 240 prostate cancer incident cases and 268 controls. Serum concentrations of BDE 47 were 37 ng/g lipid in cases and 58 ng/g lipid in controls. Other BDEs analyzed in serum included BDE 99, BDE 100, BDE 153, BDE 154, and BDE 183, but the concentrations were below the limit of detection of the analytical method (0.3–20 pg/g lipid).

PBDE concentrations in adipose tissue (sum of congeners 28, 47, 66, 100, 99, 85, 154, 153, 138, and 183) were significantly higher in 21 cases with exocrine pancreatic cancer recruited in Sweden 1996–1999 (median 3.1 ng/g lipid) than in 59 controls comprising 20 males undergoing transurethral resection for benign prostate hyperplasia and 39 females undergoing hysterectomy 1997–1998 in the same geographical area (median 1.6 ng/g lipid) (Hardell et al. 2007). Case-control analysis found that the risk of pancreatic cancer was not significantly increased with lipid PBDE (OR 3.90, 95% CI 0.93–16.3) using median concentration in controls as a cut-off after adjustment for age, sex, and BMI at tissue sampling, but that the increase in risk was significant when the BMI adjustment was performed for the year before tissue sampling (OR 7.67, 95% CI 1.53–38.5, body weight 1 year before tissue sampling obtained by questionnaire).

Serum concentrations of PBDEs (10 tri- to hepta-BDEs) were not associated with risk of thyroid cancer in a nested case-control study in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, a large multicenter clinical trial in the United States (Aschebrook-Kilfoy et al. 2015). The study included 104 case of thyroid cancer and 208 controls matched to cases by race, sex, birth date, center, and blood collection date. Median lipid adjusted concentrations of ΣPBDEs (sum of BDE 47, 99, 100, and 153) were 12.8 ng/g for cases and 19.4 ng/g for controls. For ΣPBDEs, the OR for the fourth versus the first

quartile was 0.62 (95% CI 0.29–1.30). Restricting the analyses to cases with the papillary subtype (75% of the cases) did not alter the findings.

A case-control study found no evidence of an association between adipose concentrations of PBDE (BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and their sum) and breast cancer risk in women from the San Francisco Bay area of California (Hurley et al. 2011). The study population included 78 cases with histologically confirmed invasive breast cancer and 56 controls with benign histological changes undergoing surgical breast biopsies. PBDE concentrations in both cases and controls were relatively high (median values of 56.32 and 72.25 ng/g lipid in cases and controls, respectively, for total BDE). Another case-control study of 170 native Alaskan women reported that cases (n=75) had significantly higher serum levels of BDE 47 (38.8 ng/g lipid) than controls (n=95, 25.1 ng/g lipid) (Holmes et al. 2014). The result of univariate logistic regression analysis showed increase risk that approached statistical significance (OR 1.79, 95% CI 0.97–3.32); no statistical significance was apparent in multivariable analysis (OR 1.58, 95% CI 0.75–3.33).

Animal Studies. Information on carcinogenic effects of PBDEs in animals is limited to results of chronic bioassays of decaBDE mixtures in rats and mice (Kociba et al. 1975; Norris et al. 1975a; NTP 1986). As summarized below, these studies provide limited evidence for the carcinogenicity of decaBDE in animals. No carcinogenicity studies of octaBDE or pentaBDE were located in the available literature.

NTP evaluated the carcinogenicity of commercial-grade decaBDE (94–97% pure, no detected brominated dioxins or furans) in Sprague-Dawley rats (50/sex/dose) and B6C3F1 mice (50/sex/dose) that were exposed in the diet for 103 weeks and observed for an additional 0–1 weeks (NTP 1986). Comprehensive gross and histological examinations were performed on all animals in all dose groups including those that were moribund or died during the study. Reported estimated dose levels in the rats were 1,120 and 2,240 mg/kg/day in males and 1,200 and 2,550 mg/kg/day in females. Incidences of liver neoplastic nodules in low- and high-dose male rats (7/50 and 15/49, respectively) and high-dose female rats (9/50) were significantly greater than in controls (1/50 in both males and females) ($p \leq 0.03$, Fisher Exact test) and showed positive dose-related trends ($p < 0.001$, Cochran-Armitage trend test). Incidences of hepatocellular carcinoma alone (1/50, control males; 1/50, low-dose males; 1/49, high-dose males; 0/50, control females; 2/49, low-dose females; and 0/50, high-dose females) were not significantly increased in the treated rat groups compared to controls. The increased incidences of neoplastic nodules were considered as “some evidence of carcinogenicity” in both sexes. However, although it was concluded that there was some evidence of carcinogenicity in male and female rats based on “neoplastic nodules,”

this is a poorly defined and understood term that is no longer used by NTP to characterize hepatoproliferative lesions in rats. A dose-related trend for mononuclear cell leukemia was observed in treated male rats but was not considered to be biologically significant because of a high incidence in control animals.

Reported estimated doses in the mice were 3,200 and 6,650 mg/kg/day in males and 3,760 and 7,780 mg/kg/day in females (NTP 1986). Hepatocellular adenoma or carcinoma (combined) occurred at significantly increased incidences in low-dose male mice (22/50, p=0.002) and high-dose male mice (18/50, p=0.019) in comparison to controls (8/50) and showed a positive dose-related trend (p=0.021). Incidences of hepatocellular carcinoma alone were not significantly increased in either the low- or high-dose male mice. Slightly elevated incidences of thyroid gland follicular cell adenoma or carcinoma (combined) were additionally observed in exposed male mice but the increases were not statistically significant (control, 0/50; low dose, 4/50; high dose, 3/50). Incidences of follicular cell hyperplasia were significantly increased in male mice as summarized in the subsection on Endocrine Effects in Section 3.2.2.2. No significantly increased incidences of neoplastic lesions were observed in the female mice. NTP (1986) concluded that the significant increase in liver tumors and equivocal increase in thyroid tumors represented equivocal evidence of carcinogenicity in male mice. The evidence of carcinogenicity in the male mice was considered limited by an early loss of control animals. Losses of control male mice were significant during the first year of the study, but were subsequently comparable to the dosed mice; the early losses were presumed to be due to fighting among animals in both control and treatment groups.

The carcinogenicity of decaBDE was also evaluated in Sprague-Dawley rats (25/sex/dose) that were exposed to dietary doses of 0, 0.01, 0.1, or 1.0 mg/kg/day for approximately 2 years (702 days for males, 735 days for females) (Kociba et al. 1975; Norris et al. 1975a). The commercial mixture was comprised of 77.4% decaBDE, 21.8% nonaBDE, and 0.8% octaBDE and therefore differs from typical decaBDE formulations containing ≥97% decaBDE. Comprehensive histological examinations showed no exposure-related neoplastic effects. The ability of this study to detect carcinogenic changes is limited by the very low dose levels in comparison to those tested in the NTP (1986) bioassay.

Summary. With the exception of one small case-control study reporting possible associations between adipose PBDE concentrations and risk of pancreatic cancer, there is no evidence for carcinogenicity of PBDEs in human studies. There is limited evidence of carcinogenicity in animals in a NTP bioassay with decaBDE (significantly increased incidences of neoplastic liver nodules in rats and combined

hepatocellular adenomas and carcinomas in mice). The Cancer Effect Levels (CELs) for decaBDE in the NTP (1986) study are recorded in Table 3-3 and plotted in Figure 3-3.

3.2.3 Dermal Exposure

A few studies have examined groups of chemical workers involved in the manufacture and distribution of PBDEs (Bahn et al. 1980; Brown et al. 1981; Chanda et al. 1982; Landrigan et al. 1979; Rosenman et al. 1979; Stross et al. 1981). Although the route of exposure (inhalation relative to dermal) of these workers has not been well defined, they appear to have had a high potential for dermal exposure (Anderson et al. 1978). Results from these studies are discussed in this section, as well as in Section 3.2.1. Dermal exposure may not be an important route of concern for PBDEs because dermal absorption is likely to be low, particularly for the highly brominated congeners, based on *in vitro* dermal absorption assays (Hughes et al. 2001; Roper et al. 2006).

3.2.3.1 Death

No reports of death in humans after dermal exposure to PBDEs were located in the available literature.

No deaths occurred in rabbits that were observed for 14 days following a single ≤2,000 mg/kg dermal dose of decaBDE, octaBDE, or pentaBDE (IRDC 1974, 1975a, 1975b). The PBDEs were applied to clipped intact skin, covered with an occlusive barrier, and washed from the treatment site 24 hours later.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, or renal effects in humans or animals after dermal exposure to PBDEs.

Systemic effects that have been observed in humans and animals following dermal exposure to PBDEs are described below. The highest NOAEL and all LOAEL values from each reliable study for systemic end points in each species and duration category are recorded in Table 3-4.

Endocrine Effects. There is suggestive evidence of hypothyroidism in a small group of workers who were occupationally exposed to decaBDE (Bahn et al. 1980) as summarized above and detailed in Section 3.2.1.2.

Table 3-4 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL		Reference Chemical Form	Comments
				Less Serious	Serious		
ACUTE EXPOSURE							
Systemic							
Rabbit (New Zealand)	24 hr	Bd Wt	2000 mg/kg			IRDC 1975a OctaBDE (technical)	
Rabbit (New Zealand)	24 hr	Bd Wt	2000 mg/kg			IRDC 1975b PentaBDE (technical)	

Note on chemical form: The chemical forms included technical octaBDE and pentaBDE mixtures (exact compositions were not reported).

Bd Wt = body weight; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

No studies were located regarding endocrine effects in animals after dermal exposure to PBDEs.

Dermal Effects. No studies evaluating dermal exposure of PBDEs were located.

There was no evidence of primary irritation in intact skin of rabbits that were dermally exposed to a former commercial decaBDE mixture (500 mg as dry solid was applied to clipped skin and occluded for 24 hours) (IRDC 1974). A similar application of 77.4% decaBDE (containing 21.8% nonaBDE and 0.8% octaBDE) (dry solid, amount not reported), octaBDE (500 mg as dry solid), or pentaBDE (0.5 mL as a viscous liquid) was also non-irritating to intact rabbit skin (IRDC 1975a, 1975b).

OctaBDE and pentaBDE were non-sensitizing in maximization tests in guinea pigs (Microbiological Associates Inc. 1996). The induction doses consisted of three pairs of interscapular region intradermal injections of (1) a 50:50 solution of Freund's adjuvant and corn oil, (2) 2.5% octaBDE or 5% pentaBDE solutions in corn oil, and (3) 2.5% octaBDE or 5% pentaBDE in the 50:50 corn oil/Freund's adjuvant solution. Control groups received the same regimen without PBDEs. After 7 days, the PBDE-treated animals received topical applications of neat octaBDE or pentaBDE on the previously treated interscapular sites. Two weeks later, the animals were challenged with topical doses of neat octaBDE or pentaBDE on the left flank. Subsequent examination of the test sites at 24, 48, 72, 96, or 120 hours after the challenge dose showed no erythema or edema responses in any of the animals, indicating that the PBDEs did not cause delayed contact hypersensitivity.

A 10% chloroform solution of 77.4% decaBDE (containing 21.8% nonaBDE and 0.8% octaBDE) did not induce bromacne when applied to the ear of rabbits for 30 days (Norris et al. 1975a). A slight erythematous response and slight exfoliation were the only observed effects. No additional information was reported on the design and results of this acnegenesis study.

Ocular Effects. No studies were located regarding ocular effects in animals after dermal exposure to PBDEs.

Ocular effects were investigated in rats that had 100 mg decaBDE (solid), 100 mg octaBDE (solid), or 0.1 mL pentaBDE (viscous liquid) instilled into the conjunctival sac (IRCD 1974, 1975a, 1975b). The eyes were examined for irritation after 24, 48, and 72 hours and 7 days and corneal injury after 72 hours. There were no exposure-related effects with decaBDE or octaBDE, although pentaBDE caused slight evidence of corneal damage in one of six rats (IRDC 1975b).

Body Weight Effects. No studies were located regarding body weight effects in humans after dermal exposure to PBDEs.

There were no adverse effects on body weight in rabbits that were observed for 14 days following a single ≤2,000 mg/kg dermal dose of decaBDE, octaBDE, or pentaBDE (IRDC 1974, 1975a, 1975b). The PBDEs were applied to clipped intact skin, covered with an occlusive barrier, and washed from the treatment site after 24 hours.

No studies were located regarding the following effects in humans or animals after dermal exposure to PBDEs:

3.2.3.3 Immunological and Lymphoreticular Effects
3.2.3.4 Neurological Effects
3.2.3.5 Reproductive Effects
3.2.3.6 Developmental Effects
3.2.3.7 Cancer

3.3 GENOTOXICITY

Limited information exists regarding the *in vivo* genotoxicity of PBDEs. The frequency of micronuclei in peripheral lymphocytes was significantly higher in 23 Chinese workers who dismantled electronic waste (median PBDE serum concentrations 382 ng/g lipid weight) compared with 26 unexposed workers (median PBDE serum concentrations 158 ng/g lipid weight) (Yuan et al. 2008). Multivariate logistic regression analysis of several risk factors showed that only history of engaging in dismantling electronic waste was a significant predictor of micronuclei frequencies. The investigators also noted that pollutants other than PBDEs also may have played a role in the increased frequency of micronuclei. No evidence of oxidative DNA damage was found in peripheral lymphocytes from exposed workers. A study of 84 healthy Korean subjects from the general population reported that serum concentrations of BDE 47 and BDE 99 were not associated with telomere length in peripheral lymphocytes (Shin et al. 2010); actual concentrations of PBDEs were not provided. Telomers are complex structures consisting of repeat DNA sequences and associated proteins located at the end of chromosomes which protect chromosomes from end-to-end fusions.

Dosing of mice with up to 1,250 mg/kg/day pentaBDE (mixed congeners) by gavage once per day for 3 consecutive days did not increase the frequency of micronuclei in blood or bone marrow cells (Witt et al. 2008). In another gavage study, dosing of pregnant mice with ≥10 mg/kg/day decaBDE (the lowest dose

tested) on GDs 0–17 resulted in significant sperm chromatin DNA damage in male offspring examined at 71 days of age, as indicated by DNA denaturation induction and increased DNA fragmentation index in the sperm chromatin structure analysis (Tseng et al. 2013). The investigators suggested that hydrogen peroxide, which was increased in sperm cells, may have been involved in induction of oxidative DNA damage. A summary of *in vivo* genotoxicity studies of PBDEs is presented in Table 3-5.

Results from *in vitro* assays for gene mutation in various *Salmonella typhimurium* strains and in *Escherichia coli* WP2 *uvr*A conducted with 2,2',4,4',5-pentaBDE and decaBDE yielded negative results in the presence or absence of metabolic activation (Evandri et al. 2003; NTP 1986). Assays conducted in mammalian cells yielded mixed results. Tests of decaBDE (BDE 209) for sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells were negative and the same was reported for gene mutation in mouse lymphoma L5178Y cells (NTP 1986). These tests were conducted with and without metabolic activation. However, decaBDE induced DNA damage (Comet assay) in human SK^{-}N-MC neuroblastoma cells (Pellacani et al. 2012) and SW 480 colon carcinoma cells (Curcic et al. 2014). Several congeners (BDE 47, 99, 253, 183, and 209) induced micronuclei in human mammary carcinoma cells (Barber et al. 2006). Both BDE 32 and BDE 47 caused DNA damage (Comet assay) in HepG2 hepatocellular carcinoma cells (Saquib et al. 2016). The congener 2,2',4,4'-tetraBDE (BDE 47) also induced micronuclei formation and DNA damage (Comet assay) in human neuroblastoma cells (Gao et al. 2009; He et al. 2008a; Pellacani et al. 2012) and DNA damage (Comet assay) in rat primary hippocampal neurons (He et al. 2008b). However, BDE 47 did not cause DNA damage in normal human hepatocytes, as assessed by the Comet assay (An et al. 2011). BDE 47 also induced gene recombination in Chinese hamster SPD8/V79 cells, but not Sp5/V79 cells (Helleday et al. 1999). In the same study, 3,4-diBDE (BDE 12) and 2-monoBDE (BDE 1) produced positive results for gene recombination in both Chinese hamster SPD8/V79 cells and Sp5/V79 cells (Helleday et al. 1999). Experiments conducted by Ji et al. (2011) in chicken DT40 cell lines showed that BDE 47 and 2,2,4',5-tetraBDE (BDE 49) could induce DNA damage (double strand breaks; identified by γ-H2AX focus formation) and that BDE 47 could also induce chromosomal aberration. Ji et al. (2011) also reported that tetraBDEs had a greater genotoxic potential than PBDEs with a higher number of bromine substitutes and that hydroxylated analogs of tetraBDEs were more genotoxic than tetraBDEs. These investigators suggested that DNA damage caused by tetraBDEs and hydroxylated analogs is mediated through ROS, which leads to replication blockage and subsequent chromosomal breaks. However, Song et al. (2009) did not find evidence of DNA damage (Comet assay) in human adreno cortical carcinoma cells exposed to the hydroxylated metabolite OH-BDE-47 or OH-BDE-85. DNA damage by PBDEs could be mediated via covalent binding of PBDE quinone metabolites, as both Lai et al. (2011) and Huang et al. (2015) have

Table 3-5. Genotoxicity of Polybrominated Diphenyl Ethers (PBDEs) *In Vivo*

Species (test system)	Compound	End point	Results	Reference
Human peripheral lymphocytes	Total PBDEs	Micronucleous assay	±	Yuan et al. 2008
Human peripheral lymphocytes	Total PBDEs	Oxidative DNA damage	−	Yuan et al. 2008
Human peripheral lymphocytes	BDE 47	Telomere length	−	Shin et al. 2010
Human peripheral lymphocytes	BDE 99	Telomere length	−	Shin et al. 2010
Mouse blood and bone marrow	PentaBDE (unspecified)	Micronucleous assay	−	Witt et al. 2008
Mouse sperm	BDE 209	DNA damage (sperm chromatin structure analysis)	+	Tseng et al. 2013

+ = positive result; – = negative result; ± = inconclusive result; BDE = brominated diphenyl ether

reported that PBDE quinone metabolites covalently bind to DNA *in vitro* to form DNA adducts. These metabolites would result from oxidation of hydroxylated metabolites to dihydroxylated metabolites by microsomal cytochrome P450s. A summary of the *in vitro* genotoxicity data for PBDEs is presented in Table 3-6.

Overall, the information available from *in vivo* and *in vitro* studies with a few PBDE congeners is insufficient to make generalizations regarding the genotoxicity of PBDEs.

3.4 TOXICOKINETICS

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

No studies were located regarding absorption of PBDEs in humans after inhalation exposure.

Evidence for the inhalation absorption of lower-brominated PBDEs in animals was provided by observations of systemic toxicity in rats that were intermittently exposed to a commercial octaBDE product (bromine content 78.7%) as dust aerosol for 13 weeks (Great Lakes Chemical Corporation 2000). The absorption of the lower-brominated BDE congeners was indicated by the occurrence of hepatic, thyroid, and ovarian effects in rats following exposure to 16 or 202 mg/m^3 for 6 hours/day, 5 days/week, for 13 weeks.

No studies were located that quantified absorption of inhaled PBDEs, but Staskal et al. (2005) reported absorption efficiencies of 91% in mice given single intratracheal doses of ^{14}C-BDE 47 in corn oil. This estimate was derived by comparing the radioactivity profiles in urine, feces, and tissues 5 days after intratracheal or intravenous administration of 1 mg/kg doses of ^{14}C-BDE 47.

3.4.1.2 Oral Exposure

Human Data. No information was located regarding absorption of PBDEs in humans following controlled oral exposure.

Table 3-6. Genotoxicity of Polybrominated Diphenyl Ethers (PBDEs) *In Vitro*

Species (test system)	Compound	End point	Results		Reference
			With activation	Without activation	
Prokaryotic organisms:					
Salmonella typhimurium, TA98, TA100, TA1535, TA1537	BDE 209	Gene mutation	–	–	NTP 1986
S. typhimurium, TA98, TA100	BDE 99	Gene mutation	–	–	Evandri et al. 2003
Escherichia coli, WP2 uvrA	BDE 99	Gene mutation	–	–	Evandri et al. 2003
Mammalian cells:					
Human L02 normal hepatocytes	BDE 47	DNA damage (Comet assay)	No data	–	An et al. 2011
Human MCF-7 mammary carcinoma cells	BDE 47, 99, 153, 183, 209	Micronuclei	No data	+	Barber et al. 2006
Human SW 480 colon carcinoma cells	BDE 209	DNA damage (Comet assay)	No data	+	Curcic et al. 2014
Human SH-SY5Y neuroblastoma cells	BDE 47	DNA damage (Comet assay)	No data	+	Gao et al. 2009
Human SH-SY5Y neuroblastoma cells	BDE 47	Micronuclei	No data	+	He et al. 2008a
Human SH-SY5Y neuroblastoma cells	BDE 47	DNA damage (Comet assay)	No data	+	He et al. 2008a
Human SK-N-MC neuroblastoma cells	BDE 47	DNA damage (Comet assay)	No data	+	Pellacani et al. 2012
Human SK-N-MC neuroblastoma cells	BDE 209	DNA damage (Comet assay)	No data	+	Pellacani et al. 2012
Human HepG2 hepatocellular carcinoma cells	BDE 32, 47	DNA damage (Comet assay)	No data	+	Saquib et al. 2016
Human H295R adrenocortical carcinoma cells	OH-BDE-47 OH-BDE-85	DNA damage (Comet assay)	NA	–	Song et al. 2009
Rat primary hippocampal neurons	BDE 47	DNA damage (Comet assay)	No data	+	He et al. 2008b
Mouse lymphoma L5178Y cells	BDE 209	Gene mutation	–	–	NTP 1986
Chinese hamster Sp5/V79 cells	BDE 47	Gene recombination	No data	–	Helleday et al. 1999
Chinese hamster SPD8/V79 cells	BDE 47	Gene recombination	No data	+	Helleday et al. 1999
Chinese hamster Sp5/V79 cells	BDE 12	Gene recombination	No data	+	Helleday et al. 1999

Table 3-6. Genotoxicity of Polybrominated Diphenyl Ethers (PBDEs) *In Vitro*

Species (test system)	Compound	End point	Results		Reference
			With activation	Without activation	
Chinese hamster SPD8/V79 cells	BDE 12	Gene recombination	No data	+	Helleday et al. 1999
Chinese hamster Sp5/V79 cells	BDE 1	Gene recombination	No data	+	Helleday et al. 1999
Chinese hamster SPD8/V79 cells	BDE 1	Gene recombination	No data	+	Helleday et al. 1999
Chinese hamster ovary cells	BDE 209	Sister chromatid exchange	–	–	NTP 1986
Chinese hamster ovary cells	BDE 209	Chromosomal aberrations	–	–	NTP 1986
Other cells					
Chicken DT40 cell line	BDE 47	DNA damage (γ-H2AX focus formation)	No data	+	Ji et al. 2011
	BDE 49	DNA damage (γ-H2AX focus formation)	No data	+	Ji et al. 2011
	BDE 47	Chromosomal aberrations	No data	+	Ji et al. 2011

+ = positive result; — = negative result; BDE = brominated diphenyl ether; DNA = deoxyribonucleic acid; NA = not applicable

Animal Data

Overview: Information regarding oral absorption in animals is available from studies of commercial PBDE mixtures and individual ^{14}C-labeled tetra-, penta-, hexa-, and decaBDE congeners. As summarized below, the most recent and best available estimates of oral absorption efficiencies following gavage administration in lipophilic vehicles indicate a range of 70–85% for tetra- (BDE 47), penta- (BDE 99, BDE 100), and hexa- (BDE 153, BDE 154) congeners, and 10–26% for decaBDE (BDE 209).

Information on oral absorption of the commercial pentaBDE mixture DE-71 and the commercial octaBDE mixture DE-79 is available from studies in which male Sprague-Dawley rats were fed diets containing 0 or approximately 32–33 ng/day (≈120 ng/kg/day) of either mixture in peanut oil for 21 days (Hakk et al. 2001; Huwe et al. 2002b, 2007). The doses were designed to mimic environmental exposure concentrations. Liver, carcass, and feces were analyzed for major congeners in the penta- and octaBDE formulations 24 hours after the final feeding; urine was not evaluated. The study of the pentaBDE mixture (DE-71) assessed the following six congeners: BDE 47, BDE 85, BDE 99, BDE 100, BDE 153, and BDE 154 (Hakk et al. 2001; Huwe et al. 2007). Based on liver, carcass, and unrecovered concentrations of congeners, and assuming that excretion in the urine was negligible, absorption is estimated to have been 44.3% for penta congener BDE 85 and 84.3–92.4% for the other tetra- to hexaBDE congeners. The study of the octaBDE mixture (DE-79) assessed the following eight congeners: BDE 153, BDE 154, BDE 183, BDE 190, an unknown heptaBDE, and three unknown octaBDEs (Huwe et al. 2002b). Based on liver, carcass, and unrecovered concentrations of congeners, and assuming that excretion in the urine was negligible, absorption is estimated to have been 84.2–95.1% for the hexaBDEs, 68.5–79.1% for the heptaBDEs, and 55.7–83.3% for the octaBDEs.

Early studies with ^{14}C-decaBDE (BDE 209) indicated that gastrointestinal absorption efficiency was low (~9 or <1%) in rats (El Dareer et al. 1987; Norris et al. 1973, 1975b; NTP 1986). Following treatment with a single 1 mg/kg dose of ^{14}C-decaBDE in corn oil, administered as a low purity commercial mixture (77.4% decaBDE, 21.8% nonaBDE, 0.8% octaBDE) by gavage, 90.6 and >99% of the dose was eliminated in the feces within 24 and 72 hours post-dosing, respectively (Norris et al. 1973, 1975a). An oral absorption efficiency estimate of about 9% is indicated, assuming that fecal radioactivity excreted in 24 hours was nonabsorbed, and fecal radioactivity excreted between 24 and 72 hours was from biliary excretion of absorbed material. Two feeding studies were conducted in which rats were exposed to a commercial mixture as unlabeled decaBDE (92% pure) on days 1–7 and ^{14}C-decaBDE (98.9% pure) on day 8, followed by unlabelled decaBDE on days 9, 9–10, or 9–11 (El Dareer et al. 1987; NTP 1986). In

the first study, dietary concentrations ranged from 238 to 51,100 ppm (six levels) (≈20–4,500 mg/kg/day). Recovery of radioactivity in the feces ranged from 91.3±4.0 to 101±4% of the administered dose and was not related to dose level. In the second study, rats were exposed to dietary concentrations of 277 or 48,000 ppm (≈20 or 4,300 mg/kg/day). Recovery of radioactivity in the feces ranged from 82.5±4.7 to 86.4±8.5% of the dose and was not related to dose level or time of sacrifice (24, 48, or 72 hours after ^{14}C-decaBDE intake). For both dose levels, the percent of ^{14}C dose remaining in the gut contents (<4%) and gut tissue (<0.04%) decreased with time. Of the radioactivity recovered, >99% was in the feces and gut contents. Based on a comparison of average tissue concentrations following intravenous and oral administration, NTP (1986) estimated that oral absorption was 0.33±0.19% at the highest dietary level (50,000 ppm).

More recent studies indicated that absorption efficiency of ^{14}C-decaBDE (BDE 209) in rats can be influenced by vehicle and have reported absorption efficiencies in the 10–26% range using lipophilic vehicles (Hakk et al. 2002b; Morck and Klasson Wehler 2001; Morck et al. 2003; Riu et al. 2008; Sandholm et al. 2003). In normal and bile duct-cannulated male Sprague-Dawley rats administered single 3-µmol/kg (≈3 mg/kg) doses of ^{14}C-decaBDE (>98% pure) in Lutrol F127/soya phospholipone (34:16, w/w)/water, radioactivity in feces collected for 72 hours accounted for about 90% of the administered dose in conventional rats (Morck and Klasson Wehler 2001; Morck et al. 2003). In bile duct-cannulated rats, averages of 88 and 9.5% of the dose were recovered in feces and bile, respectively, within 3 days. Radioactivity recovered in urine was <0.1% of the dose in normal and bile-duct cannulated rats. The radioactivity in bile indicates that at least 10% of the dose was absorbed (Morck and Klasson Wehler 2001; Morck et al. 2003). Hakk et al. (2002b) reported similar results in another study in which four bile duct-cannulated male Sprague-Dawley rats were orally administered single 3-µmol/kg doses of ^{14}C-decaBDE (>98% pure) in Lutrol 127, soyaphospholipone, and water. Radioactivity in bile and urine collected for 72 hours accounted for 9.2 and <0.1% of the administered dose, respectively. Sandholm et al. (2003) reported an oral bioavailability of 26% for decaBDE from plasma concentration-time curves for 144 hours following gavage and intravenous administration of single 2-µmol/kg doses of unlabeled decaBDE (purity >98%) in a DMA/polyethylene glycol/water vehicle (4:4:1) to Sprague-Dawley rats. DecaBDE concentrations in plasma samples were quantified by gas chromatography/mass spectrometry (GC/MS). Oral bioavailability was calculated by dividing the area under the plasma concentration-time curve for oral exposure (AUC_{oral}) by the $AUC_{i.v.}$. Qualitative analysis by GC/MS of pooled plasma samples determined 3 major metabolites among 13 hydroxylated metabolites: a hydoxy-octaDDE, a hydroxyl-nonaBDE and a hydoxy-methoxy-hexaBDE. The presence of these phenolic metabolites in the plasma samples indicates that the oral bioavailability (and hence oral absorption efficiency) of decaBDE

may have been higher than the calculated value of 26% based on parent-compound plasma concentration-time curves (Sandholm et al. 2003). Riu et al. (2008) reported that >19% of administered radioactivity was recovered in tissues (including fetuses) and carcasses, 24 hours after oral administration of 2-mg/kg doses of ^{14}C-labeled decaBDE (>99.8% radiopurity) dissolved in peanut oil to pregnant Wistar rats on GDs 16, 17, 18, and 19. This finding indicates an absorption efficiency of about 20%, assuming that radioactivity recovered in the feces (about 66% of the administered dose) was not absorbed. Because biliary excretion of decaBDE has been demonstrated in rats, this value may underestimate the actual oral absorption that occurred in this study. However, the aqueous fraction (which would contain hydroxylated metabolites and conjugates) accounted for only 4% of the radioactivity in the collected feces, and 97% of the radioactivity in the organic-solvent fraction of the feces was determined by high-performance liquid chromatography (HPLC) to be unchanged decaBDE.

Estimates of oral absorption efficiencies for BDE 47 in rats and mice have ranged from about 75 to 95%, depending on species and employed measurement techniques (Örn and Klasson-Wehler 1998; Sanders et al. 2006a; Staskal et al. 2005). A single 14.5-mg/kg (30-µmol/kg) gavage dose of ^{14}C-BDE 47 in corn oil was well absorbed by male Sprague-Dawley rats and male C57Bl mice (Örn and Klasson-Wehler 1998). Approximately 5% of the dose in rats and 7% of the dose in mice was excreted as parent congener in the feces in 24 hours. The investigators concluded that these values represented the non-absorbed doses, indicating that absorption of BDE 47 was 93–95%. Later studies with male F344 rats and male B6C3F1 mice reported oral absorption efficiency estimates for BDE 47 of 75% in rats and 85% in mice (Sanders et al. 2006a). Sanders et al. (2006a) used a more refined technique that compared profiles of radioactivity in urine, feces, and tissues 24 hours after gavage and intravenous administration of single 1-µmol/kg doses of ^{14}C-BDE 47 in corn oil. Using a similar technique comparing radioactivity profiles in excreta and tissues 5 days after gavage and intravenous administration of single 1-mg/kg doses of ^{14}C-BDE 47 in corn oil, Staskal et al. (2005) reported that oral absorption efficiency was approximately 82% in female C57BL/6J mice.

Evidence for extensive oral absorption of pentaBDE congeners (BDE 99 and BDE 100) in rats and mice comes from studies that administered single oral doses of about ~28 µmol/kg ^{14}C-BDE 100 (~98% pure) in peanut oil to male Sprague-Dawley rats (Hakk et al. 2006), about 2.2 mg/rat (~15 µmol/kg) of ^{14}C-BDE 99 (~98% pure) in corn oil to male Sprague-Dawley rats (Hakk et al. 2002a), or 1 µmol/kg body weight of ^{14}C-BDE 99 (~96% pure) in corn oil to male F344 rats and male B6C3F1 mice (Chen et al. 2006). Seventy-two hours after administration of BDE 100, about 73 and 41% of the administered radioactivity remained in tissues of conventional and bile-duct cannulated rats, respectively; fecal

radioactivity accounted for about 20 and 26% of administered radioactivity in conventional and bile-duct cannulated rats, respectively (Hakk et al. 2006). Assuming that radioactivity excreted in feces within 24 hours was non-absorbed, reported values of 11.5% in conventional rats and 16.8% in bile duct-cannulated rats indicate oral absorption for BDE 100 of about 89 and 84%, respectively. In the studies with BDE 99, radioactivity in 24-hour feces accounted for 22.3 and 52.5% of administered radioactivity in conventional and bile-cannulated rats, respectively, indicating absorption efficiencies for BDE 99 of at least 78 and 48% (Hakk et al. 2002a). Chen et al. (2006) compared radioactivity profiles in 24-hour excreta and tissues after gavage and intravenous administration of ^{14}C-BDE 99 to arrive at estimated oral absorption efficiencies of approximately 85% in both male rats and male mice.

Results from rat and mouse studies with ^{14}C-labeled hexaBDEs (BDE 153 and BDE 154) administered as single doses in oil vehicles also indicate extensive oral absorption (Hakk et al. 2009; Sanders et al. 2006b). Hakk et al. (2009) estimated an oral absorption efficiency for BDE 154 of about 77% in male Sprague-Dawley rats, based on radioactivity profiles in 72-hour excreta and tissues after gavage administration of single 11.3-µmol/kg ^{14}C-BDE 154 (>98% pure) to conventional and bile duct-cannulated rats. Sanders et al. (2006b) estimated oral absorption efficiencies for BDE 153 of about 70% in male F344 rats and male B6C3F1 mice, based on comparison of radioactivity profiles in 24-hour excreta and tissues after gavage and intravenous administration of single 1-µmol/kg doses ^{14}C-BDE 153 (96% pure).

3.4.1.3 Dermal Exposure

No information was located regarding dermal absorption of PBDEs in humans.

A dermal absorption efficiency of 62% was reported for female mice exposed to an occluded dermal dose of 1 mg/kg ^{14}C-BDE 47) (Staskal et al. 2005). Profiles of radioactivity in urine, feces, and tissues 5 days after dermal or intravenous administration were compared to estimate dermal absorption efficiency. Approximately 15% of the administered dose remained at the site of application 5 days after application. No other *in vivo* studies of dermal absorption with PBDE congeners or mixtures were located.

In *in vitro* studies with human breast skin and mouse dorsal skin samples exposed to ^{14}C-BDE 147 (radiochemical purity=96.5%) for 24 hours in a flow-through diffusion cell apparatus, reported mean absorption efficiencies (percent of applied radioactivity collected in receptor compartment) were 1.88% for human skin (n=10) and 14.58% for rat skin (n=12) (Roper et al. 2006).

Limited dermal absorption of ^{14}C-decaBDE by mouse skin was indicated in an *in vitro* study in which ^{14}C-decaBDE dissolved in tetrahydrofuran was applied (three dose levels) to dorsal skin excised from adult hairless female mice, and fractions of receptor fluid were collected over a 24-hour period (Hughes et al. 2001). Transfer of radioactivity to the receptor fluid was minimal, accounting for only 0.07–0.34% of the applied radioactivity. Two to 20% of the radioactivity was found in the skin. The highest percentage of the dose in the skin was associated with application of the lowest dose. Washing the skin with solvent 24 hours after application removed 77–92% of the applied dose.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No information was located regarding distribution of PBDEs in humans following controlled inhalation exposure.

The distribution of bromine was examined in tissues of rats after inhalation exposure to octaBDE (Great Lakes Chemical Corporation 1978). Groups of rats were exposed to 0, 1.2, 12, 120, or 1,200 mg/m^3 of dusts of octaBDE 8 hours/day for 14 days. At necropsy, sections of the lungs, adipose tissue, and liver were collected for bromine analysis using a neutron activation technique. The results showed concentrations of bromine in the lungs and adipose tissue significantly higher in all groups relative to controls; the amounts of bromine detected were concentration-related. In the liver, the concentration of bromine was also elevated in all groups relative to controls except in the 1.2 mg/m^3 exposure group; the elevated concentrations in the liver were not as marked as in the lungs or in adipose tissue.

3.4.2.2 Oral Exposure

Human Studies.

Overview: No studies were located that examined tissue distribution of PBDEs in humans following controlled oral exposure. Evidence for the transfer of PBDEs from pregnant mothers to the developing fetus and for the transfer of PBDEs from maternal blood to breast milk and then to nursing infants comes from a number of studies of PBDE concentrations in maternal and cord serum samples and breast milk samples from groups of non-occupationally exposed women. Although the contributions of different possible exposure routes experienced by these women are uncertain, it is thought that ingestion of PBDEs

in dust and food represented a significant exposure route. In general, the tetra- and penta-brominated PBDEs have been the predominant congeners detected in maternal and cord serum samples and breast milk samples, but some recent studies assaying for a wider range of PBDE congeners have found evidence for distribution of hepta-, octa-, or decaBDEs into cord serum and breast milk. In evaluation of these studies, the location where the study was performed (e.g., Asia, Europe or North America) is very important, as the patterns of exposure to various congeners were different due to different usage patterns of commercial mixtures (see Section 6.5, General Population and Occupational Exposure, for more information).

A number of studies have examined concentrations of PBDEs or hydroxylated PBDEs in maternal and cord blood samples in non-occupationally exposed groups of women from the United States (Chen et al. 2013; Mazdai et al. 2003; Qiu et al. 2009), France (Antignac et al. 2009, 2008), China (Li et al. 2013a), Japan (Kawashiro et al. 2008), Spain (Vizcaino et al. 2011), the Netherlands (Meijer et al. 2008), and Korea (Wan et al. 2010). In the most recent of the U.S. studies, BDE 28, BDE 47, BDE 99, BDE 100, and BDE 153 were detected in 90, 90, 95, 85, and 100% of 20 maternal and 65, 65, 80, 90, and 85% of 20 matched cord serum samples, respectively (Chen et al. 2013). Percentages of samples above the limit of detection were lower for BDE 209 (55% maternal and 40% cord) and BDE 154 (25% maternal and 5% cord). Chemical analyses for other PBDE congeners were not conducted in this study. Median concentrations of congeners in maternal serum samples (ng/g lipid) were in the following order: BDE 47 > BDE 153 > BDE 209 > BDE 99 > BDE 100 > BDE 28 > BDE 154. In cord serum samples, the order was: BDE 47 > BDE 99 > BDE 209 > BDE 28 > BDE 153 > BDE 100 > BDE 154. Concentrations of four hydoxylated PBDEs (6-OH-BDE-47, 5-OH-BDE-47, 4'-OH-BDE-49 and 6'-OH-BDE-99) were also determined. Geometric mean concentrations (ng/g lipid) of total BDEs and total OH-BDEs in cord samples were higher than those in maternal serum samples (~52% and 42 higher for OH-BDEs and BDEs, respectively). Equal or higher concentrations of total OH-BDEs or total BDEs in cord serum, compared with maternal serum, were found in 85 and 80% of the matched mother-neonate pairs. The results suggest that each of the seven BDEs and four OH-BDEs can cross the placenta and distribute to the fetus, and that internal exposure via blood is equal to or higher in fetuses, compared with their mothers. In contrast, BDE 209 was the most abundant congener detected in both maternal and cord blood samples from China, where industrial production of BDE 209 may result in exposure (Li et al. 2013a).

Similar evidence for transplacental transfer of a set of PBDE congeners more enriched in higher-brominated PBDEs was reported in a recent study that measured concentrations of 19 PBDE congeners in maternal and cord serum samples from 29 mother-neonate pairs from a Wenzhou region of China that is

the major region for electronics dismantling and recycling in China (Li et al. 2013a). In both maternal and cord serum samples, the congeners with the highest geometric mean concentrations (ranging from 3.32 to 1.78 ng/g lipid in maternal samples) were BDE 207 > BDE 208 > BDE 209 > BDE 28. Geometric means for total cord serum concentrations of lower-brominated congeners (BDE 17 to BDE 154), higher-brominated congeners (BDE 183 to BDE 209), and all BDEs were higher than respective concentrations in maternal blood by about 15, 47, and 41%, respectively (Li et al. 2013a).

Frederiksen et al. (2010) utilized a human *ex vivo* placenta perfusion system to study the kinetics of placental transfer of BDE 47, BDE 99, and BDE 209 to the fetus. Placentas were perfused for 4 hours with 1 ng/mL concentrations of the non-labeled congeners. Samples of maternal and fetal compartments were taken during the perfusion, and concentrations in samples were determined by GC/MS. Placental transfer of BDE 47 and BDE 99 was demonstrated, and transfer of BDE 47 was faster and more extensive than BDE 99. Transfer of BDE 209 across the placenta to the fetal compartment was not demonstrated with the detection limits of the techniques employed. Frederiksen et al. (2010) proposed that future perfusion studies with BDE 209 should use ^{14}C-labeled BDE 209 to increase sensitivity.

Evidence for the transfer of PBDEs from maternal blood to breast milk and hence to nursing infants comes from a number of studies examining PBDE concentrations in breast milk samples. Most studies of PBDEs in breast milk samples through 2002 measured concentrations of only lower-brominated congeners (tetra, penta and hexaBDEs) used in many commercial products up to that time: BDE 47, BDE 99, BDE 100, BDE 153, and BDE 154 (Hites 2004). Additionally, higher brominated PBDEs (e.g., decaBDE; BDE 209) were often omitted from early human biomonitoring due to the inability to accurately measure them at that time. In these early studies, BDE 47 was the congener detected at the highest concentration. A number of more recent studies have included chemical analysis for a greater number of PBDE congeners (including hepta-, octa- and deca-brominated congeners) in breast milk samples collected in the United States (Daniels et al. 2010; Park et al. 2011; Schecter et al. 2010, 2006), France (Antignac et al. 2009, 2008), and Philippines (Malarvannan et al. 2013). The detection of higher-brominated congeners in some of these recent studies indicates that both lower and higher-brominated congeners can be distributed to breast milk (Antignac et al. 2008, 2009; Malarvannan et al. 2013; Park et al. 2011; Schecter et al. 2010).

Further support for the transfer of PBDEs from mothers to nursing children comes from a report that average concentrations of BDE 47 and BDE 99 were statistically significantly ($p < 0.05$) increased by ~5-fold in serum samples from 4-year-old Spanish children (n=202) who had been breastfed, compared

with formula-fed 4-year-old children (n=42) (Carrizzo et al. 2007). This study analyzed serum samples for 13 PBDE congeners including tri- (BDE 17, BDE 28), tetra- (BDE 47, BDE 66, BDE 71), penta- (BDE 85, BDE 99, BDE 100), hexa- (BDE 138, BDE 153, BDE 154), and hepta-brominated congeners (BDE 183, BDE 190). BDE 47 and BDE 99 were the predominant congeners detected in both breastfed and formula-fed children; concentrations of BDE 47 and BDE 99 in breastfed children were about 16 and 6 times higher than the congener with the next highest concentration, BDE 100. The following congeners were not detected in the collected serum samples: BDE 17, BDE 28, BDE 66, BDE 71, BDE 85, BDE 183, and BDE 190. This study did not control for potential differences in other PBDE exposure pathways, such as ingestion of contaminated dusts or food.

Animal Studies.

Overview: Tissue distribution studies in animals orally exposed to ^{14}C-labeled BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 209 indicate that decaBDE is distributed among tissues somewhat differently than tetra-, penta- and hexaBDEs. While lower-brominated BDE congeners, following absorption and an initial wide distribution, are preferentially accumulated in adipose tissues, absorbed decaBDE is less readily distributed to adipose tissues and appears to preferentially distribute to highly perfused tissues. Although less likely to partition to adipose tissues, decaBDE was still found in low quantities in adipose tissues in these studies, and has been shown to transfer from dams to fetuses and neonates from exposure during gestational and nursing periods. Two studies with female Sprague-Dawley rats given oral doses of nonlabeled BDE 209 in corn oil from GD 7 to postpartum day (PPD) 4 or 8 demonstrated maternal transfer to developing fetuses and neonates by examining whole-body BDE 209 concentrations in offspring, but another study with similarly exposed Sprague-Dawley rats found no clear evidence for maternal transfer by examining BDE 209 concentrations in blood from dams and offspring.

Results from studies of rats or mice orally exposed to ^{14}C-labeled decaBDE (BDE 209) indicate that decaBDE is not as readily distributed to adipose tissues as the lower-brominated BDEs and more readily distributed to highly perfused tissues (El Dareer et al. 1987; Morck and Klasson-Wehler 2001; Morck et al. 2003; Norris et al. 1975a; NTP 1986; Riu et al. 2008). In male rats administered single gavage doses of 1 mg/kg of a ^{14}C-labeled commercial decaBDE mixture (77.4% pure containing 21.8% nonaBDE and 0.8% octaBDE), radioactivity could be detected on day 1 in all sampled tissues (adipose, skin, liver, heart, adrenals, spleen, pancreas) (Norris et al. 1975a). On day 16 after dosing, radioactivity was only detected in adrenals and spleen (0.01 and 0.06% of the administered dose per gram of tissue, respectively). In F344 rats fed diets containing a commercial mixture as unlabeled decaBDE (92% pure) on days 1–7,

^{14}C-decaBDE (98.9% pure) on day 8, and unlabelled decaBDE on days 9, or 9–10, or 9–11, the levels of radioactivity remaining in tissues 72 hours after the exposure period ended had the following order: gastrointestinal tract > liver > kidney > lung > skin > adipose (El Dareer et al. 1987; NTP 1986). In Sprague-Dawley rats given single 3-µmol/kg (≈3 mg/kg) gavage doses of ^{14}C-decaBDE (>98% pure) in Lutrol F127/soya phospholipone (34:16, w/w)/water, concentrations of radioactivity remaining in tissues 3 days after dose administration had the following order (^{14}C nmol equivalents/g lipid concentrations in parentheses): plasma (22 nmol/g) > liver (14.9 nmol/g) > heart ~ small intestine wall ~ adrenal (ranging from 5.6 to 4.0 nmol/g) > lung ~thymus ~ kidney (ranging from 2.1 to 1.8 nmol/g) > adipose (0.17 nmol/g) (Morck and Klasson-Wehler 2001; Morck et al. 2003). In pregnant Wistar rats given single 2-mg/kg doses of ^{14}C-decaBDE (>99.8% pure) in peanut oil daily on GDs 16–19, concentrations of radioactivity on a wet weight basis (µg ^{14}C-decaBDE equivalents/g tissue) were highest in adrenals (33 µg/g), ovaries (16 µg/g), and liver (11 µg/g); intermediate in kidneys > stomach > heart ~ placentas > lung > spleen > plasma > uterus > carcass (ranging from 3.90 to 1.11 µg/g); and lowest in adipose tissue (0.79 µg/g), fetuses (0.46 µg/g), brain (0.11 µg/g), and amniotic fluid (0.11 µg/g) (Riu et al. 2008).

Results from studies of rats and mice exposed to oral doses of ^{14}C-labeled BDE 47 indicate wide distribution to tissues following absorption with preferential accumulation in fatty tissues, contrasting the preferential distribution of decaBDE to highly perfused tissues (Örn and Klasson-Wehler 1998; Sanders et al. 2006a; Staskal et al. 2005, 2006a). Five days after gavage administration of single 30-µmol/kg doses of ^{14}C-BDE 47 in corn oil to male Sprague-Dawley rats, concentrations of radioactivity had the following order (^{14}C-BDE 47 equivalent nmol/g wet tissue): adipose (706) > lung (12) > kidney (11) > liver (5) > brain (1.9) > plasma (0.76) (Örn and Klasson-Wehler 1998). A similar order of wet tissue concentrations (nmol/g wet tissue) was observed in male C57Bl mice exposed to ^{14}C-BDE 47: adipose (79) > liver (7) > lung (5) > kidney (3) > brain (1) > plasma (not detected) (Örn and Klasson-Wehler 1998). In another study that gave single 1-µmol/kg doses of ^{14}C-BDE 47 to male and female F344 rats in corn oil, the following order of amounts of radioactivity remaining in tissues was observed 24 hours after dose administration (percentages of administered dose for male rats): adipose (24.6%) > skin (13.0%) > muscle (3.0%) > liver (1.3%) > blood (0.2%) > brain ~ kidney ~ lung (0.1% each) (Sanders et al. 2006a). A similar order was observed in female rats, but the amount of radioactivity in adipose in females (37%) was higher than in male rats (24.6%) (Sanders et al. 2006a). Tissue distribution in male and female B6C3F1 mice exposed to ^{14}C-BDE 47 was similar to distribution in F344 rats, with females showing higher accumulation of radioactivity in adipose than male mice (Sanders et al. 2006a). Staskal et al. (2005) reported similar patterns for tissue distribution in female C57BL/6J mice 5 days after administration of single oral doses of 0.1, 1.0, 10, or 100 mg/kg ^{14}C-BDE 47 in corn oil. For all doses,

the reported order of remaining radioactivity in tissues was: adipose (8–14% of administered dose) > skin and muscle (~2–3%) > liver (~1%) > brain ~ kidneys ~ lungs (<0.05% each) (Staskal et al. 2005). The same tissue distribution pattern was also observed in female C57BL/6J mice given nine 1-mg/kg/day doses of unlabeled BDE 47 in corn oil followed by single 1-mg/kg doses of ^{14}C-BDE 47 (Staskal et al. 2006a). Using percent of dose/g of tissue as the dose metric to compare single-dose levels in rats reported by Staskal et al. (2005) with repeated-dose levels in tissues, the tissue concentrations from the repeated-dose scenario with 1 mg/kg were comparable to tissue concentrations from single 10-mg/kg doses and about 2 times greater than concentrations from single 1-mg/kg doses (Staskal et al. 2006a). The results indicate the potential for incremental accumulation of BDE 47 in fat with continued exposure.

Results from studies of ^{14}C-BDE 99 (Chen et al. 2006; Hakk et al. 2002a) and ^{14}C-BDE 100 (Hakk et al. 2006) indicate an initial wide distribution of penta-brominated congeners to tissues following absorption with preferential accumulation in fatty tissues, similar to results from studies with BDE 47. In male F344 rats given single doses of 0.1 µmol/kg ^{14}C-BDE 99 (~96% pure) in corn oil, levels of radioactivity remaining in tissues 24 hours after dose administration had the following order (percent of administered dose): adipose (20.8%) > skin (7.0%) > muscle (5.2%) > liver (2.1%) > blood = brain (0.3% each) > lung = brain (0.1% each) (Chen et al. 2006). Similar orders of tissue concentrations were seen 24 hours after oral administration in male rats given single 1, 10, 100, or 1,000 µmol/kg doses, and in female F344 rats and male and female B6C3F1 mice given single 1 µmol/kg doses (Chen et al. 2006). Preferential accumulation in adipose also was found in male Sprague-Dawley rats 24 hours after administration of 14.5 µmol/kg doses of ^{14}C-BDE 99 (>98% pure) in corn oil (Hakk et al. 2002a). In male Sprague-Dawley rats 72 hours after administration of single 25.2 µmol/kg doses of ^{14}C-BDE 100 (>95% pure) in peanut oil, the adipose, gastrointestinal tract, and skin had the highest concentrations of radioactivity (>35 nmol/g fresh tissue weight), liver and lung had intermediate concentrations (~10–15 nmol/g), and other tissues, including lung, adrenals, testes, and muscle, had the lowest concentrations (<10 nmol/g) (Hakk et al. 2006).

Preferential distribution and accumulation in adipose also has been found for hexa-brominated BDEs in studies with rats or mice exposed orally to ^{14}C-BDE 153 (Sanders et al. 2006b) and ^{14}C-BDE 154 (Hakk et al. 2009). In male F344 rats given single doses of 1 µmol/kg ^{14}C-BDE 153 (~96% pure) in corn oil, levels of radioactivity remaining in tissues 24 hours after dose administration had the following order of percent of administered dose: adipose (16.7%) > muscle (13.4%) > skin (8.3%) > liver (5.8%) > blood (0.9%) > kidney (0.4%) > lung = brain (0.2% each) (Sanders et al. 2006b). Similar orders of tissue concentrations were observed in similarly exposed female F344 rats and male and female B6C3F1 mice

(Sanders et al. 2006b). During repeated daily applications of 1-µmol/kg ^{14}C-BDE 153 doses to male rats, supralinear increases in concentrations of radioactivity (nmol ^{14}C equivalents/g wet tissue) were observed in adipose and skin (e.g., average concentrations in adipose after 1, 3, and 10 consecutive doses were 1.57, 9.21, and 30.38 nmol/g, respectively) (Sanders et al. 2006b). This observation suggests that adipose and skin serve as sinks for radioactivity initially distributed to other tissues. In male Sprague-Dawley rats sampled 72 hours after administration of single 12.3 µmol/kg doses of ^{14}C-BDE 154 (>95% pure) in peanut oil, levels of radioactivity remaining in tissues had the following order (percent of administered dose): carcass (24.3%) > gastrointestinal tract (3.77%) > adipose (1.82%) > liver (0.68) > thymus ~ testis ~ lung ~ adrenal ~ kidney ~ plasma ~ heart ~ spleen (≤0.1% each) (Hakk et al. 2009). Expression of the data on a concentration basis (nmol ^{14}C equivalents/g wet tissue weight) showed the highest concentrations in lipid rich tissues: adrenals (29.56 nmol/g), adipose (21.79 nmol/g), skin (7.55 nmol/g), gastrointestinal tract (4.58 nmol/g), and thymus (4.48 nmol/g). Concentrations in carcass, lung, and liver were intermediate (3.58, 2.26, and 1.80 nmol/g), and concentrations in testis, kidney, heart, spleen, and muscle ranged from 1.01 to 0.45 nmol/g. The concentration in plasma was very low (0.04 nmol/g) (Hakk et al. 2009).

Maternal transfer of decaBDE (BDE 209) has been demonstrated in female Sprague-Dawley rats given oral 5 µmol/kg/day doses of nonlabeled BDE 209 in peanut oil from GD 7 to PPD 4 (Cai et al. 2011). BDE 209 concentrations in blood of dams increased with duration of exposure: mean concentrations (standard error [SE]) were 358.17 (210.49) and 701.17 (63.43) µg/g lipid weight on GD 15 and PPD 4, respectively (Cai et al. 2011). Whole-body concentrations of BDE 209 in fetuses and neonates also increased with duration: mean BDE 209 concentrations (and SE) were 20.53 (7.9), 28.95 (3.57), and 45.04 (10.23) µg/g lipid on GD 15, GD 21, and PPD 4, respectively (Cai et al. 2011). Nona-brominated congeners (BDE 208, BDE 207, and BDE 206) were detected in dam blood samples and whole-bodies of fetuses and neonates at lower concentrations than BDE 209 and higher concentrations than octa-brominated BDEs (BDE 196, 197/204, 198/203). In a related study by the same group of investigators, female Sprague-Dawley rats were exposed to 5 µmol/kg/day doses of nonlabeled BDE 209 from GD 7 to PPD 8 and from PPD 1 to 8 (Zhang et al. 2011). Whole-body BDE 209 concentrations in pups exposed during gestation and lactation were about 2 times greater than concentrations of pup exposed only during lactation, suggesting that BDE 209 exposure and accumulation can occur during gestational and nursing periods (Zhang et al. 2011).

In another study with female Sprague-Dawley rats given 1, 10, 100, 300 or 1,000 mg/kg/day of nonlabeled BDE 209 in corn oil from GD 7 to PPD 4, measurements of BDE 209 concentrations in blood

samples of dams and offspring gave no clear evidence of maternal transfer, but whole-body concentrations in offspring were not measured (Biesemeier et al. 2010). BDE 209 concentrations in blood (reported as ng/mL blood) collected on GD 20 were generally higher in exposed dams than in exposed fetuses, but mean concentrations of dams and fetuses in the 100-, 300-, and 1,000-mg/kg groups did not increase with increasing dose. Mean BDE 209 concentrations in maternal or offspring blood collected on PPD 4 also did not increase with increasing dose levels, but mean BDE 209 concentrations in exposed groups of PPD 4 pups were mostly higher than mean concentrations in dams from the same exposed groups (Biesemeier et al. 2010).

Further evidence for different tissue distributions of decaBDE and lower-brominated congeners comes from a study in which neonatal NMRI mice were given single 0.7 mg/kg doses of ^{14}C-decaBDE or 0.8 mg/kg doses of ^{14}C-BDE 99 in a 20% fat emulsion to simulate milk on PND 3, 10, or 19 (Eriksson et al. 2002b; Viberg et al. 2003a). Neonatal mice exposed to ^{14}C-BDE 209 on PND 3, 10, or 19 had about 0.48, 0.40, and 0.06% of the total administered radioactivity in the brain, 24 hours after dose administration (Viberg et al. 2003a). Seven days after exposure, radioactivity in the brain had increased approximately 2-fold in mice exposed on PND 3 or 10 (to 0.74 and 1.05% of the administered dose), but remained the same as previously measured in mice exposed on PND 19 (0.06% of administered dose). Mice exposed to ^{14}C-BDE 99 on PND 3, 10, or 19 had about 0.37, 0.51, and 0.51% of the administered dose in the brain, 24 hours after dose administration; 7 days after dose administration, decreased levels in brains were seen in mice exposed on PNDs 3, 10, and 19 (about 0.18, 0.28, and 0.15% of the administered dose) (Eriksson et al. 2002b).

3.4.2.3 Dermal Exposure

No studies were located regarding distribution of PBDEs in humans or animals after controlled dermal exposure.

3.4.3 Metabolism

Oxidative hydroxylation of PBDEs is a principal metabolic transformation that occurs in humans and laboratory animals. Hydroxylated PBDEs have been identified in samples of human biological fluids, including blood (Athanasiadou et al. 2008; Hovander et al. 2002; Lacorte and Ikonomou 2009; Qiu et al. 2009; Rydén et al. 2012; Wan et al. 2009, 2010; Wang et al. 2012; Yu et al. 2010a) and breast milk (Lacorte and Ikonomou 2009). Hydroxylated PBDEs also have been identified in feces or bile of laboratory rodents exposed to ^{14}C-labeled tetra-, penta-, hexa- or decaBDEs, including:

- rats orally exposed to BDE 47 (Marsh et al. 2006; Orn and Klasson-Wehler 1998; Sanders et al. 2006a);
- female mice given single intravenous doses of BDE 47, BDE 99, BDE 100, or BDE 153 (Staskal et al. 2006b)
- conventional or bile-duct cannulated male rats given oral or intravenous doses of BDE 99 (Chen et al. 2006; Hakk et al. 2002a);
- conventional or bile-duct cannulated rats given oral doses of BDE 154 (Hakk et al. 2009); and
- conventional or bile-duct cannulated rats given oral doses of BDE 209 (Morck et al. 2003; Riu et al. 2008).

Hydroxylated metabolites have also been identified in feces and carcasses of male rats fed a commercial pentaBDE mixture (DE-71) for 21 days (Huwe et al. 2007), in plasma of rats given single intraperitoneal injections of an equimolar mixture of BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 183 (Malmberg et al. 2005), and in plasma of mice after oral and subcutaneous exposure to DE-71 for 34 days (Qiu et al. 2007). Oxidative hydroxylation of PBDEs also has been demonstrated and studied in *in vitro* metabolic systems with human liver microsomes or primary hepatocytes (Cheng et al. 2008; Erratico et al. 2012, 2013; Feo et al. 2013; Lupton et al. 2009; Stapleton et al. 2009) and rat liver microsomes or primary hepatocytes (Dong et al. 2010; Erratico et al. 2011).

Metabolic cleavage of the ether bond in PBDEs to form brominated phenols and debromination to form lower-brominated PBDEs are other metabolic fates of PBDEs in mammals. Evidence for cleavage of the ether bond includes the identification of:

- glucuronide- and sulfate-conjugates of 2,4-dibromophenol in male rats given single oral doses of ^{14}C-BDE 47 (2,2',4,4'-tetraBDE) (Sanders et al. 2006a);
- unconjugated 2,4,5-tribromophenol in feces and urine and glucuronide-, sulfate-, and glutathionyl-conjugates of 2,4,5-tribromophenol in bile and urine in conventional and bile duct-cannulated male rats given single oral doses of ^{14}C-BDE 99 (2,2',4,4',5-pentaBDE) (Chen et al. 2006); and
- 2,4-dibromophenol and 2,4,5-tribromophenol as metabolites of BDE 47 and BDE 99 following in vitro incubation with human liver microsomes (Erratico et al. 2013, 2012).

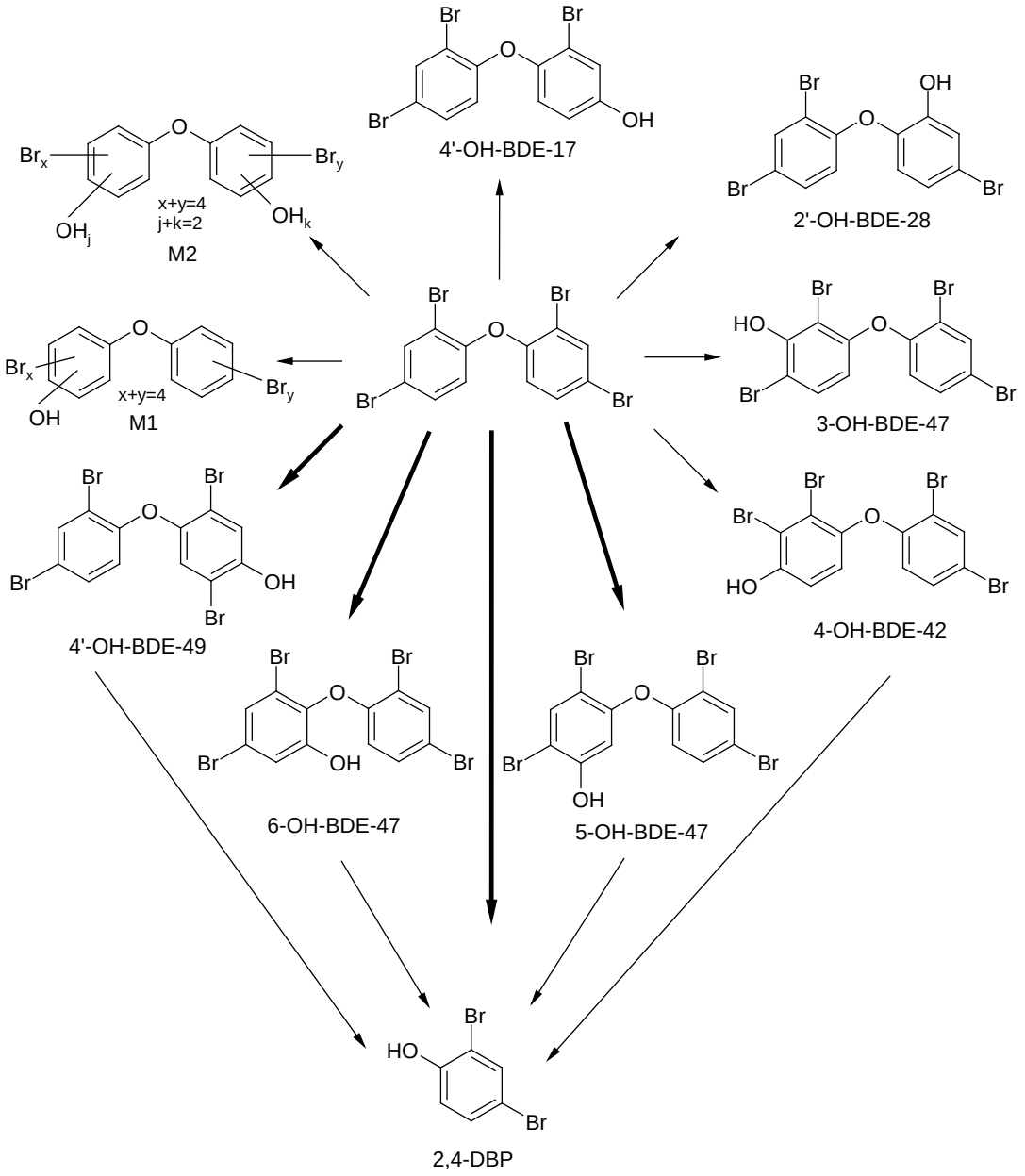
Evidence for debromination includes the identification of:

- hydroxylated triBDEs in feces of rats given single oral doses of ^{14}C-BDE 47 (2,2',4,4'-tetraBDE) (Marsh et al. 2006);
- hydroxylated tetraBDEs in feces of male rats given single oral doses of ^{14}C-BDE 99 (2,2',4,4',5-pentaBDE) (Hakk et al. 2002a);
- mono- and di-hydroxylated tetraBDEs in feces of rats given single oral doses of ^{14}C-BDE 100 (2,2',4,4',6-pentaBDE) (Hakk et al. 2006);
- hydroxylated tetra- and pentaBDEs in feces of rats given single oral doses of ^{14}C-BDE 154 (2,2',4,4',5,6'-hexaBDE) (Hakk et al. 2009);
- several hydroxylated BDEs with five to seven bromines per molecule in feces of rats given single doses of ^{14}C-BDE 209 (Morck et al. 2003); and
- several hydroxylated BDEs with eight or nine bromines per molecule in male rats given single doses of ^{14}C-BDE 209 (Sandholm et al. 2003).

Information from *in vivo* toxicokinetic studies with rodents exposed to the best studied congeners (i.e., BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 209) is inadequate to describe detailed metabolic pathways, but is adequate to propose that cytochrome P450s are likely to be involved in the formation of hydroxylated metabolites and hydoxylated debrominated metabolites of BDE 47 (Sanders et al. 2006a), BDE 99 (Chen et al. 2006), BDE 100 and BDE 154 (Hakk et al. 2006, 2009), and BDE 209 (Morck et al. 2003; Sandholm et al. 2003). Recent *in vitro* studies with human or rat liver microsomes or hepatocytes, and human or rat recombinant CYPs, provide more detailed information adequate for proposing metabolic pathways for BDE 47, BDE 99, and BDE 100 in humans showing CYP2B6 mediation for hydroxylation, debromination, and ether bond cleavage for BDE 47, hydroxylation and ether bond cleavage for BDE 99, and hydroxylation for BDE 100 (see Figures 3-4, 3-5, and 3-6); however, no clear metabolic pathways were identified for BDE 153 or BDE 209 using these methods.

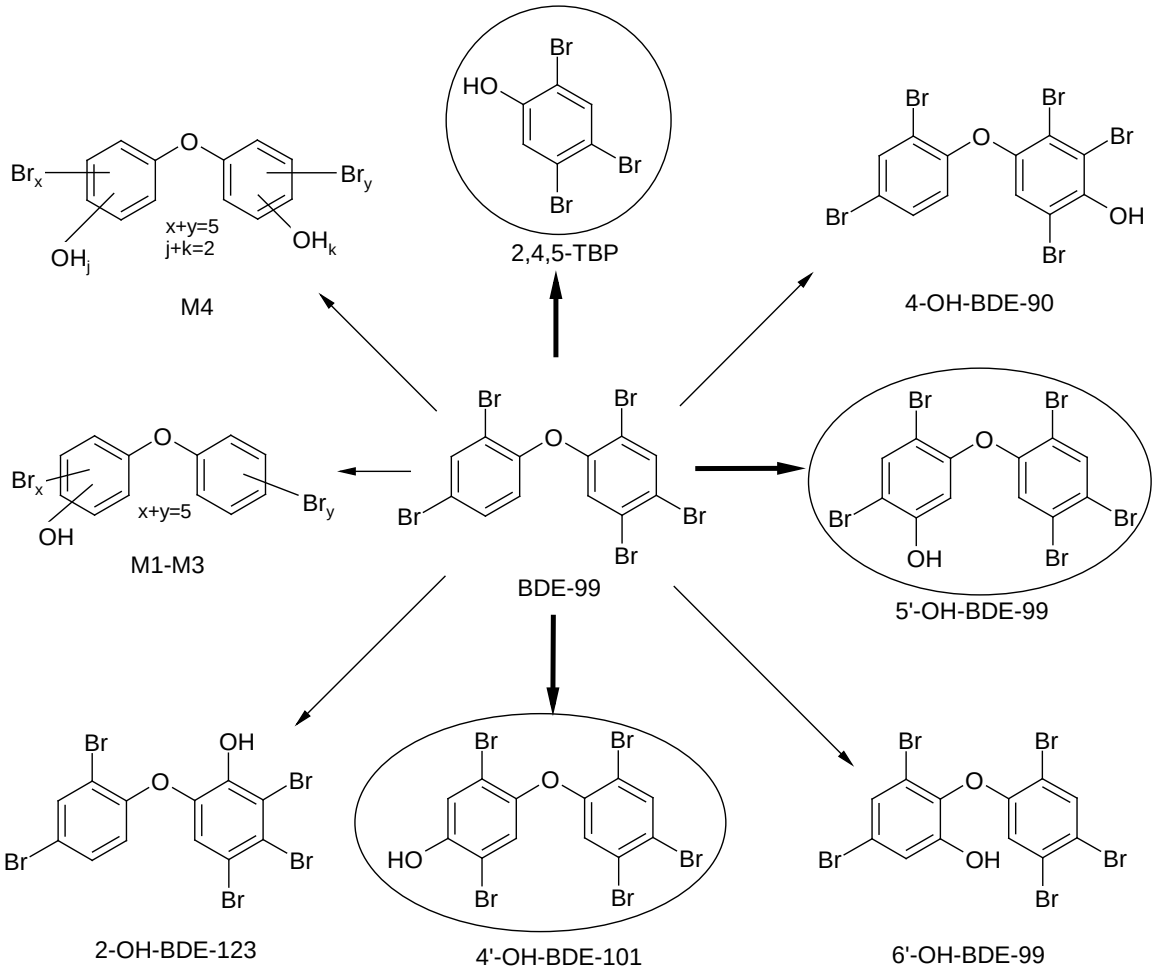
- BDE 47 was metabolized, principally by CYP2B6, in human liver microsomes (Erratico et al. 2013; Feo et al. 2013; Lupton et al. 2009), producing 5-OH-BDE-47, 6-OH-BDE-47, an unidentified dihydroxylated tetrabrominated BDE and 2,4-dibromophenol as major metabolites, and several minor metabolites including three other hydroxylated tetrabrominated BDEs and two hydroxylated tribrominated BDEs (Erratico et al. 2013; see Figure 3-4). The predominance of CYP2B6 involvement was demonstrated by inhibition of the production of all metabolites by a specific antibody to CYP2B6 (Erratico et al. 2013) and comparison of capabilities of 11 or 12 recombinant human CYPs (Erratico et al. 2013; Feo et al. 2013).

Figure 3-4. Structures and General Metabolic Scheme for Hydroxylated Metabolites of BDE 47 Produced by Human Liver Microsomes*



*M1 and M2 refer to general structures of unidentified hydroxylated and dihydroxylated tetrabrominated BDEs. Structures of other metabolites were determined with authentic chemical standards and ultra-performance liquid chromatography-mass spectrometry techniques. Bold arrows indicate major metabolites. CYP2B6 is proposed to be involved in production of all metabolites, based on inhibition of BDE 47 metabolism by a specific antibody to CYP2B6, and higher rates of BDE 47 metabolism in human liver microsomes incubated with specific human recombinant CYP2B6, compared with 11 other human recombinant CYPs.

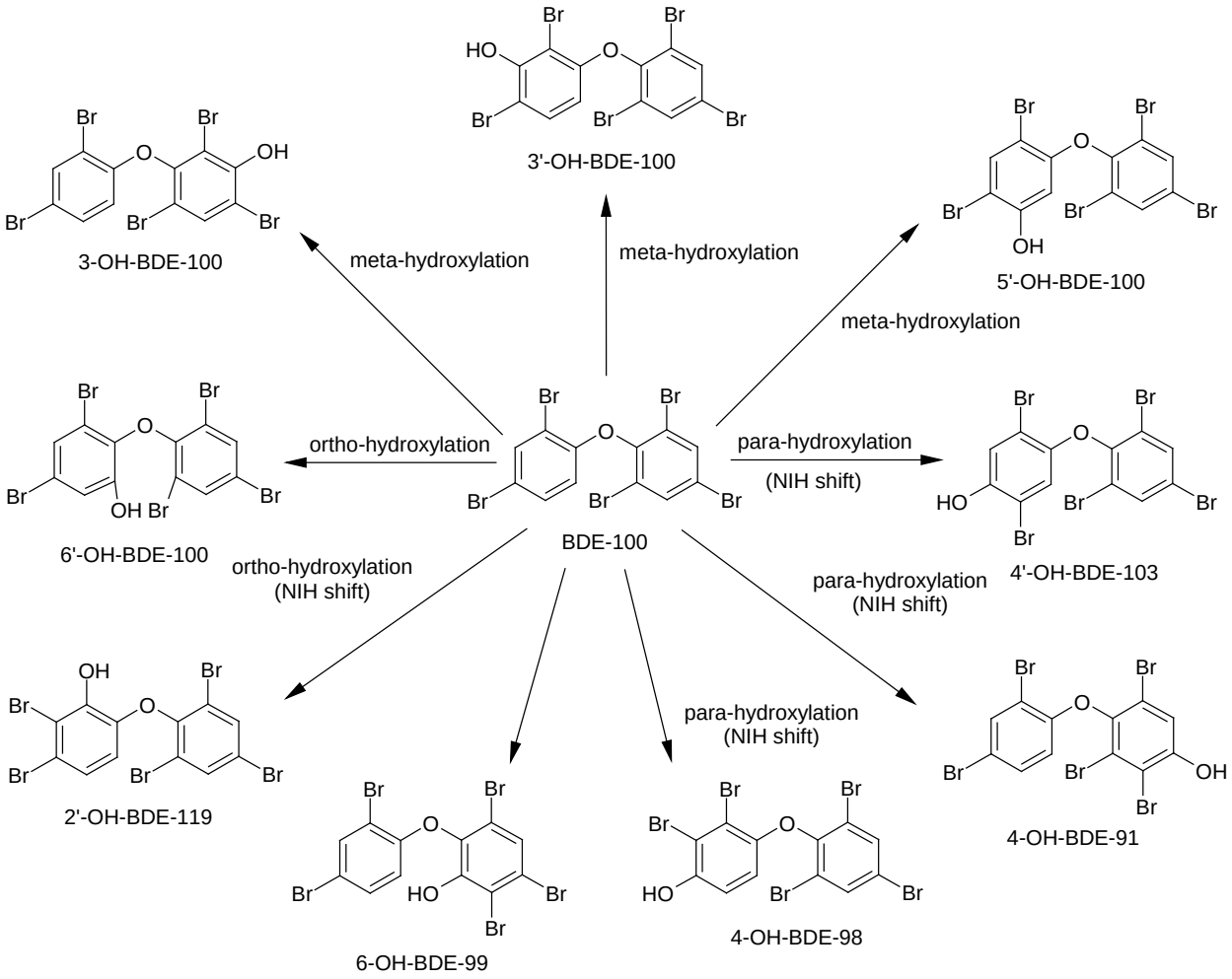
Figure 3-5. Structures and General Metabolic Scheme for Hydroxylated Metabolites of BDE 99 Produced by Human Liver Microsomes*



*M1-3 and M4 refer to general structures of unidentified hydroxylated and dihydroxylated pentabrominated BDEs. Structures of other metabolites were determined with authentic chemical standards and ultra-performance liquid chromatography-mass spectrometry techniques. CYP2B6 is proposed to be involved in production of all metabolites, based on inhibition of BDE 99 metabolism by a specific antibody to CYP2B6, and higher rates of BDE 99 metabolism in human liver microsomes incubated with human recombinant CYP2B6, compared with11 other human recombinant CYPs.

Source: Erratico et al. 2012

Figure 3-6. Structures and General Metabolic Scheme for Hydroxylated Metabolites of BDE 100 Produced by Human Liver Microsomes and Human CYP2B6*



*Structures of 3-OH-BDE-100, 5'-OH-BDE-100, 6'OH-BDE-100, and 4'-OH-BDE-103 were determined using commercial reference standards and ultra-performance liquid chromatography-mass spectrometry techniques. The two remaining mono-OH-pentaBDE metabolites were hypothesized using mass spectral fragmentation characteristics of derivatized OH-BDEs. Additional information based on theoretical boiling point calculations using COnductor-like Screening MOdel for Realistic Solvents (COSMO-RS) and experimental chromatographicretention times were used to identify the hypothesized metabolites as 2′-hydroxy-2,3′,4,4′,6-pentabromodiphenyl ether (2′-OHBDE-119) and 4-hydroxy-2,2′,4′,5,6-penta-bromodiphenyl ether (4-OH-BDE-91), respectively. CYP2B6 is proposed to be involved in production of all metabolites, based on inhibition of BDE 99 metabolism by a specific antibody to CYP2B6, and higher rates of BDE 100 metabolism in human liver microsomes incubated with human recombinant CYP2B6, compared with nine other human recombinant CYPs.

Source: Gross et al. 2015

- BDE 47 was metabolized by liver microsomes from phenobarbital (PB)-induced and dexamethasone (DEX)-induced rats, producing five hydroxylated tetrabrominated BDEs with PB-induction and two hydroxylated tetrabrominated BDEs with DEX induction (Erratico et al. 2011). The major metabolites identified were 4'-OH-BDE-49 and 3-OH-BDE-47 (with PB or DEX induction), and 4-OH-BDE-42 (PB induction only). No evidence for debromination or ether bond cleavage was found. These data and a comparison of metabolic capabilities of 14 recombinant rat CYP enzymes indicate that rat liver metabolism of BDE 47 involves CYP1A1, CYP2A2, and CYP3A1 (Erratico et al. 2011),

- BDE 99 was metabolized, principally by CYP2B6, in human liver microsomes (Erratico et al. 2012; Lupton et al. 2009; Stapleton et al. 2009), producing 2,4,5-tribromophenol, 5'-OH-BDE-99 and 4'-OH-BDE-101 as major metabolites and seven minor hydroxylated pentaBDEs (4-OH-BDE-90, 6'-OH-BDE-99, and 2-OH-BDE-123, three unidentified monohydroxy pentabrominated BDEs, and one dihydroxy pentabrominated BDE) (Erratico et al. 2012; see Figure 3-5). No evidence for debromination of BDE 99 was found. CYP2B6 was the only CYP among a panel of 12 human recombinant CYPs showing metabolic activity with BDE 99, and a specific antibody to CYP2B6 inhibited the production of all BDE 99 metabolites by human liver microsomes (Erratico et al. 2012).

- BDE 99 was metabolized to hydroxylated metabolites in primary rat hepatocytes (Dong et al. 2010) and in liver microsomes from DEX- and PB-induced rats (Erratico et al. 2011). Liver microsomes from DEX- and PB-induced rats produced 4-OH-BDE-99 as the major metabolite and lesser amounts (in decreasing order) of 5'-OH-BDE-99, 6'-OH-BDE-99, 2,4,5-tribromo-phenol, 4'-OH-BDE-101, and 2-OH-BDE-123. No evidence for debromination was found. These data and a comparison of metabolic capabilities of 14 recombinant rat CYP enzymes indicated that rat liver metabolism of BDE 99 involves CYP1A1, CYP2A2, CYP2B1, and CYP3A1 (Erratico et al. 2011).

- BDE 100 was metabolized by recombinant human P450s and pooled human liver microsomes (Gross et al. 2015). As with BDE 47 and BDE 99, human CYP2B6 was found to be the predominant enzyme responsible for nearly all formation of six mono-OH-pentaBDE and two di-OH-pentaBDE metabolites. Four metabolites were identified as 3-hydroxy-2,2′,4,4′,6-pentabromodiphenyl ether (3-OH-BDE-100), 5′-hydroxy-2,2′,4,4′,6-pentabromodiphenyl ether (5′-OHBDE-100), 6′-hydroxy-2,2′,4,4′,6-penta-bromodiphenyl ether (6′-OH-BDE-100), and 4′-hydroxy-2,2′,4,5′,6-pentabromodiphenyl ether (4′-OH-BDE-103) through use of reference standards (see Figure 3-6). The two remaining mono-OH-pentaBDE metabolites were hypothesized using mass spectral fragmentation characteristics of derivatized OH-BDEs, which allowed prediction of an ortho-OH-pentaBDE and a para-OH-pentaBDE positional isomer. Additional information based on theoretical boiling point calculations using COnductor-like Screening MOdel for Realistic Solvents (COSMO-RS) and experimental chromatographic retention times were used to identify the hypothesized metabolites as 2′-hydroxy-2,3′,4,4′,6-penta-bromodiphenyl ether (2′-OHBDE-119) and 4-hydroxy-2,2′,4′,5,6-pentabromodiphenyl ether (4-OH-BDE-91), respectively (Simpson et al. 2015). Kinetic studies of BDE 100 metabolism using P450 2B6 and HLMs revealed K_m values ranging from 4.9 to 7.0 μM and 6−10 μM, respectively, suggesting a high affinity toward the formation of OH-BDEs. Compared to the metabolism of BDE 47 and BDE 99 reported in previous studies, BDE 100 appears to be more slowly metabolized by P450s due to the presence of a third ortho-substituted bromine atom.

- BDE 153 was not metabolized by human liver microsomes under conditions that produced hydroxylated metabolites from BDE 47 (a dihydroxylated BDE 47 and 2,4-dibromophenol), and BDE 99 (2,4,5- tribromophenol, a dihydroxlated BDE 99, and 1,3-dibromo benzene) (Lupton et al. 2009).

- BDE 209 was not metabolized by human primary liver hepatocytes under conditions that produced 2,3,5-tribromophenol, two hydroxylated pentabrominated BDE metabolites and an unidentified hydroxylated tetrabrominated metabolite from BDE 99 (Stapleton et al. 2009).

In summary, Feo et al. (2013) and Gross et al. (2015) characterized the in vitro metabolism of BDE 47 and BDE 100 by pooled human liver microsomes and recombinant human CYPs, identifying a number of hydroxylated BDE metabolites; however, no brominated phenols were detected by the methods utilized by these investigators. In other studies, CYP2B6-mediated metabolism of BDE 47 and BDE 99 produces multiple hydroxylated metabolites via hydroxylation and ether bond cleavage, based on *in vitro* studies with human liver microsomes or hepatocytes and human recombinant CYPs (Erratico et al. 2012, 2013). The major metabolites of BDE 47 and BDE 99 formed by human liver microsomes were not the same as those identified using rat liver microsomes (Erratico et al. 2013, 2012, 2011). It is important to note that all studies consistently identified CYP2B6 as the primary human CYP responsible for the formation of hydroxylated metabolites of BDE 47, BDE 99, and BDE 100 (Erratico et al. 2012, 2013; Feo et al. 2013; Gross et al. 2015), while different classes of CYP enzymes appear to be involved in *in vitro* rat liver metabolism of BDE 47 and BDE 99: CYP1A1, CYP2A2, and CYP3A1 for BDE 47 and CYP1A1, CYP2A2, CYP2B1, and CYP3A1 for BDE 99 (Erratico et al. 2011). Production of hydroxylated metabolites of BDE 153 (Lupton et al. 2009) and BDE 209 (Stapleton et al. 2009) has not been demonstrated with human liver microsomes or hepatocytes, respectively. It is uncertain if these latter findings are reflective of a limited *in vivo* capacity of humans to metabolize these BDE congeners or because the proper *in vitro* conditions for metabolizing these congeners were not provided. Currently, studies of metabolism of BDE 47 and BDE 99 with *in vitro* human and rat systems have found evidence of metabolic oxidative debromination only with BDE 47 in human liver microsomes. In contrast, a number of *in vivo* studies have found evidence for oxidative debromination in feces collected from rats exposed to BDE 47 (Marsh et al. 2006), BDE 99 (Hakk et al. 2002a), BDE 100 (Hakk et al. 2006), BDE 154 (Hakk et al. 2009), and BDE 209 (Morck et al. 2003; Sandholm et al. 2003).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

No studies were located regarding excretion of PBDEs in humans or animals after inhalation exposure.

3.4.4.2 Oral Exposure

Oral Exposure Elimination Overview. Apparent half-lives of PBDE congeners in blood of PBDE-exposed workers during non-exposed vacation periods ranged from 15 days for BDE 209, 18–39 days for nonabrominated congeners, and 37–94 days for octabrominated congeners. The detection of PBDEs in human breast milk samples indicates that elimination via milk is an elimination route for women, but several studies examining PBDE concentrations during lactation do not provide a clear account of the degree to which PBDEs are cleared from the body during lactation. Results from animal studies given single oral doses of ^{14}C-labeled PBDE congeners or PBDE mixtures indicate that biliary excretion into the feces is the principal route of elimination in rats, and that the urine and feces are principal routes of elimination of orally absorbed PBDEs in mice.

No studies were located regarding excretion of PBDEs in humans after controlled oral exposure.

Apparent half-lives of several PBDEs in blood were estimated for eight PBDE-exposed workers (four electronics dismantlers and four workers in a factory making flame-retarded rubber) from measurements of hepta-, octa-, nona- and decaBDE concentrations in blood collected during 28–29-day vacation periods without occupational exposure (Thuresson et al. 2006). After fitting the data to a single-phase exponential model, calculated apparent half-lives were: 15 days for BDE 209; 28, 39, and 18 days for nonabrominated congeners BDE 208, BDE 207, and BDE 206; 37, 72, 85, and 91 days for BDE 203 and three other octabrominated congeners of uncertain chemical structure; and 94 days for BDE 183.

The detection of PBDEs in human breast milk samples suggests that breast milk represents an elimination route of absorbed PBDEs in women (see Jakobsson et al. 2012 and Frederiksen et al. 2009 for reviews of PBDE levels in breast milk). Several studies have examined changes in PBDE concentrations (and other persistent lipophilic chemicals) in breast milk during lactation, but the results do not provide a clear account of the degree to which PBDEs are cleared from the body during breast feeding (Hooper et al. 2007; Jakobsson et al. 2012; LaKind et al. 2009; Thomsen et al. 2010).

Studies with rats given single oral doses of ^{14}C-labeled PBDE congeners or PBDE mixtures indicate that ingested PBDEs are principally excreted in the feces with <2% of administered radioactivity excreted in the urine within 3 days of dose administration. This pattern has been observed in male and female rats exposed to BDE 47 (Orn and Klasson-Wehler 1998; Sanders et al. 2006a), male and female rats exposed

to BDE 99 (Chen et al. 2006; Hakk et al. 2002a), male rats exposed to BDE 100 (Hakk et al. 2006), male and female rats exposed to BDE 153 (Sanders et al. 2006), male rats exposed to BDE 154 (Hakk et al. 2009), pregnant female and male rats exposed to BDE 209 (Morck et al. 2003; Riu et al. 2008), and male and female rats exposed to a mixture with 77.4% ^{14}C-BDE 209, 21.8% nonabrominated BDE, and 0.8% octabrominated (Norris et al. 1973, 1975b). Studies with bile duct-cannulated male rats indicated that radioactivity excreted in feces of conventional rats included bile duct-delivered metabolites (i.e., absorbed material) and unabsorbed compound. Cumulative biliary excretion represented about 3.9% of administered dose with BDE 99 (Hakk et al. 2002a), 1.7% with BDE 100 (Hakk et al. 2006), 1.3% with BDE 154, and about 10% with BDE 209 (Morck et al. 2003).

A different elimination pattern has been observed in mice, especially with BDE 47. In mice given single oral doses of ^{14}C-labeled PBDE congeners, fecal and urinary elimination were principal routes of elimination for BDE 47 (Orn and Klasson-Wehler 1998; Sanders et al. 2006a), whereas fecal elimination appeared to be more important than urinary elimination with BDE 99 (Chen et al. 2006) and BDE 153 (Sanders et al. 2006b). Male C57Bl mice excreted 20% of administered radioactivity in feces and 33% in urine within 5 days of administration of ^{14}C-BDE 47 (Orn and Klasson-Wehler 1998). Male and female B6C3F1 mice excreted about 30 and 20% of administered ^{14}C-BDE 47 dose in urine and about 22% and 25% in feces, within 24 hours (Sanders et al. 2006a). In B6C3F1 mice given ^{14}C-BDE 99, males excreted 7.8% in urine and 27.1% in feces within 24 hours, and females excreted 4.1% in urine and 32.4% in feces (Chen et al. 2006). Twenty-four hours after administration of ^{14}C-BDE 153, male B6C3F1 mice excreted 1% of administered dose in urine and 31.5% in feces, and females excreted 0.3% in urine and 26.0% in feces (Sanders et al. 2006b).

Complementary studies with female C57BL/6J given single intravenous 1-mg/kg doses of ^{14}C-labeled BDE 47, BDE 99, BDE 100, or BDE 153 also indicate that the degree of urinary excretion in mice is congener specific (Staskal et al. 2006b). Cumulative percentages of administered radioactivity excreted in urine within 5 days were 40, 16, 6, and 2% for BDE 47, BDE 99, BDE 100, and BDE 153, respectively. Relatively greater amounts of parent compound were found in urine from BDE 47-exposed mice, compared with mice exposed to the other congeners. Ratios of cumulative percentage dose excreted as parent compound or metabolite in urine were 1.5, 0.5, 0.4, and 0.2 for BDE 47, BDE 99, BDE 100, and BDE 153, respectively. In feces, respective ratios of parent compound:metabolite were 0.7, 0.2, 0.2, and 0.3 for these congeners, respectively.

The mechanism for the relatively high elimination of BDE 47 in the urine of mice is unknown, but has been hypothesized to involve binding to mouse major urinary protein (m-MUP) in the blood (Sanders et al. 2006a; Staskal et al. 2006b) and other membrane transporting polypeptides (Emond et al. 2013; Pacyniak et al. 2010, 2011).

3.4.4.3 Dermal Exposure

No studies were located regarding excretion of PBDEs in humans or animals after dermal exposure.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The

numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

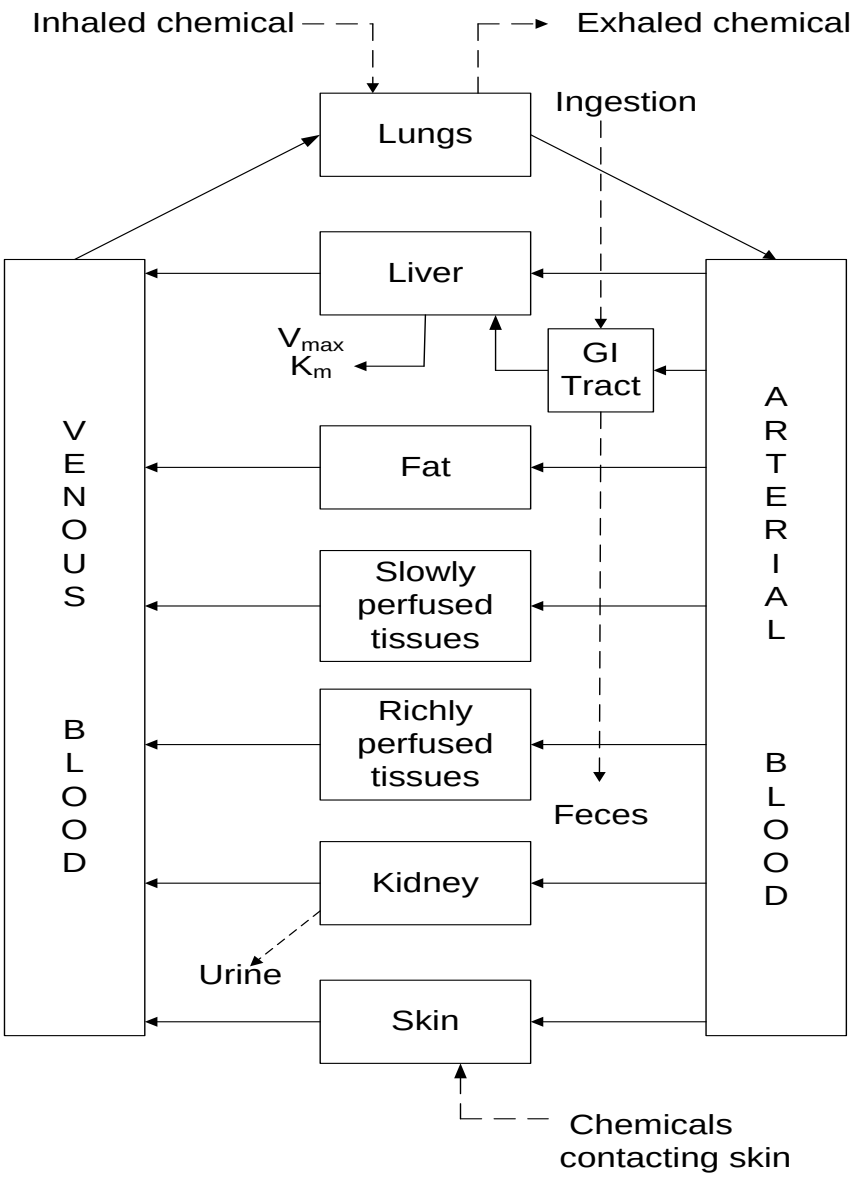
PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-7 shows a conceptualized representation of a PBPK model.

If PBPK models for PBDEs exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Emond et al. (2010) developed a PBPK model for BDE 47 in male and female (nonpregnant and pregnant) adult rats. The model included eight compartments: liver, brain, adipose tissue, kidney, placenta, fetus, blood, and the remaining body. The model was calibrated with tissue concentration data from adult male and maternal-fetal toxicokinetic studies. Other data sets were then used to evaluate the model's performance. Model evaluations indicated that simulated BDE 47 tissue concentrations in adult male, adult female, and fetal compartments were within the standard deviations of the empirical data.

Emond et al. (2013) developed a PBPK model for BDE 47 in adult mice to describe the distribution of BDE 47 in tissues and its elimination in feces and urine, and to evaluate the role of transporters in elimination of BDE 47. The structure of the model was similar to the rat model developed by Emond et al. (2010), without the gestational submodel. In addition, binding to transporters proposed to facilitate

Figure 3-7. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: Krishnan and Andersen 1994

urinary excretion in mice were added: mouse major urinary protein (m-MUP) in blood and P-glycoprotein, a membrane transporter in brain, liver, and kidneys. The model was used to investigate the roles that m-MUP and P-glycoprotein may play in BDE 47 elimination in mice.

No reports were located on the development of PBPK models for PBDEs in humans.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. Information regarding oral absorption in animals is available from studies of commercial PBDE mixtures and individual ^{14}C-labeled tetra-, penta-, hexa-, and decaBDE congeners. The most recent and best available estimates of oral absorption efficiencies following gavage administration in lipophilic vehicles indicate a range of 70–85% for tetraBDE (BDE 47), pentaBDE (BDE 99, BDE 100), and hexaBDE (BDE 153, BDE 154) congeners, and 10–26% for decaBDE (BDE 209) (Hakk et al. 2001, 2002b, 2006, 2009; Huwe et al. 2002b, 2007; Morck and Klasson Wehler 2001; Morck et al. 2003; Örn and Klasson-Wehler 1998; Riu et al. 2008; Sanders et al. 2006a, 2006b; Sandholm et al. 2003; Staskal et al. 2005). Underlying mechanisms for oral absorption (e.g., active transport, diffusion, protein binding) have not been described.

Studies using *in vitro* gastrointestinal digestion models have evaluated bioaccessibility of PBDEs in environmentally relevant sources. In a study by Yu et al. (2010b), the bioaccessibility of lower-brominated PBDEs in 13 types of food (fish, meat, rice, flour, and vegetables) ranged from 2.6 to 41.3% in food. Dietary fat was the most important factor affecting the bioaccessibility of PBDEs, with bioavailability increasing with increased fat content, likely due to the lipophilic nature of PBDEs (Yu et al. 2010b). Bioavailability was also increased with increasing carbohydrate content, potentially due to the formation of micelles (Yu et al. 2010b). However, bioavailability decreased with increasing protein and fiber content, potentially due to adsorption to dietary fiber and ionic strength effect of amino acids leading to decreased partition of PBDEs in the aqueous phase (Yu et al. 2010b). Lepom et al. (2010) evaluated the bioaccessibility of PBDEs in ingested dust, which is expected to be the predominant source of human exposure in the United States (EPA 2010). In this study, the bioavailability of PBDEs in ingested dust was <50%, with higher bioavailability for the lower-brominated PBDEs (27–42%) compared with BDE 209 (10%) (Lepom et al. 2010). A similar study by Abdallah et al. (2012) showed comparable results for the bioaccessibility of PBDEs in ingested dust, with higher bioavailability for the lower-brominated PBDEs (32–58%) compared with BDE 209 (14%).

A dermal absorption efficiency of 62% was reported for female mice exposed to an occluded dermal dose of 1 mg/kg ^{14}C-BDE 47) (Staskal et al. 2005). *In vitro* studies have evaluated diffusion of PBDEs across human, rat, and mouse skin. For ^{14}C-BDE 47, the mean absorption efficiencies (percent of applied radioactivity collected in receptor compartment) were 1.88% for human skin and 14.58% for rat skin (Roper et al. 2006). For ^{14}C-decaBDE, mean absorption efficiency for mouse skin *in vitro* was reported to be only 0.07–0.34% of the applied dose (Hughes et al. 2001). Underlying mechanisms for dermal absorption (e.g., active transport, diffusion, protein binding) specific to PBDEs have not been described.

Distribution. Evidence for the transfer of PBDEs from pregnant mothers to the developing fetus and for the transfer of PBDEs from maternal blood to breast milk and then to nursing infants comes from a number of studies of PBDE concentrations in maternal and cord serum samples and breast milk samples from groups of non-occupationally exposed women (Antignac et al. 2009, 2008; Chen et al. 2013; Kawashiro et al. 2008; Li et al. 2013b; Malarvannan et al. 2013; Mazdai et al. 2003; Meijer et al. 2008; Park et al. 2011; Qiu et al. 2009; Schecter et al. 2010; Vizcaino et al. 2011; Wan et al. 2010). In general, the tetra- and penta-brominated PBDEs have been the predominant congeners detected in maternal and cord serum samples and breast milk samples, but some recent studies analyzing a wider range of PBDE congeners have found evidence for distribution of hepta-, octa-, or decaBDEs into cord serum and breast milk. Frederiksen et al. (2010) utilized a human *ex vivo* placenta perfusion system to study the kinetics of placental transfer of BDE 47, BDE 99, and BDE 209 to the fetus. Placentas were perfused for 4 hours with 1 ng/mL concentrations of the nonlabeled congeners. Samples of maternal and fetal compartments were taken during the perfusion, and concentrations in samples were determined by GC/MS. Placental transfer of BDE 47 and BDE 99 was demonstrated, and transfer of BDE 47 was faster and more extensive than BDE 99. Transfer of BDE 209 across the placenta to the fetal compartment was not demonstrated with the detection limits of the techniques employed. Frederiksen et al. (2010) proposed that future perfusion studies with BDE 209 should use ^{14}C-labeled BDE 209 to increase sensitivity.

Tissue distribution studies in animals orally exposed to ^{14}C-labeled BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 209 indicate that decaBDE is distributed among tissues somewhat differently than tetra-, penta- and hexaBDEs. While lower-brominated BDE congeners, following absorption and an initial wide distribution, are preferentially accumulated in adipose tissues, absorbed decaBDE is less readily distributed to adipose tissues and appears to preferentially distribute to highly perfused tissues (Chen et al. 2006; El Dareer et al. 1987; Eriksson et al. 2002b; Hakk et al. 2002a, 2006; Morck and Klasson-Wehler 2001; Morck et al. 2003; Norris et al. 1975a; NTP 1986; Örn and Klasson-

Wehler 1998; Riu et al. 2008; Sanders et al. 2006a, 2006b; Staskal et al. 2005, 2006a; Viberg et al. 2003a). Although less likely to partition to adipose tissues, decaBDE was still found in low quantities in adipose tissues in these studies, and has been shown to transfer from dams to fetuses and neonates from exposure during gestational and nursing periods (Cai et al. 2011; Zhang et al. 2011).

Metabolism. Oxidative hydroxylation of PBDEs is a principal metabolic transformation that is thought to occur in humans and laboratory animals. Hydroxylated PBDEs have been identified in samples of human biological fluids including blood (Athanasiadou et al. 2008; Hovander et al. 2002; Lacorte and Ikonomou 2009; Qiu et al. 2009; Rydén et al. 2012; Wan et al. 2009, 2010; Wang et al. 2012; Yu et al. 2010a) and breast milk (Lacorte and Ikonomou 2009). Hydroxylated PBDEs also have been identified in feces or bile of laboratory rodents exposed to ^{14}C-labeled tetra-, penta-, hexa- or decaBDEs (Chen et al. 2006; Hakk et al. 2002a, 2009; Marsh et al. 2006; Morck et al. 2003; Orn and Klasson-Wehler 1998; Riu et al. 2008; Sanders et al. 2006a; Staskal et al. 2006b). Oxidative hydroxylation of PBDEs also has been demonstrated and studied in *in vitro* metabolic systems with human liver microsomes or primary hepatocytes (Cheng et al. 2008; Erratico et al. 2012, 2013; Feo et al. 2013; Lupton et al. 2009; Stapleton et al. 2009) and rat liver microsomes or primary hepatocytes (Dong et al. 2010; Erratico et al. 2011). Metabolic cleavage of the ether bond in PBDEs to form brominated phenols and debromination to form lower-brominated PBDEs are other metabolic fate processes for PBDEs in mammals (Chen et al. 2006; Erratico et al. 2013, 2012; Hakk et al. 2002a, 2006, 2009; Marsh et al. 2006; Morck et al. 2003; Sanders et al. 2006a; Sandholm et al. 2003).

Information from *in vivo* toxicokinetic studies with rodents exposed to the best studied congeners (i.e., BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 209) is inadequate to describe detailed metabolic pathways, but is adequate to propose that cytochrome P450s are likely to be involved in the formation of hydroxylated metabolites and hydoxylated debrominated metabolites. *In vitro* studies with human liver microsomes or hepatocytes and human recombinant CYPs indicate that CYP2B6-mediated metabolism of BDE 47, BDE 99, and BDE 100 produced multiple metabolites via hydroxylation (Erratico et al. 2012, 2013; Feo et al. 2013; Gross et al. 2015) and ether bond cleavage (Erratico et al. 2012, 2013). The major metabolites of BDE 47 and BDE 99 formed by human liver microsomes were not the same as those identified using rat liver microsomes (Erratico et al. 2011, 2012, 2013). Different sets of CYP enzymes appear to be involved in *in vitro* rat liver metabolism of BDE 47 and BDE 99: CYP1A1, CYP2A2, and CYP3A1 for BDE 47 and CYP1A1, CYP2A2, CYP2B1, and CYP3A1 for BDE 99 (Erratico et al. 2011). Production of hydroxylated metabolites of BDE 153 (Lupton et al. 2009) and BDE 209 (Stapleton et al. 2009) has not been demonstrated with human liver microsomes or hepatocytes. It is

uncertain if these latter findings are reflective of a limited *in vivo* capacity of humans to metabolize these BDE congeners or because the proper *in vitro* conditions for metabolizing these congeners were not provided. While rat studies provide evidence for metabolic oxidative debromination of BDE 47, BDE 99, BDE 100, BDE 154, and BDE 209, studies with human liver microsomes only found evidence supporting the oxidative debromination of BDE 47.

Elimination. Apparent half-lives of PBDE congeners in blood of PBDE-exposed workers during non-exposed vacation periods ranged from 15 days for BDE 209, 18–39 days for nonabrominated congeners, and 37–94 days for octabrominated congeners (Thuresson et al. 2006). The detection of PBDEs in human breast milk samples indicates that elimination via milk is an elimination route for women, but several studies examining PBDE concentrations during lactation do not provide a clear account of the degree to which PBDEs are cleared from the body during lactation (Hooper et al. 2007; Jakobsson et al. 2012; LaKind et al. 2009; Thomsen et al. 2010). Results from animal studies given single oral doses of ^{14}C-labeled PBDE congeners or PBDE mixtures indicate that biliary excretion into the feces is the principal route of elimination in rats (Chen et al. 2006; Hakk et al. 2002a, 2006; Morck et al. 2003; Norris et al. 1973, 1975b; Orn and Klasson-Wehler 1998; Riu et al. 2008; Sanders et al. 2006a), and that the urine and feces are principal routes of elimination of orally absorbed PBDEs in mice (Chen et al. 2006; Orn and Klasson-Wehler 1998; Sanders et al. 2006a). In mice, the importance of urinary excretion is congener-specific, with BDE 47 showing the greatest ratio of cumulative percentage dose excreted as parent compound or metabolite (Staskal et al. 2006b). The mechanism for the relatively high elimination of BDE 47 in the urine of mice is unknown, but has been hypothesized to involve binding to mouse major urinary protein (m-MUP) in the blood (Sanders et al. 2006a; Staskal et al. 2006b) and other membrane transporting polypeptides (Emond et al. 2013; Pacyniak et al. 2010, 2011).

3.5.2 Mechanisms of Toxicity

Overview. As summarized in Section 2.2 (Summary of Health Effects) and detailed in Chapter 3 (Health Effects), the main targets of concern following PBDE exposure in humans are the developing nervous and reproductive systems, the developing and mature endocrine system, the liver, and the male reproductive system. Other potential targets are the female reproductive system, the adult nervous system, and the developing and adult immune system; however, evidence for these end points is limited. Numerous studies have been conducted to identify potential mechanisms of toxicity for PBDE exposure. These studies include evaluations of general mechanisms (e.g., hepatic enzyme induction, AhR-mediated effects) as well as target-specific mechanisms. For specific targets, the majority of mechanistic studies

have focused on endocrine disruption and neurological effects; however, definitive mechanisms underlying these effects have not been elucidated. For other effects, including reproductive toxicity, immunotoxicity, and hepatotoxicity, only limited mechanistic data are available. Mechanistic data relevant to toxic effects of PBDEs are reviewed below.

General Mechanisms of Toxicity. PBDEs share some toxicological properties with other structurally similar polyhalogenated aromatic compounds, particularly PBBs, PCBs, PCDDs, and PCDFs (ATSDR 1994, 1998, 2000). Although these chemicals are structurally similar in two dimensions, PBDEs (and polychlorinated diphenyl ethers or PCDEs) differ from the other classes on a three-dimensional basis. In particular, the oxygen bridge of the ether linkage in the diphenyl ether molecule increases the distance between the biphenyl rings, introduces a 120° bend in the alignment of the biphenyl rings, and serves as a barrier to rotation that inhibits the two aromatic rings from assuming a coplanar configuration (Hardy 2002a; Howie et al. 1990). Furthermore, halogen substitution of the *ortho* positions in the diphenylether molecule, as occurs for some congeners, pushes the aromatic rings to be orthoganol to each other (i.e., offset by 90°) (Hardy 2002a). Because the toxicity of TCDD and related compounds is related to their ability to assume a coplanar configuration for binding to AhR, this suggests that PBDEs are unlikely to display similar toxic potency (Hardy 2002a). Assays conducted by Chen et al. (2001) to compare AhR binding affinity and EROD activity of PBDE congeners and mixtures found that activities were very low relative to TCDD and related compounds, but also that, unlike for PCBs, AhR binding affinity was not correlated with planarization energies of the congeners (the calculated energy needed to force coplanarity of the PBDE molecule). The researchers speculated that the large size of the bromine atoms may distort the AhR binding site so that coplanar configuration is not required. However, even in studies of chlorinated analogs (PCDEs), it was found that increasing *ortho* substitution is less effective in decreasing the activity of these congeners than for PCBs (Howie et al. 1990). The authors attributed this difference to the increased bond length between the phenyl rings in PCDEs relative to PCBs.

In other words, the ether bridge makes PBDEs more non-coplanar in nature, which reduces AhR binding affinity relative to similar compounds, but also less sensitive to the influence of *ortho* substitutions that inhibit AhR binding of PBBs or PCBs. This has implications not only for dioxin-type toxicities, which are mediated by the AhR pathway, but also for non-dioxin-type effects. For example, Chen et al. (2001) found that the induction of CYP1A1 by PBDEs is AhR-mediated, as it is for numerous organochlorines, even though PBDEs do not readily adopt the coplanar conformation usually considered characteristic of AhR ligands. Structure-activity relationships have been incompletely elucidated for non-dioxin-like effects of PBDEs such as neurotoxicity. However, based on limited available data, it can be speculated

that di-*ortho*-substituted PBDEs might follow the neurotoxic potency of *ortho*-PCBs (Eriksson et al. 2002b; Kodavanti and Derr-Yellin 2002; Mariussen and Fonnum 2002, 2003).

There are also geometrical differences in PCBs, PBBs, and PBDEs due to the higher atomic weight and considerably larger molecular volume of bromine compared to chorine (Hardy 2000a, 2002a). These differences contribute to dissimilar physical/chemical properties that can influence the relative bioavailability, absorption, tissue accumulation, receptor interactions, and toxicities of the chemicals. For example, a comparison of a series of isosteric 3,3',4,4'-tetrahalobiphenyls in rats showed that relative toxicity (growth rate and thymic atrophy), AhR binding affinity, and aryl hydrocarbon hydroxylase (AHH) and EROD induction potencies increased with increasing bromine substitution (Andres et al. 1983). Possible explanations for this effect included the increased polarizability of bromine versus chlorine and differences in the electronic, hydrophobic, and hydrogen bonding characteristics of bromine and chlorine (Andres et al. 1983).

The enzyme induction properties of PBDEs have been studied to a lesser extent than the enzyme induction properties of other structurally similar chemicals. Existing information suggests that PBDEs can be classified as mixed-type inducers of hepatic microsomal monooxygenases, although the mixed induction properties of the commercial mixtures are likely due to contamination with polybrominated-*p*-dibenzodioxins (PBDDs) and polybrominated dibenzofurans (PBDFs) (Darnerud et al. 2001; de Wit 2002; Hardy 2002b). Few studies have examined the structure-induction relationships for PBDEs. Chen et al. (2001) examined the ability of 12 PBDE congeners and 3 commercial mixtures to induce EROD activity in chick and rat hepatocytes, in liver cell lines from rainbow trout, rat, and human, and in a human intestinal cell line. The number of bromine substitutions in the congeners tested ranged from 3 to 7. In all cell types, BDE 77, 2,2',4,4',6-pentaBDE, BDE 66, and BDE 126 were the strongest inducers. BDE 153 and 2,2',3,4,4',5',6-heptaBDE were weak inducers in all cell types, whereas BDE 66 and BDE 85 were very weak inducers in rat hepatocytes and inactive in the other cells. BDE 47 and 2,2'4,4',5-pentaBDE, which are prominent in the environment, were not inducers in any cell line, and neither were BDE 28, 2,2',4,4',5,6'-hexaBDE, or the penta-, octa-, or decaBDE mixtures. For those congeners that had measurable EROD induction activity, their relative potencies were 10^{-3}–10^{-6} that of 2,3,7,8-TCDD. In general, the EROD induction activity paralleled the strength of the AhR binding with the notable exception of BDE 85, which despite its relatively strong AhR binding affinity (see above), showed no evidence of activating the AhR to its DRE binding form and was only a weak EROD inducer.

As discussed in the introduction to this section, bromination at the *ortho* position does not appear to significantly change the biological effects of PBDE molecules. Structure-activity studies have shown that some PBDE congeners can bind to the AhR, although binding affinities and induction of AhR-mediated responses are very weak or negligible, particularly for commercial PBDE mixtures and environmentally relevant congeners.

For example, Meerts et al. (1998) indirectly examined the AhR-mediated (dioxin-like) properties of 17 PBDE congeners in a recombinant H4II rat hepatoma cell line showing AhR-mediated expression of a luciferase reporter gene. The tested congeners varied from dibromo- to heptabromo- substituted compounds, and with the exception of BDE 15 and BDE 77, all had at least one *ortho* substitution. Seven of the congeners showed luciferase expression, indicating their ability to activate the AhR. The only discernable pattern of receptor activation that appeared to emerge from these results was that greater receptor activation was obtained with the penta- and hexaBDEs than with tri- and tetraBDEs.

Another study also examined the AhR induction potency of PBDE congeners using the *in vitro* luciferase assay with H4IIE-luc recombinant rat hepatoma cells (Villeneuve et al. 2002). Only 1 of 10 tested congeners (BDE 126) induced a significant response for AhR-mediated gene expression in the H4IIE-luc cells, but the magnitude of induction was 87% less compared with the response induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). With the exception of BDE 105, which induced a response of 1.7% of the TCDD maximum, no other congener, including the environmentally prominent congeners, BDE 47, BDE 99, and BDE 153, yielded a response greater than 1% of TCDD. Overall, the tested PBDE congeners were at least 200,000 times less potent than TCDD for inducing AhR-mediated gene expression in this test system. Using the same test system (H4IIE-luc recombinant rat hepatoma cells), Behnisch et al. (2003) reported AhR activities 5–6 orders of magnitude lower than TCDD for BDE 77, BDE 105, BDE 126, BDE 119, BDE 190, and BDE 209; BDE 47, BDE 66, BDE 85, BDE 99, BDE 100, BDE 153, and BDE 183 were inactive. Similarly, Hamers et al. (2006) reported AhR activities 6 orders of magnitude lower than TCDD for 7/19 PBDE congeners tested (BDE 38, BDE 49, BDE 79, BDE 99, BDE 181, BDE 183, and BDE 190), as well as the hydroxylated metabolite, 6OH-BDE-47. In another study, several hydroxylated and methoxylated PBDEs (19 out of 34 tested) were also shown to activate the AhR receptor in the H4IIE-luc assay, with potencies 4–12 orders of magnitude less than TCDD (Su et al. 2012).

Chen et al. (2001) studied the affinities of a series of 18 PBDE congeners and 3 commercial PBDE mixtures for rat hepatic AhR by using competitive AhR-ligand and EROD induction assays. The analysis

showed that both the congeners and octa- and pentaBDE commercial mixtures had binding affinities of 10^{-2}–10^{-5} times that of 2,3,7,8-TCDD. The congener with the highest affinity among the tested congeners was BDE 85, although its relative binding affinity was only 2% that of 2,3,7,8-TCDD. No binding activity could be determined for the decaBDE mixture. In contrast with PCBs, the binding affinities did not appear to relate to the planarity of the molecule, which according to Chen et al. (2001), was possibly due to the fact that the large size of bromine atoms expands the receptor binding site. The dioxin-like activity of the PBDE congeners and commercial mixtures was subsequently more completely characterized, by determining whether they act as AhR agonists or antagonists at sequential stages of the AhR signal transduction pathway leading to CYP1A1 expression in rat hepatocytes (Chen and Bunce 2001). BDE 77, BDE 119, and BDE 126 were moderately active towards dioxin-response element (DRE) binding and induced responses of both CYP1A1 mRNA and CYP1A1 protein analogous to the maximal response of TCDD, although at concentrations 3–5 orders of magnitude greater than TCDD. These congeners showed additive behavior towards DRE binding with TCDD (i.e., an increased response compared to TCDD alone), whereas most of the other congeners antagonized the action of TCDD. BDE 100, BDE 153, and BDE 183 were very weak activators of DRE binding, and other congeners and the three commercial BDE mixtures were inactive. In particular, the environmentally prominent congeners BDE 47 and BDE 99 were among the least active with respect to dioxin-like behavior (i.e., were inactive at all stages of signal transduction), and the commercial pentaBDE mixture had negligible EROD induction activity. The PBDE congeners that bound most strongly to the AhR were also the strongest inducers of CYP1A1 mRNA and CYP1A1 protein, indicating that the induction of CYP1A1 was AhR-mediated. Considering all of the end points evaluated in the Chen et al. (2001) and Chen and Bunce (2001) studies, it was concluded that the relative induction potencies (REPs) of the most active PBDEs toward CYP1A1 are $\approx 10^{-4}$ that of TCDD, which is similar to some mono-*ortho*-PCBs and two orders of magnitude less than those of coplanar PCBs, but the REPs for the environmentally prominent congeners are essentially zero. Consistent with these findings, Behnisch et al. (2003) also reported REPs of 10^{-4}–10^{-6} that of TCDD for BDE 25, BDE 77, BDE 100, BDE 126, and BDE 183 in the H4IIE-EROD bioassay, while BDE 154, BDE 99, BDE 47, and BDE 28 were inactive.

Mechanism of Endocrine Disruption. PBDE-induced endocrine disruption is likely to involve multiple mechanisms, including altered synthesis/clearance, transport, and/or receptor binding of endocrine hormones.

The apparent lack of effect of PBDEs on serum TSH suggests that direct effects on the thyroid leading to inhibition of T_4 synthesis are unlikely. However, Wu et al. (2016) present *in vitro* data that BDE 47

inhibits iodide uptake in rat thyroid follicular FRTL-5 cells via non-competitive inhibition of the sodium/iodide symporter (NIS). Additionally, mRNA expression levels of thyroid peroxidase (TPO) was decreased by BDE 47. Together, NIS and TPO are considered critical molecules in thyroid hormone synthesis, and are targets of many thyroid disruptors (e.g., perchlorate and thiocyanate) (Wu et al. 2016). No additional data regarding the potential for PBDEs to interfere with thyroid hormone synthesis were located.

PBDEs are hepatic microsomal enzyme inducers, but there is little evidence that increased enzyme activity leads to greater clearance of thyroid hormones. The induction of hepatic UDPGT by PBDEs has been demonstrated in several studies (Fowles et al. 1994; Hallgren and Darnerud 2002; Hallgren et al. 2001; Skarman et al. 2005; Stoker et al. 2004; Szabo et al. 2009; Zhou et al. 2001, 2002) and this could increase the UDPGT-catalyzed deactivation and excretion of T_4 (i.e., the conjugation of T_4 with glucuronic acid). An indication that increased UDPGT activity may not be the main mechanism for the reduced T_4 levels is provided by Hallgren et al. (2001), who found that exposure to ≥18 mg/kg/day pentaBDE for 14 days caused serum T_4 reductions in both mice and rats with no effect on UDPGT activity in the mice, and increased UDPGT in the rats only at higher dose levels. In contrast, the decreases in serum T_4 correlated with the induction of microsomal phase I enzymes (EROD and MROD). Increased microsomal enzyme activity (discussed above) could also increase the formation of hydroxylated PBDE metabolites that can bind to T_4 plasma transport proteins. This would serve to increase the number of occupied sites on T_4-binding proteins and subsequently result in decreased serum levels of T_4; however, this mechanism is not fully elucidated.

Several studies have demonstrated that PBDE metabolites compete with T_4 for binding thyroid hormone transport proteins (TTR, thyroxine-binding globulin [TBG]). Meerts et al. (1998, 2000) tested 17 PBDE congeners and 3 hydroxylated PBDEs for possible interaction with T_4 binding to human TTR, a plasma transport protein of thyroid hormones, in an *in vitro* competitive binding assay. None of the pure congeners competed with T_4 for binding to human TTR without metabolic activation. Incubation of the congeners with rat liver microsomes induced by PB (CYP2B enriched), β-napthoflavone (CYPIA enriched), or clofibrate (CYP4A3 enriched) indicated that 9 of the 17 pure congeners generated metabolites (not identified) that were able to displace T_4 from TTR (>60% competition): BDE 15, BDE 28, BDE 30, BDE 47, BDE 51, BDE 75, BDE 77, BDE 100, and BDE 119. Testing of the three known hydroxylated PBDEs, used for their structural resemblance with the thyroid hormones 3,5-diiodothyronine (3,5-T_2), 3,3',5-triiodothyronine (T_3), and 3,3',5,5'-tetraiodothyronine (T_4) showed that the T_4-like (2,6-dibromo-4-[2,4,6-tribromophenoxy]phenol) and T_3-like (2-bromo-4-[2,4,6-tri-

bromophenoxy]phenol) hydroxylated PBDEs were 1.42- and 1.22-fold more potent, respectively, than T_4; the T_2-like hydroxylated PBDE (4-[2,4,6-tribromophenoxy]phenol) showed low affinity for TTR (0.41-fold less potent than T_4). Consistent with these findings, Hamers et al. (2006) reported that 6OH BDE-47, but none of the 19 PBDE congeners, competed with the natural ligand T_4 for TTR binding (4-fold less potent than T_4). Additionally, Ren and Guo (2012) reported that 5 of the 11 OH-PBDEs tested bound to TTR with 1.2–2-fold greater potency than T_4 and 1 of the 11 OH-PBDEs tested bound to TBG with 1.5-fold greater potency than T_4. In another study, all OH-PBDEs tested were considered strong binders of TTR (relative potency compared with T_4 between 0.1 and 1) and moderate-to-strong binders of TBR (relative potency compared with T_4 between 0.01 and 1), including 3-OH-BDE 47, 5-OH-BDE 47, 6-OH-BDE 47, 4-OH-BDE 49, and 6-OH-BDE 99 (Marchesini et al. 2008). Parent compounds (BDE 47, BDE 49, BDE 68, and BDE 99) and MeO-PBDEs (6-MeO BDE 57, 2'-MeO-BDE 68) showed no-to-weak binding (<0.01 relative potency compared with T_4). Marsh et al. (1998) tested the affinity of 4'-hydroxyl derivatives of 1,3,5-triBDE, 1,3,3',5'-tetraBDE, and 1,3,3',5,5'-pentaBDE were tested for human thyroid hormone receptor subunits THR-α and THR-β *in vitro*. These congeners were tested because they theoretically show the highest structural similarity to T_4 and T_3. None of the hydroxylated derivatives effectively competed with the thyroid hormones for binding to either receptor (affinities were 41–>1,000 times less than for T_4 and T_3).

Studies also suggest that PBDEs and/or their metabolites can alter thyroid hormone binding to thyroid receptors. In a reporter gene assay in Chinese hamster ovary (CHO) cells, 4OH-BDE-90 was antagonistic to both THR-α and THR-β receptors, with a potency 2-fold less potent in the THR-α assay and ~30% more potent in the THR-β assay than the reference compound tetrabrominated bisphenol A (TBBPA) (Kojima et al. 2009). However, receptor antagonism was not observed for the other three OH-PBDEs tested (4OH-BDE-17, 4OH-BDE-42, 4OH-BDE-49) or any of the eight PBDEs or four MeO-PBDEs tested (Kojima et al. 2009). In another reporter gene assay in fibroblast-derived CV-1 cells, T_3-dependent THR-α- and THR-β-responsive gene expression was decreased by BDE 100, BDE 153, BDE 154, BDE 290, and DE-71 in fibroblast-derived CV-1 cells by 35–45% (Ibhazehiebo et al. 2011). Thyroid responsive element (TRE) dissociation from TRs was also significantly increased by BDE 100, BDE 154, and BDE 290 by 30–45%; however, no changes in THR-α and THR-β cofactor recruitment were observed for 11 PBDEs, 2 OH-PBDEs, or DE-71 (Ibhazehiebo et al. 2011). In contrast, in a THR-α and THR-β cofactor recruitment assay, 4 of the 10 OH-PBDEs tested were TR agonists (2OH-BDE-28, 3OH-BDE-28, 5OH-BDE-47, 6OH-BDE-47), showing 70–90% of the maximal response induced by T_3 (Ren et al. 2013).

In general, lower-brominated PBDEs, and their hydroxylated (OH-PBDEs) and methoyxylated (MeO-PBDEs) metabolites, show estrogenic activities 5–7 orders of magnitude lower than the natural ligand 17β-estradiol (E2) in reporter gene assays, and higher-brominated PBDEs and their metabolites show anti-estrogenic activities that are 1–6 orders of magnitude lower than reference antiestrogenic compounds (Hamers et al. 2006; Kojima et al. 2009; Li et al. 2013b; Meerts et al. 2001; Mercado-Feliciano and Bigsby 2008b). A good correlation has been shown between estrogenic activity and ER binding affinity of low-brominated PBDEs (Li et al. 2013b; Mercado-Feliciano and Bigsby 2008b). One study reported no estrogenic activity in an ER-dependent gene transcriptional activation assay for "PBDEs" in human HeLa 9903 cells stably transfected with the human ERα receptor; however, the specific congener(s) were not identified (Kim et al. 2011b).

For two OH-PBDEs that have bromine substitution patterns similar to the thyroid hormones T_2 [3,5-diiodothyronine] and T_3 [3,3',5-triiodothyronine] (i.e., 4-(2,4,6-tribromophenoxy)phenol and 2-bromo-4-(2,4,6-tribromophenoxy) phenol, respectively), estrogenic activities were 2–10 times more potent than E2 (Meerts et al. 2001). The T_2-like hydoxylated PBDE 4-(2,4,6-tribromophenoxy)phenol also showed estrogenic activity in ERα- and ERβ-like human embryonic kidney cells, with maximum inductions of 50–95% of the maximum induction by E2 (Meerts et al. 2001).

Estrogenic effects were also demonstrated using cell proliferation assays in breast cancer cells exposed to the commercial pentaBDE mixture DE-71 (Mercado-Feliciano and Bigsby 2008b). Basal cell proliferation was significantly increased by up to 10-fold with DE-71 exposure (compared with increases up to 15-fold with exposure to the natural ligand E2). Co-exposure of cells to DE-71 and E2 significantly decreased proliferation relative to E2-only exposure in a concentration-dependent manner, suggesting an antagonistic effect of DE-71 on E2-induced cell proliferation (Mercado-Feliciano and Bigsby 2008b). However, neither basal nor E2-induced cell proliferation were altered in breast cancer cells exposed to BDE 47, BDE 99, BDE 100, or BDE 209 (Kwiecińska et al. 2011). Caspase-9 activity (apoptotic marker) was significantly decreased by ~40–60% in tests with all congeners compared with decreases of ~25% with exposure to E2 (Kwiecińska et al. 2011).

Estrogenic effects have also been reported in other *in vitro* and *in vivo* assays, including increased uterine mRNA expression of ERα and ERβ in adult female offspring of rats given subcutaneous injections of BDE 99 at 1 mg/kg/day (but not 10 mg/kg/day) from GD 10 to 18 (Ceccatelli et al. 2006); increased uterine mRNA and protein levels of calbindin-D9k, a biomarker for estrogenic compounds, in immature female rats exposed to BDE 47 at 50–200 mg/kg/day from PND 16 to 18 (Dang et al. 2007); increased

gene and protein expression of ERα and ERβ in porcine ovarian follicles by 5-OH-BDE-47 and 6-OH-BDE-47 (Karpeta et al. 2014); inhibition of E2 metabolism in rat liver microsomes by 11 OH-PBDEs and 1 MeO-BDE (Lai and Cai et al. 2012); inhibition of E2 sulfation (E2SULT) in V79 cells by 6-OH-BDE-47, BDE 19, BDE 47, and BDE 49 (Hamers et al. 2006); and increased production of vitellogenin in trout hepatocytes exposed to BDE 47, BDE 99, or BDE 205 (Nakari and Pessala 2005). However, estrogenic effects were not observed with exposure to BDE 47, BDE 99, or BDE 205 in recombinant yeast assays with human estrogen receptor (hER) (Nakari and Pessala 2005). In a human cohort study, total serum PBDE in adult females were positively associated with ERα and ERβ mRNA expression levels in the blood; however, BDE 47 serum levels were negatively associated with ERα and ERβ mRNA expression levels (Karmaus et al. 2011). Similarly, ERβ gene and protein expression were decreased in porcine ovarian follicles exposed go BDE 47; however, no change was observed in ERα gene or protein expression levels (Karpeta et al. 2014).

Anti-androgenic and anti-prostagenic activity was observed in reporter gene assays in human osteoblast cells following exposure to 16 different PBDE congeners, 6OH-BDE-47, and 2 commercial PBDE mixtures (DE-71, Octa LM) (Hamers et al. 2006). The most potent antiandrogens, BDE 19 and BDE 100, were 21 and 13 times less potent than the reference antiandrogenic drug flutamide. BDE 19 was also the most potent antiprostagen, and was 3 orders of magnitude lower in potency than the reference compound RU-486 (Hamers et al. 2006). Neither antiandrogenic nor antiprogestagenic activity were observed for BDE 169, BDE 206, or BDE 209 (Hamers et al. 2006). Antiandrogenic activity was also observed in two of five PBDEs tested (BDE 47, BDE 100) and the commercial pentaBDE mixture DE-71 tested in human breast cells (Stoker et al. 2005), and in five of eight PBDEs tested, three of four OH-PBDEs tested, and four of four MeO PBDEs (all low-brominated) tested in CHO cells (Kojima et al. 2009). In contrast, Christen et al. (2010) reported that BDE 100 and BDE 155 enhanced DHT-dependent activation of androgen receptor (AR)-responsive gene expression in human breast cells (no other congeners tested). However, findings were not concentration-dependent, with the greatest enhancement (~50%) at 10 nM (highest concentration used was 10 μM) (Christen et al. 2010). This suggests that very low concentrations of PBDEs may be androgenic, while higher concentrations appear to be antiandrogenic. No changes in AR gene or protein expression were observed in porcine ovarian follicular cells exposed to BDE 47, 5-OH-BDE-47 or 6-OH-BDE-47 (Karpeta et al. 2014). Anti-progestagenic activity was also observed in an *in vivo* study, where adult female offspring of rats given subcutaneous injections of PBDE 99 at 1 or 10 mg/kg/day from GD 10 to 18 had significantly decreased uterine mRNA expression levels of progesterone receptor (Ceccatelli et al. 2006).

The ability of the commercial pentaBDE mixture DE-71 and the pure congeners, BDE 47, BDE 99, BDE 100, BDE 153, and BDE 154 to compete with 1.0 nM [^{3}H]R1881 for binding to the rat AR was evaluated using a cytosolic extract prepared from rat ventral prostate tissue (Stoker et al. 2005). Relative binding curves suggested that all of the compounds competed with [^{3}H]R1881 for binding to the AR, with inhibition up to 80 and 98% for DE-71 and BDE 100, respectively. In order to determine if this inhibition was competitive, additional *in vitro* binding tests for BDE 100 were conducted to calculate the inhibition constant (K_i). These experiments showed that BDE 100 was a competitive inhibitor with a K_i value of 1 μM (Stoker et al. 2005).

Anti-glucocortogenic activity was observed in 3/8 PBDEs (PBDE 85, PBDE 99, PBDE 100), 2/4 OH-PBDEs (4OH-BDE-17, 4OH-BDE-49), and 1/4 MeO-PBDEs (4MeO-BDE-49) in a glucocorticoid receptor-mediated reporter gene assay with CHO cells (Kojima et al. 2009). Anti-glucocortogenic potencies were 3 orders of magnitude lower than for the reference compound RU-486.

In summary, the mechanistic studies show that PBDEs and/or their metabolites are capable of acting as thyroid hormone transporters or receptors and are weakly estrogenic, anti-androgenic, anti-prostagenic, and anti-glucocortogenic. However, these findings were not always consistent between different congeners, metabolites, and studies. Therefore, mechanisms of endocrine disruption by PBDEs have not been fully elucidated.

Mechanisms of Neurotoxicity. As detailed in Section 3.2.2.4 Neurological Effects, developmental exposure to PBDEs has been associated with altered neurodevelopment and behavior later in life in both humans and animals. The mechanisms for these behavioral and cognitive effects have not been elucidated; however, proposed mechanisms include neuroendocrine disruption (including altered thyroid hormone homeostasis), alterations in neurotransmitter systems (cholinergic, dopaminergic, glutamatergic, and/or gabaergic), altered calcium homeostatic mechanisms, altered intracellular communication, oxidative stress, and cell death. Additionally, monohydroxylated metabolites are more potent than the parent BDE in several of the mechanistic assays, suggesting that bioactivation by oxidative metabolism contributes to the neurotoxic potential of PBDEs.

Since altered thyroid hormone levels have been reported in both animals and humans (see Section 3.2.2.2 Systemic Effects, Endocrine subsection), one possible mechanism of neurotoxicity involves the well-documented key role of thyroid hormones in brain development. In support of this mechanism, BDE 99 down-regulates the transcription of the thyroid receptors α1 and α2 (TRα, TRα2) in cultured rat cerebellar

granular cells, leading to disruption in the expression of T_3-mediated genes, including decreased brain-derived neurotrophic factor (BDNF) (Blanco et al. 2011). BDE 99 and hydroxylated PBDE metabolites (3-OH-BDE-47, 6-OH-BDE-47, and 5'-OH-BDE-99) have also been shown to significantly decrease the activity of the selenodeiodinase type 2 iodothyronine deiodinase (DIO2), which converts T_4 to T_3 in the brain, in primary human astrocytes and H4 glioma cells; BDE 47 did not affect DIO2 activity (Roberts et al. 2015). Decreased DIO2 activity associated with exposure to BDE 99 and OH-PBDEs was attributed to multiple mechanisms, including observed downregulation of *DIO2* mRNA, competitive inhibition of DIO2, and enhance post-translational degradation of DIO2. Ibhazehiebo et al. (2011) showed that the TR-antagonist, BDE 209 (see *Mechanisms of Endocrine Disruption* section above), inhibits T_4-induced dendritic arborization in cultured rat cerebellar Purkinje cells. However, BDE 47, which was not found to be a TR-antagonist (see *Mechanisms of Endocrine Disruption* section above), did not alter dendritic arborization (Ibhazehiebo et al. 2011). Study authors indicate that other PBDEs and OH-PBDEs were evaluated for dendritic developmental effects; however, results were not reported (Ibhazehiebo et al. 2011). Additionally, Schreiber et al. (2010) showed that reduced migration and differentiation observed in cultured fetal human neural progenitor cells (hNPCs) following exposure to BDE 47 and BDE 99 was prevented with co-exposure to T_3. These findings indicate that *in vitro* neurodevelopmental changes were due to disruption of cellular thyroid hormone signaling. However, BDE 209 did not significantly interfere with the T_3-mediated response in either a human embryonic kidney reporter cell line expressing mouse TRα (HEK293-Gal4TRα) or a mouse cerebellar neural reporter cell line expressing TRα (C17.2α-HRLuc), with or without metabolic activation (Guyot et al. 2014). Preliminary studies also did not show altered T_3-mediated responses in C17.2α-HRLuc cells incubated with a commercial PBDE mixture (BDE-CM, AccuStandard, Inc.; according to manufacturer datasheet, the mixture contains equal parts BDE 28, 47, 99, 100, 153, 154, 183, and 209) (Guyot et al. 2014). Additionally, only slight, but statistically significant, changes were observed in the expression of T_3-responsive genes in C17.2α cells following exposure to BDE 209 and T_3, compared with T_3 exposure alone (Guyot et al. 2014).

Some studies suggest that the neurological effects of PBDEs might be related to alterations in cholinergic functions. For example, neonatal exposure to a single oral dose of BDE 99 (8 mg/kg) on PND 10 or a single oral dose of BDE 209 (≥5.76 mg/kg) on PND 3 altered the behavioral response to cholinergic agents (nicotine or paraoxen) in adult mice (Buratovic et al. 2014; Johansson et al. 2008; Viberg et al. 2002, 2007). Neonatal exposure to nicotine and adult exposure to BDE 99 (single 8 mg/kg oral dose at age 5 months) also affected behavior in mice, although the change was not seen in mice only exposed to BDE 99 as adults or mice only exposed to nicotine as neonates (Ankarberg et al. 2001). Additionally, the densities of cholinergic nicotinic receptors in the brain hippocampus and/or cortex were decreased by 7–

31% in adult mice exposed once to BDE 99 or BDE 153 at ≥0.8 mg/kg on PND 10 (Fischer et al. 2008; Viberg and Eriksson 2011;Viberg et al. 2003a, 2004b, 2005) and increased transcription of cholinergic receptors nAchR-β2, AChR5, and nAChR-α4 were observed in the cortex of 2-month-old mice exposed to BDE 99 at 12 mg/kg on PND 10 (Hallgren et al. 2015). However, no exposure-related effects were observed in any cholinergic parameters in 6- or 27-week-old mink from mink sows exposed to dietary pentaBDE at 0, 0.01, 0.05, or 0.25 mg/kg/day from pre-mating day 28 to PNW 6 (Bull et al. 2007). In rat neuroendocrine pheochromocytoma (PC12) cells and rat neuroblastoma (B35) cells, BDE 209 significantly decreased the Ach-evoked response *in vitro* (Hendriks et al. 2014). Additionally, *in vitro* exposure to BDE 99 led to altered neurotransmitter phenotype differentiation in rat PC12 cells, resulting in a decreased number of cholinergic cells and a greater number of dopaminergic cells; this was not observed with exposure to BDE 47 (Dishaw et al. 2011; Slotkin et al. 2013). BDE 47 also did not modulate human $\alpha_4\beta_2$ nicotinic acetylcholine (nACh) receptor function (expressed in *Xenopus* oocytes); however, its hydroxylated metabolite, 6-OH-PBDE-47, was a nACh antagonist (Hendriks et al. 2010).

Other studies have reported alterations in the dopaminergic system. Alterations have been observed in the striatum of mice exposure to 30 mg/kg/day of the pentaBDE mixture DE-71 for 30 days via gavage, including reductions in dopamine levels, altered dopamine handling (i.e., altered dopamine breakdown into DOPAC and HVA), and reductions in the striatal dopamine transporter (DAT) and vesicularmonoamine transporter 2 (VMAT2) (Bradner et al. 2013). DE-71 also inhibited the *in vitro* uptake of the neurotransmitter dopamine into rat brain synaptic vesicles; however, inhibition was not observed with commercial mixtures of octaBDE (DE-79) or decaBDE (DE-83R) (Mariussen and Fonnum 2002, 2003; Mariussen et al. 2003). Similarly, DE-71 reduced synaptosomal dopamine concentrations and increased medium dopamine concentrations in striatal synaptosomes derived from PND 7–21 rats (Dreiem et al. 2010). However, in contrast to the cholinergic system, acute neonatal exposure to BDE 99 (12 mg/kg) on PND 10 did not significantly affect dopaminergic gene transcription in the cortex or hippocampus on PND 11 or at 2 months of age; genes evaluated included tyrosine hydroxylase (*TH*), *DAT*, and dopamine receptors D1, D2, and D5 (*DRD*1, *DRD*2, and *DRD*5) (Hallgren and Viberg 2016).

Additional evidence suggests that alterations in the glutamatergic system may also contribute to observed neurological effects following PBDE exposure. Neonatal exposure to BDE 209 via gavage doses of 20 mg/kg/day from PND 3 to 10 resulted in significant upregulation of NR1 mRNA in the frontal cortex and hippocampus of PND 11 and PND 60 mice, as well as decreased binding of the regulatory complex REST/NRST (Repressor Element Silencing Transcription Factor/Neuron-Restrictive Silencer Factor) to the NR1 promotor (Verma et al. 2015). Additionally, extracellular glutamate levels (along with oxidative

stress and cell death) were significantly elevated in cultured mouse cerebellar neurons following *in vitro* exposure to BDE 47 (Costa et al. 2016). Antagonists of ionotropic glutamate receptors, but not metabotropic, prevented BDE 47-induced oxidative stress and cell death, providing additional evidence for a role of glutamatergic signaling in PBDE neurotoxicity (Costa et al. 2016). In rats, exposure to BDE 47 at doses ≥0.1 mg/kg/day for 30 days via gavage resulted in altered mRNA expression of the glutamate receptor subunits NR(1), NR(2)B, and NR(2)C (Yan et al. 2012).

Limited evidence suggests that alterations in the gabaergic system may also contribute to observed neurological effects following PBDE exposure. A commercial pentaBDE mixture (DE-71) caused a slight inhibition of *in vitro* uptake of the neurotransmitter GABA into rat brain synaptic vesicles, but commercial mixtures of octaBDE (DE-79) or decaBDE (DE-83R) did not alter GABA uptake (Mariussen and Fonnum 2003). Additionally, the metabolite, 6-OH-PBDE-47, was a partial agonist for the human $GABA_A$ receptor expressed in *Xenopus* oocytes; however, its parent compound (BDE 47) did not modulate $GABA_A$ receptor activity (Hendriks et al. 2010).

Effects of PBDEs on the function and development of the nervous system could also involve disruption of calcium homeostatic mechanisms and intracellular signaling events. In human neural progenitor cells (hNPCs), *in vitro* exposure to BDE 47 or 6-OH-BDE-47 led to transient increases in intracellular Ca^{+2} levels due to increased influx of extracellular Ca^{+2} as well as intracellular release from the endoplasmic reticulum (Gassmann et al. 2014). Based on additional studies using multiple inhibitors/stimulators of presumably involved signaling pathways, the increase in extracellular influx appears to be due to interference with the cell membrane, rather than alterations of Ca^{+} ion channel dynamics, and the increased ER release was associated with activation of protein lipase C and inositol 1,4,5-trisphophate receptor, independent of the ryanodine receptors (Gassmann et al. 2014). A series of studies evaluated the effects of *in vitro* exposure to PBDEs and their metabolites on calcium homeostasis in rat PC12 cells (Dingemans et al. 2008, 2010a, 2010b). Collectively, these studies show that hydroxylated metabolites of PBDEs lead to increased basal Ca^{+2} levels due to Ca^{+2} release from the endoplasmic reticulum and mitochondria as well as decreased depolarization-evoked Ca^{+2} levels. BDE 47 was shown to have similar, but less potent effects, while no effects on calcium homeostasis were observed with BDE 49, BDE 99, BDE 100, BDE 153, or methylated metabolites (Dingemans et al. 2008, 2010a, 2010b). In a similar study, BDE 209 did not alter calcium homeostasis in rat PC12 or B35 cells (Hendriks et al. 2014). BDE 49, OH-BDE-47, and 4'OH-BDE-49 have also been shown to be potent modulators of ryanodine receptors type 1 and 2, which regulate essential aspects of Ca^{+2} signaling; BDE 47 was without activity in this assay (Kim et al. 2011c; Pessah et al. 2010). In culture systems, BDE 47 and BDE 209 were shown

to increased intracellular Ca^{+2} content in mouse cerebellar granule cells and rat neonatal hippocampal neurons, respectively (Chen et al. 2010; Costa et al. 2016). *In vitro* exposure to DE-71 or BDE 47 also stimulated arachidonic acid release in rat cerebellar granule neurons; this effect was not seen with DE-79 (Kodavanti 2003; Kodavanti and Derr-Yellin 2002). The release of arachidonic acid appeared to be mediated by the activation of both Ca^{+2}-dependent and Ca^{+2}-independent cytosolic phospholipase A_2. *In vitro* exposure to DE-71and BDE 47 also caused translocation of protein kinase C, as indicated by increased phorbol ester binding; DE-79 did not induce this effect (Kodavanti and Derr-Yellin 2002; Rao et al. 2003). Other effects of penta mixture DE-71 and tetra congener BDE 47 included decreases in intracellular calcium buffering by microsomes and mitochondria (Kodavanti and Derr-Yellin 2002). The tetra- congener BDE 47 was generally more potent than the DE-71 mixture (mainly comprised of tetra- and penta- congeners) in these tests. All commercial mixtures and congeners tested (DE-71, DE-79, BDE 47, BDE 77, BDE 99, BDE 153) elevated phosphorylated extracellular signal-regulated kinase (pERK) ½, with congeners having a greater effect than mixtures (Fan et al. 2010). pERK ½ is a widely studied MAPK cascade known to be involved in learning and memory.

Neurotoxicity may be mediated by cell death, perhaps in response to oxidative stress, as increased apoptosis and upregulation of apoptotic proteins and markers of oxidative have been observed in the hippocampus, cortex, and cerebellum of rats following developmental exposure to PBDEs (He et al. 2009; Chen et al. 2014; Cheng et al. 2009; Costa et al. 2015; see Section 3.2.2.6, Developmental Effects for more details). In support, ROS were increased in rat cerebellar granular cells exposed to 25 µM BDE 99, and this increase was correlated with a decrease in the gene expression of the anti-apoptotic protein Bcl-2 (Blanco et al. 2011). Another study found that DE-71 was more toxic than octa- and deca-congeners in inducing cell death and free radical formation in cerebellar granule cells (Reistad et al. 2002). In cultured rat cortical cells, a high concentration of BDE 99 (30 µM) induced cell death without any apparent increase in caspase-3 activity (Alm et al. 2008). BDE 47 also induced apoptosis in primary cultured rat hippocampal neurons; changes in oxidative stress parameters included increased ROS levels, malondialdehyde content, glutathione peroxidase levels and decreased glutathione and superoxide dismutase levels (He et al. 2008c). BDE 47-induced apoptosis in human SH-SY5Y was shown to be mediated via the mitochondrial p53 pathway, as evidenced by up-regulation of p53 and Bax, down-regulation of Bcl-2 and Bcl-2/Bax ratio, enhancement of Cyt c release from mitochondria into the cytosol, and activation of caspase-3, as well as by ultrastructural abnormalities of mitochondria (Zhang et al. 2013c). In mouse cerebral granule cells, PBDE exposure caused decreased cell viability, induced apoptotic cell death, and increased ROS and lipid peroxidation following exposure to BDE 47, BDE 99, BDE 100, BDE 153, and BDE 209 (Costas et al. 2015, 2016; Huang et al. 2010). Huang et al. (2010)

reported a congener potency ranking of BDE 100 > BDE 47 > BDE 99 > BDE 153 >> BDE 209. Similarly, in cultured rat neonatal hippocampal neurons, BDE 209 decreased cell viability and increased the rate of apoptosis, ROS levels, malondialdehyde (MDA) content, and NO content (MAPKs) (Chen et al. 2010). In rat B35 cells, increased ROS generation in the absence of decreased cell viability was observed following exposure to BDE 209; no changes in ROS generation or cell viability were observed in rat PC12 cells (Hendriks et al. 2014).

Proteomic and genomic studies have identified multiple systems/pathways in the brain that can be altered following PBDE exposure; however, strong conclusions regarding mechanisms of neurotoxicity from these studies cannot be made at this point. In laboratory animals, *in vivo* exposure to ≥12 mg/kg/day of DE-71, BDE 99, BDE 203, BDE 206, or BDE 209 for 1–30 days has been shown to alter expression of proteins involved in mediating GABA and glutamate neurotransmission in the frontal cortex; neuronal survival, growth, and synaptogenesis in the cortex, hippocampus, cerebellum, and striatum; cytoskeletal proteins in the cortex and hippocampus; oxidative stress and apoptosis in the cerebellum and hippocampus; and metabolism and energy production in the hippocampus and cerebellum (Alm et al. 2008, 2006; Buratovic et al. 2014; Bradner et al. 2013; Kodavanti et al. 2015; Verma et al. 2015; Viberg 2009a, 2009b; Viberg and Eriksson 2011; Viberg et al. 2008). In mice exposed to 0.45 mg/kg/day for 28 days, combined analysis of proteomic and genomic data using biological network analysis indicated disturbances in the following functional categories: nervous system development and function, neurological disease, and behavior (Rasinger et al. 2014). Gene ontogeny analysis showed significant changes in mitochondrion morphogenesis, Wnt receptor signaling pathway, L-glutamate transport, and calcium ion transport into cytosol, while proteomics identified differential expression of dynamin 1, calcium/calmodulin-dependent protein kinase II delta, and alpha 4a tubulin (Rasinger et al. 2014). In culture systems, proteomic analysis of neonatal hippocampal neural stem/progenitor cells following exposure to BDE 47 and/or BDE 209 showed differential expression of 19 proteins, including proteins involved in metabolism, signal transduction, transcription, transport, and cell structures (Song et al. 2014). Key proteins showing downregulation were cofilin-1, which is associated with cell cycle and neuronal migration, and vimentin, which is implicated in nervous system repair mechanisms.

Epigenetic changes may also contribute to neurotoxicity following exposure to PBDEs. Byun et al. (2015) examined this hypothesis in mice exposed perinatally to BDE 47 by evaluating DNA methylation patterns in mitochondrial genes involved in respiration (cytochrome c oxidase I, II, and III), nuclear genome methylation markers (5hmc, L1Rn), and nuclear candidate genes related to behavioral and brain functions (BDNF, corticotropin releasing hormone receptor 1, melanocortin 2 receptor, nuclear receptor

subfamily 3 group C member 1, and alpha-synuclein). Significant changes in methylation were not observe in DNA methylation patterns for the majority of genes examined, although slight (<2-fold), but statistically significant, methylation decreases were observed in cytochrome c oxidase II, L1Rn, BDNF, and nuclear receptor subfamily 3 group C member 1 (NR3CL) in mice exposed to BDE 47, compared with control (Byun et al. 2015). However, global DNA methylation was significantly decreased in female, but not male, offspring of mouse dams exposed to tetraBDE from premating day 28 to PND 21 (Woods et al. 2012).

Mechanisms of Reproductive Toxicity. As discussed above (see *Mechanisms of Endocrine Disruption*), PBDEs have been shown to be weakly estrogenic and anti-androgenic. Additionally, altered steroidogenesis has been observed *in vitro.* Limited data are available regarding other potential mechanisms of reproductive toxicity, such as altered placental function due to increased prostaglandin synthesis and impaired mitochondrial function in sperm cells.

Testosterone secretion was increased by up to 3-fold in cultured rat Leydig cells exposed to a mixture of tetra- and pentaBDEs (PBDE-710) (Wang et al. 2011c) or BDE 47 alone (Zhao et al. 2011). These studies suggest that PBDEs may stimulate testosterone secretion by acting directly on Leydig cells to activate the cAMP pathway and increased expression of steroidogenic acute regulatory protein (StAR), as evidence by increased gene and protein expression of StAR (Wang et al. 2011c, Zhao et al. 2011) as well as increased intracellular levels of cAMP, increased PKAα nuclear translocation, and increased activity of CYP 11A1 (Wang et al. 2011c). In support, the observed increase in testosterone secretion was blocked in the presence of the adenylyl cyclase inhibitor SQ22536 (Wang et al. 2011c). However, gene expression of StAR was not upregulated in mouse Leydig cells exposed to BDE 47 (testosterone secretion was not evaluated) (Schang et al. 2016).

Testosterone production was also significantly increased by ~2–4.5-fold in porcine ovarian follicles exposed *in vitro* to BDE 47, BDE 99, or BDE 100 (Karpeta et al. 2011). Androstenedione (A4) and progesterone production was also significantly increased, but responses were not concentration-dependent. No exposure-related changes were observed in E2 production. Potential mechanisms underlying increased testosterone production were judged to be congener-specific. For BDE 47, observed increases in 17β-HSD protein expression and activity and decreases in CYP19 (aromatase) activity suggest that increased testosterone production is due to increased conversion of A4 to testosterone by 17β-HSD and decreased conversion of testosterone to E2 by CYP 19. For BDE 100, observed increases in CYP17 protein expression and activity and decreases in CYP19 activity suggest increased testosterone

production is due to increased conversion of progesterone to A4 by 17β-HSD (which is later converted into testosterone) and decreased conversion of testosterone to E2 by CYP 19. BDE 99 did not alter protein expression or activity of 17β-HSD, CYP 17, or CYP 19. The study authors suggest that the mechanism of action for BDE 99 is prior to the secretion of progesterone (i.e., StAR or 3β-HSD). In contrast, OH-PBDEs (5OH-BDE-47, 6OH-BDE-47) did not alter testosterone or A4 production in porcine ovarian follicles; however, E2 production was significantly increased for both metabolites by 2.4–2.9-fold (Karpeta et al. 2013). Additionally, the OH-PBDEs increased protein expression and activity of CYP 19, suggesting that the increase in E2 may be due to increased aromatase-mediated conversion of testosterone to E2. Similarly, changes in E2 secretion in mid-phase porcine luteal cells (increased) and late-phase porcine luteal cells (decreased) following *in vitro* exposure to BDE 47 was significantly correlated with CYP 19 activity, although neither 5-OH-BDE-47 nor 6-OH-BDE-47 altered E2 secretion (Gregoraszczuk et al. 2015). In an *in vivo* study, CYP 19 activity in the ovaries of female rats was not altered with exposure to BDE 209 doses up to 60 mg/kg/day for 28 days via gavage; however, CYP 17 activity in the adrenal glands was significantly decreased by up to 97% in females, but not males (Van der Ven et al. 2008a).

Progesterone production was significantly increased by 1.3–2.1-fold in mid-phase porcine luteal cells exposed to BDE 47 (≥250 ng/mL) or BDE 99 (≥50 ng/mL) for 24 hours, but not 48 hours (Gregorasczuk et al. 2012). Exposure to BDE 100 did not alter progesterone levels at concentrations up to 500 ng/mL at either time point. Following exposure for 24 hours, cells exposed to BDE 99 also showed a significant increase in the activity and protein level of 3β-HSD (which converts pregnenolone into progesterone); no changes in protein levels or activity of CYP11A1 (which converts 25-hydroxycholesterol into progesterone) were observed for any congener. Increased gene expression of 3β-HSD was also observed in mouse Leydig cells exposed to BDE 47; however, no exposure-related changes were observed in progesterone secretion (Schang et al. 2016). Another study did not observe increased progesterone production in early-, mid-, or late-phase porcine luteal cells exposed to BDE 47 for 24 hours at doses up to 50 ng/mL (lower than the lowest effective dose in the previous study) (Gregorasczuk et al. 2015). However, metabolites of BDE 47 (5-OH-BDE-47 and 6-OH-BDE-47) significantly decreased progesterone secretion in mid- and late-phase cells, respectively; 6-OH-BDE-47 also significantly inhibited 3β-HSD activity in late-phase cells (Gregorasczuk et al. 2015). Gregorasczuk et al. (2012) also measured activity of caspases 3, 8, and 9 in porcine luteal cells collected during the middle luteal phase following exposure to BDE 47, 99, or 100. Increased activation of both intrinsic (caspase 9) and extrinsic (caspase 8) apoptotic pathways was observed at 24 hours, with additional activation of caspase 3 at 48 hours, was observed with all congeners, indicating premature apoptosis of middle luteal cells

(apoptosis should occur during late luteal phase). The study authors proposed that the combined effects of altered steroidogenesis and premature apoptosis observed following *in vitro* exposure to PBDEs could negatively impact the estrous cycle by shortening the luteal phase, and potentially fecundability.

Several studies have also evaluated steroidogenesis in human adrenocortical carcinoma cells following exposure to PBDEs or their metabolites. The parent compound, BDE 47, did not alter testosterone, androstenedione, E2, pregnenolone, 17α-OH-pregnenolone, progesterone, or 17α-OH- progesterone levels in human adrenocortical carcinoma cells (van den Dungen et al. 2015). As observed in porcine follicular cells, increased E2 production accompanied by increased CYP 19 gene expression and activity was observed in human adrenocortical carcinoma cells following exposure to the methyoxylated metabolites 6Cl-2MeO-BDE-68 and 6MeO-BDE852/10 (He et al. 2008c). However, other tested metabolites (6Cl-2OH-BDE-7, 5Cl-6OH-BDE-47, 2MeO-BDE-28) showed decreased E2 production (He et al. 2008c). Testosterone production was increased by 2/10 tested MeO-PBDEs (6MeO-BDE85, 6MeO-BDE137), but 0/10 tested OH-PBDEs (He et al. 2008c). The activity of the steroidogenic enzyme CYP 19 was also evaluated in cultured H295R human adrenocortical carcinoma cells exposed to 19 PBDEs, 5 OH-PBDEs, and one methoxylated (MeO-)PBDE (Canton et al. 2005). Two low-brominated PBDEs (BDE 19, BDE 28) induced CYP 19 by 200%, while two higher-brominated PBDEs (BDE 206, BDE 209) inhibited CYP 19 by 61–64%. 6OH-BDE-99 and 6MeO-BDE-47 also inhibited CYP 19 by 46–67%. The 6OH-BDE-47 metabolite showed minimal inhibition accompanied by cytotoxicity. In another study evaluating 11 OH-PBDEs and 11 MeO-PBDEs, all OH-PBDEs inhibited CYP 19 (Canton et al. 2008). The most potent aromatase inhibitors were 6OH-BDE-49 and 3OH-BDE-47, which decreased CYP 19 activity by 54 and 27%, respectively. No CYP 19 inhibition was observed with the MeO-PBDEs.

Park and Loch-Caruso (2015) evaluated the effects of BDE 47 exposure on prostaglandin E2 synthesis in first trimester human extravillous trophoblast cells (HTR-8/SVneo), as prostaglandin E2 is a pro-inflammatory regulator of trophoblast cellular functions critical for successful placentation. Significantly increased prostaglandin E2 release was following *in vitro* exposure to BDE 47. mRNA expression of enzymes involved in prostaglandin synthesis and catabolism were also significantly altered, including increases in prostaglandin synthase 2 and COX-2 (rate-limiting enzyme of prostaglandin synthesis) and decreased 15-hydroxyprostaglandin dehydrogenase and prostaglandin E synthase. Prostaglandin changes were completely blocked following treatment with a COX-2 inhibitor, confirming that prostaglandin upregulation was COX-dependent. Additionally, significant ROS generation was observed following BDE 47 exposure. The peroxyl radical scavenger, α-tocopherol, blocked both ROS generation and

prostaglandin release; however, it had no effect on COX-2 mRNA levels. The study authors proposed that generation of ROS in the placenta following BDE 47 exposure stimulates prostaglandin E2 production via post-translational modifications of a COX-2-dependant pathway, and may lead to placental dysfunction (Park and Loch-Caruso 2015).

Following 8-week oral exposure to BDE 47, increased apoptosis was observed in early leptotene spermatocytes of adult male rats (Huang et al. 2015). Proteomic analysis of testicular tissue indicated differential expression of 64 proteins, including 20 proteins related to apoptosis. Of these 15/20 apoptotic proteins were located in the mitochondria. Protein expression data from cultured testicular mouse cells (GC1-spg cells) were consistent with *in vivo* results, showing significant decreases in Uqcrc1, Atp5b, Tufm, Sucla2, and Lap3, indicating mitochondrial dysfunction and apoptosis, and a significant decrease in the anti-apoptotic factor Bcl-2 (Huang et al. 2015).

Mechanisms of Immunotoxicity. As discussed in Section 3.2.2.3, Immunological and Lymphoreticular Effects, limited evidence from animal studies suggest that PBDEs may cause immunosuppression (Darnerud and Thuvander 1998; Feng et al. 2016b; Fowles et al. 1994; Watanabe et al. 2008, 2010b). Lundgren et al. (2009) suggested that decreased immune response to infection may be due to suppression of cytokines by PBDE exposure. In mice infected with human coxsackievirus B3 (CVB3), serum levels of IL-12, MIP-1β, RANTES, IFN-γ, and KC were markedly decreased (or completely lacking) in mice exposed to BDE 99 or Bromkal 70-5 DE (37% BDE 47, 35% BDE 99), compared with infected controls.

No information regarding potential mechanisms of immunotoxicity for PBDEs were located. Howie et al. (1990) examined the immunotoxic potencies of various polychlorinated diphenyl ether congeners on the inhibition of the plaque-forming splenic cell response to sheep red blood cell antigen in mice. The observed potency order (2,3,3',4,4',5-hexaCDE > 3,3',4,4',5-pentaCDE > 2,3',4,4',5-pentaCDE > 3,3',4,4'-tetraCDE > 2,2',4,4',5,5'-hexaCDE > 2,2',4,5,5'-pentaCDE > 2,2',4,4',5,6'-hexaCDE) generally paralleled the congener-specific potencies for induction of hepatic microsomal AHH and EROD. Worth noting is the fact that the resulting ranking order of potency did not follow the order that would have been expected for a response known to be AhR-mediated, such as the inhibition of the plaque-forming splenic cell response to challenge with sheep red blood cells antigen. For example, the laterally substituted congeners 3,3',4,4'-tetraCDE and 3,3',4,4',5-pentaCDE were less immunotoxic than their respective monoortho-substituted analogs; this was true also for their enzyme induction potencies. These findings showed that increasing *ortho*- substitution is less effective in reducing the “dioxin-like” activity of these compounds. Howie et al. (1990) suggested that the ether bridge in the polychlorinated diphenyl ether

molecules increases the bond length between the two phenyl rings, thus diminishing the effects of *ortho* substituents on the biochemical and toxic potencies of these compounds. However, these findings may or may not be relevant to immunotoxic activities of PBDEs because of the considerable difference in the molecular size of brominated and chlorinated analogues, which may influence receptor-mediated effects, as well as potential toxicokinetic differences.

Mechanisms of Hepatotoxicity. As discussed in Section 3.2.2.2 Systemic Effects (hepatic subsection), liver effects have been reported in adult, pregnant, and developing animals exposed to lower-brominated PBDEs. Evidence for hepatic effects in animals following exposure to decaBDE is less consistent.

Existing information suggests that PBDEs can be classified as mixed-type inducers of hepatic microsomal monooxygenases (see General Mechanisms of Toxicity Section). Observed effects of liver enlargement and hepatocellular hypertrophy are consistent with enzymatic induction. However, oxidative stress, inflammatory processes, and induction of apoptosis may also contribute to hepatotoxicity of PBDEs. In 12-week gavage studies in mice, administration of troxerutin, an antioxidant and anti-inflammatory agent, prevented tetraBDE-induced hepatotoxicity (increased liver weight, increased serum ALT, hepatocyte hypertrophy and vacuolization, and inflammatory cell infiltration) (Zhang et al. 2015a, 2015b). Moreover, tetraBDE-mediated induction of protein and genetic markers of oxidative stress, inflammation, proteasomal subunits, ER-stress pathways, and apopotisis are prevented by troxerutin. Zhang et al. (2015b) proposed that upregulation of NF-kB via the tetraBDE-induction of histone 3 acetylation at lysine 9 (H3K9) is due to tetraBDE-mediated decreases in SirT1 (which mediates deacetylation), and that this upregulation of NF-kB leads to the observed increases in mRNA levels of inflammation-related genes. Additionally, Zhang et al. (2015a) suggested that oxidative stress leads to apoptosis via proteasome dysfunction-mediated ER stress. Taken together, the therapeutic mechanism of actions for troxerutin could include antioxidant actions and restoration of proteasomal function, as well as the activation of SirT1. These are supported by similar decreases in markers of oxidative stress following vitamin E treatment as well as the blunting of troxerutin-mediate effects with concurrent EX527 injections (a SirT1-specific inhibitor) or epoxomicin injections (a selective proteasome inhibitor) (Zhang et al. 2015a, 2015b). *In vitro* studies also report elevated ROS levels, depleted GSH levels, mitochondrial damage and dysfunction, and apoptosis in HepG2 human hepatocellular carcinoma cells exposed to BDE 47 (Liu et al. 2015; Saquib et al. 2016; Yeh et al. 2015). As observed *in vivo*, known antioxidants (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) protect cultured HepG2 human hepatocellular carcinoma cells from BDE-47-induced oxidative stress and mitochondrial dysfunction (Yeh et al. 2015). However, Pereira et al. (2014) suggest that apoptosis may be mediated via direct

interaction of PBDEs with mitochondria, rather than secondary to PBDE-mediated oxidative stress. In isolated rat liver mitochondria, BDE 154 directly interacted with the mitochondrial membrane, permeabilizing the membrane and leading to ATP depletion. These events occurred in the absence of ROS accumulation in the mitochondria, suggesting that BDE 154 impairs mitochondrial bioenergetics and permeabilizes the mitochondria, potentially leading to cell death, in the absence of oxidative stress (Pereira et al. 2014). BDE 47 and BDE 99 have also been shown to directly interact with the mitochondrial membrane in isolated rat liver mitochondria, resulting in altered mitochondrial bioenergetics and ATP depletion (Pazin et al. 2015).

3.5.3 Animal-to-Human Extrapolations

Residues of PBDEs in humans reflect multiple exposure pathways and congener-specific elimination and thus, in general, represent steady-state body burdens that do not match the congener profiles in the original exposure sources. For example, profiles of PBDE congeners in human milk do not resemble the pattern of any of the previously used commercial mixtures, which were predominantly pentaBDE, octaBDE, and/or decaBDE, as illustrated by the finding that the major PBDE congener in milk from Swedish mothers was 2,2',4,4'-tetraBDE (BDE 47), which comprised approximately 55% of the total PBDEs (Darnerud et al. 1998). As discussed in Chapter 6, residue analyses indicate that tetra- to hexa-congeners predominate in humans, aquatic mammals, birds, fish, and other biota, indicating that the biological fate of PBDE congeners is qualitatively similar in various animal species. The wildlife residue data also indicate that different species have different tissue ratios of congeners, possibly reflective of interspecies differences in metabolic capabilities as well as potential differences in exposure. The likelihood of interspecies differences in the quantitative disposition of PBDEs is illustrated by the observation that metabolism and urinary excretion of a single oral dose of BDE 47 was significantly slower in rats than in mice (Orn and Klasson-Wehler 1998; Staskal et al. 2006b).

While alterations in thyroid hormone levels are consistently altered in in PBDE-exposed animals, human data are less consistent. Humans are possibly less sensitive than rats to effects of PBDEs on circulating levels of thyroid hormones. This difference is thought to derive from the rat thyroid having a smaller store of iodinated thyroglobulin that is more easily depleted when the availability of iodide is limited, and from a more rapid clearance of T_4 from the rat circulation; the latter resulting from rats not having a high affinity binding protein for T_4 in serum analogous to TBG in humans (Capen 1997). If the production of T_4 and T_3 is impaired sufficiently to deplete the thyroid of stored iodinated thyroglobulin, the thyroid cannot produce or secrete amounts of T_4 and T_3 needed to support physiological demands, circulating

levels of T_4 (free T_4) and T_3 decrease, and a state of thyroid hormone insufficiency ensues. TTR is the major thyroid hormone binding protein in rats, but not in man. In most mammals, including humans, TBG is the principal thyroid hormone binding protein; 74% of the total bound-T_4 is bound to TBG, and TTR and albumin bind only 11 and 15%, respectively, of the total (Schussler 2000). In contrast to most mammals, the rat utilizes TTR as the major T_4 plasma binding protein; approximately 75% of T_4 in rat serum is bound to TTR and only 25% to albumin. Both circulating T_3 and T_4 are highly protein bound with only a small fraction of their total present as free hormone, and this high degree of protein binding serves to maintain equilibrium between the extracellular and intracellular pools of these hormones (O'Connor et al. 1999).

Less is known about the relative sensitivities of humans and experimental animals to developmental effects of PBDEs. Outstanding uncertainties include potential differences in kinetics of maternal-fetal and maternal-infant transfer of PBDEs, as well as potential differences in the degree to which the fetus of the human, in comparison to experimental animals, is dependent on maternal thyroid hormone for development, particularly during the period of gestation prior to the onset of fetal hormone production.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens

(Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Concern has been raised that many industrial chemicals, including PBDEs, are endocrine-active compounds capable of having widespread effects on humans and wildlife (Colborn et al. 1993; Crisp et al. 1998; Daston et al. 1997; Safe and Zacharewski 1997). Particular attention has been paid to the possibility of these compounds mimicking or antagonizing the action of estrogen. Estrogen influences the growth, differentiation, and functioning of many target tissues, including female and male reproductive systems, such as mammary gland, uterus, vagina, ovary, testes, epididymis, and prostate. In addition, there is evidence that some of these environmentally-persistent chemicals alter the thyroid hormone system, which is a very important system for normal structural and functional development of sexual organs and the brain.

As discussed in Chapter 2 and Section 3.2.2.2. (Systemic Effects), the thyroid is a target of concern following exposure to PBDEs, with adverse effects including altered thyroid hormone expression (predominantly reduced serum T_4) and histological changes in the thyroid indicative of glandular stimulation (e.g., follicular cell hyperplasia similar to that induced by a hypothyroid state). Additionally, as discussed in Chapter 2 and Section 3.2.2.6 (Developmental Effects), the developing nervous system and the developing reproductive system are also targets of concern. Since these effects could be mediated by the neuroendocrine axis, several studies have tested PBDEs and their metabolites in *in vitro* endocrine disruption screens and *in vivo* gene expression assays. These studies, and their results, are summarized in Section 3.5.2 (Mechanisms of Toxicity) in the subsections on Mechanisms of Endocrine Disruption (thyroid hormone assays; anti-estrogenic, -androgenic, -progestagenic, and -glucocortogenic assays) and Mechanisms of Reproductive Toxicity (steroidogenesis assays). While results are not always consistent between studies, the data collectively indicate that there is a potential for some PBDEs to disrupt thyroid and other endocrine system functions in humans.

Additionally, a few studies specifically evaluated neuroendocrine disruption *in vitro*. BDE 47 and BDE 99 altered neurodevelopment of cultured fetal hNPCs (Schreiber et al. 2010). The migration distance of the hNPCs was reduced by up to 30% by BDE 47 and up to 35% by BDE 99. Differentiation of hNPCs into neurons was reduced up to 50% by BDE 47 and up to 68% by BDE 99, and differentiation of hNPCs into oligodendrocytes was reduced up to 51% by BDE 47 and up to 93% by BDE 99. Co-exposure with T_3 prevented the effects of PBDE exposure on migration and differentiation, indicating that neurodevelopmental changes were due to endocrine disruption of cellular thyroid hormone signaling. Similarly, Ibhazehiebo et al. (2011) showed that the TR-antagonist, BDE 209 (see discussion in Mechanisms of Endocrine Disruption), inhibits T_4-induced dendritic arborization in cultured rat cerebellar Purkinje cells. However, BDE 47, which was not found to be a TR-antagonist (see discussion in Mechanisms of Endocrine Disruption), did not alter dendritic arborization (Ibhazehiebo et al. 2011). Study authors indicate that other PBDEs and OH-PBDEs were evaluated for dendritic developmental effects, but results were not reported (Ibhazehiebo et al. 2011). In another study, hyperosmotic-stimulated somatodendritic vasopressin release in supraoptic tissue from the hypothalamus of rats was significantly reduced by 40–50% by BDE 46, BDE 77, and the commercial pentaBDE mixture DE-71 (no other PBDEs tested) (Coburn et al. 2007). The neuropeptide vasopressin, which is synthesized in magnocellular neuroendocrine cells, functions to maintain body fluid homeostasis, cardiovascular control, learning and memory, and nervous system development.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less

susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The fetus/infant has an immature (developing) blood-brain barrier that past literature has often described as being leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-CNS interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the blood-brain barrier, there are differences between fetuses/infants and adults which are toxicologically important. These differences mainly involve variations in physiological transport systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; as it raises a very important toxicological question as to whether these mechanisms provide protection for the developing brain or do they render it more vulnerable to toxic injury. Each case of chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Body burden data, as well as intake modeling, suggest that infants and toddlers have higher exposures to PBDEs as compared to older children or adults (EPA 2010; Lorber 2008; Trudel et al. 2011; Wong et al. 2013). Exposure during development may occur by transfer of PBDEs that have accumulated in women's bodies to the fetus across the placenta (Antignac et al. 2009, 2008; Chen et al. 2013; Kawashiro et al. 2008; Li et al. 2013a; Mazdai et al. 2003; Meijer et al. 2008; Qiu et al. 2009; Vizcaino et al. 2011; Wan et al. 2010). Placental transfer, although it may be limited in absolute amounts (Frederiksene et al. 2010), is a concern because of possible effects of PBDEs on sensitive immature tissues, organs, and systems, with potentially serious long-lasting consequences. Because PBDEs are lipophilic substances, they can additionally accumulate in breast milk and be transferred to nursing infants (Antignac et al. 2008, 2009; Malarvannan et al. 2013; Park et al. 2011; Schecter et al. 2010). Transfer of PBDEs via breast milk could be considerable and, like prenatal exposure, has the potential to contribute to altered development. Toddlers and older children are exposed to PBDEs in the same manner as the general population, primarily via ingestion of contaminated dust and consumption of contaminated foods (EPA 2010; Lorber 2008). However, exposure from these sources may be greater in young children due to: (1) greater hand-to-mouth behavior, increasing the risk of ingestion of contaminated dust and/or residues from PBDE-

treated materials; and (2) increased exposure relative to their body weight via diet due to increased caloric intake normalized to body weight and a higher general intake of animal fats. Additionally, children may be particularly vulnerable to PBDEs because, compared to adults, they are growing more rapidly and are generally expected to have lower and distinct profiles of biotransformation enzymes, as well as much smaller fat depots for sequestering these lipophilic chemicals.

In human studies, significant associations between umbilical cord and placental PBDE (BDE 28, BDE 47, BDE 99, BDE 153, BDE 183, and total) and adverse birth outcomes were reported in a comparison of 128 normal births and 25 cases of adverse birth outcomes (low birth weight, premature birth, still birth) (Wu et al. 2010; Xu et al. 2015a). Elevated maternal serum BDE 47 levels were also significantly associated with an increased risk of preterm birth in a case-control study of 197 full-term births and 82 pre-term births in Nashville, Tennessee (Peltier et al. 2015). A study of births from 20 healthy pregnant women in Taiwan found that elevated PBDE concentrations (BDE 47, BDE 99, BDE 100, and BDE 209) in breast milk was associated with significantly reduced infant birth weight, length, chest circumference, and Quetelet's index (i.e., BMI) (Chao et al. 2007). A significant negative association between breast milk PBDE concentrations (BDE 47, BDE 99, BDE 100, BDE 153, and their sum) and birth weight was also identified in a Swedish cohort of 254 women with median total PBDE levels of 2.4 ng/g lipid (Lignell et al. 2013). Several studies have also reported a negative association between one or more PBDE congeners in maternal serum and birth weight, length, and/or head circumference: a prospective reproduction study of 234 couples in Michigan and Texas (Robledo et al. 2015a), a birth cohort of 215 Chinese women with a median total PBDE level of 21.68 ng/g lipid (Chen et al. 2015), and a birth cohort of 686 Spanish women (Lopez-Espinosa et al. 2015). In other studies, no significant associations were observed between birth weight, length, or head circumference and maternal or cord serum PBDE concentrations (Foster et al. 2011; Harley et al. 2011; Mazdai et al. 2003; Miranda et al. 2015; Serme-Gbedo et al. 2016; Tan et al. 2009). In another study, a positive association between birth weight and length and colostrum PBDE (BDE 47, BDE 99, BDE 100, BDE 153, and their sum) was observed in a Tanzanian cohort of 95 women with a median total PBDE concentration of 19.8 ng/g lipid (Müller et al. 2016).

In general, available data from animal studies do not indicate that PBDEs are embryotoxic or fetotoxic at PBDE doses below doses that elicited maternal toxicity, although occasional observations of reduced pup weight were reported (Argus Research Laboratories 1985a, 1985b; Biesemeier et al. 2011; Bowers et al. 2015; Branchi et al. 2001, 2002, 2005; Breslin et al. 1989; Ellis-Hutchings et al. 2009; Fujimoto et al. 2011; Hardy et al. 2001, 2002; Kodavanti et al. 2010; Koenig et al. 2012; Life Science Research Israel

Ltd. 1987; Poon et al. 2011; Rice et al. 2007; Saegusa et al. 2012; Ta et al. 2011; Talsness et al. 2005, 2008; Tseng et al. 2008, 2013; Wang et al. 2011a; Watanabe et al. 2008, 2010b; WIL Research Laboratories 1986; Woods et al. 2012; Zhou et al. 2002). At PBDE doses that elicited maternal toxicity (15 mg/kg/day in rabbits, 50 mg/kg/day in rats), developmental effects observed included increased post-implantation loss due to late resorptions and skeletal variations commonly associated with maternal toxicity (Breslin et al. 1989; WIL Research Laboratories 1986). A few studies did report effects at doses below maternal toxicity, including delayed ossification and an increased incidence of internal variations in GD 20 rat fetuses exposed to pentaBDE at 2 mg/kg/day from GD 6 to 19 (Blanco et al. 2012), increased postimplantation loss and number of resorptions and decreased number of live fetuses/litter in mice exposed to decaBDE at ≥750 mg/kg/day from GD 7 to 9 (Chi et al. 2011), and subcutaneous edema and delayed ossification in rats exposed to a low purity (77%) decaBDE mixture at 1,000 mg/kg/day from GD 6 to 15 (Dow Chemical Co. 1985; Norris et al. 1975a).

A major target of concern in infants and children is the developing nervous system. Numerous studies have reported results suggestive of an effect of PBDE on neurodevelopment in children. PBDE levels in cord blood, maternal or infant serum, and/or breast milk have been correlated with cognitive score and adaptive behavior deficits in infants (Chao et al. 2011; Gascon et al. 2012; Shy et al. 2011); mental and physical development deficits in infants/toddlers at ages 12, 24, and 36 months (Herbstman et al. 2010); language and social developmental score deficits in toddlers at 24 months (Ding et al. 2015); increased impulsivity in toddlers at 24–36 months (Adgent et al. 2014; Hoffman et al. 2012); poor social competence and ADHD or increased attention problems in 4-year-old children (Cowell et al. 2015; Gascon et al. 2011); decreased IQ and increased hyperactivity in 5-year-old children (Chen et al. 2014); impaired fine motor coordination, verbal memory and comprehension, and sustained attention in 5–7-year-old children (Eskenazi et al. 2013; Roze et al. 2009); and poor attention and executive function deficits in 9–12-year-old children (Sagiv et al. 2015). In one birth cohort, no associations were observed between maternal serum PBDEs and neonatal behavior in 5-week-old infants (Donauer et al. 2015) or autistic behaviors at 4–5-year-old children (Braun et al. 2014); however, children from the same cohort showed associations between maternal serum PBDEs and decreased IQ and increased hyperactivity at 5 years of age (Chen et al. 2014) and executive function deficits at 5–8 years of age (Vuong et al. 2016a). Pre- and peri-natal studies in animals also consistently reported neurodevelopmental effects following exposure to lower-brominated PBDEs and decaBDE at doses ≥0.06 and ≥2.22 mg/kg/day, respectively, including neurobehavioral alterations, delayed ontogeny of reflexes, ultrastructural changes, altered nicotinic receptor density, altered electrophysiology, and altered gene and protein expression levels (Biesemeier et al. 2011; Blanco et al. 2013; Bowers et al. 2015; Branchi et al. 2001, 2002, 2005;

Buratovic et al. 2014; Chen et al. 2014; Cheng et al. 2009; Eriksson et al. 2001, 2002b, 2006; Fischer et al. 2008; Fujimoto et al. 2011; Gee and Moser 2008; He et al. 2009, 2011; Johansson et al. 2008; Koenig et al. 2012; Kuriyama et al. 2004, 2005; Rice et al. 2007; Sand et al. 2004; Ta et al. 2011; Viberg et al. 2002, 2003a, 2003b, 2004a, 2004b, 2005, 2006, 2007; Woods et al. 2012; Xing et al. 2009). Consistent reports of neurological effects have not been found in adult animal studies, and the only studies that did report neurological effects in adults observed altered neurobehavior at doses above those that cause neurodevelopmental effects in pre- and peri-natal studies (26.2 mg/kg/day of pentaBDE and ≥0.1 mg/kg/day of tetraBDE) (Driscoll et al. 2009; Yan et al. 2012).

Neurodevelopmental effects may be mediated through the neuroendocrine axis, as thyroid hormones regulate cell proliferation, migration, and differentiation during development, and maintenance of normal levels is essential to normal growth and development. In support, neuronal migration and differentiation of fetal human neural progenitor cells (hNPCs) were significantly impaired following *in vitro* exposure to tetraBDE (Schreiber et al. 2010), and decaBDE was shown to be a thyroid hormone receptor antagonist that significantly inhibited T_4-induced dendritic arborization in cultured rat cerebellar Purkinje cells (Ibhazehiebo et al. 2011). Additionally, PBDE-induced alterations in thyroid hormone binding to transport proteins and receptors have been demonstrated in several *in vitro* studies (Hamers et al. 2006; Ibhazehiebo et al. 2011; Marsh et al. 1998; Meerts et al. 1998, 2000; Ren and Guo 2012; Ren et al. 2013). In humans, evidence for thyroid hormone disruption in infants is inconclusive. Some studies reported negative associations between developmental exposure to PBDEs and infant serum or cord blood T_4 levels (Abdelouahab et al. 2013; Herbstman et al. 2010; Kim et al. 2011a); however, other studies reported no association (Kim et al. 2011d, 2012a, 2012b, 2015; Lignell et al. 2016; Lin et al. 2011; Mazdai et al. 2003; Shy et al. 2012). Associations between developmental PBDE exposure and infant serum or cord blood T_3 and TSH were similarly inconsistent (Abdelouahab et al. 2013; Eggesbo et al. 2011; Kim et al. 2011d, 2012a, 2012b, 2015; Lignell et al. 2016; Lin et al. 2011; Mazdai et al. 2003; Shy et al. 2012; Stapleton et al. 2011; Turyk et al. 2008). In contrast, reduced serum T_4 levels have been consistently reported in animals exposed to lower-brominated PBDEs during development (Bansal et al. 2014; Blanco et al. 2013; Bondy et al. 2011, 2013; Bowers et al. 2015; Ellis-Hutchings et al. 2006; Kodavanti et al. 2010; Kuriyama et al. 2007; Miller et al. 2012; Poon et al. 2011; Shah et al. 2011; Skarman et al. 2005; Szabo et al. 2009; Wang et al. 2011a; Zhang et al. 2009; Zhou et al. 2002). Some studies also reported reduced serum T_3 levels, although findings are less consistent (Blanco et al. 2013; Bondy et al. 2013; Bowers et al. 2015; Shah et al. 2011; Zhang et al. 2009). Consistent changes in thyroid hormones were not found in animals exposed to decaBDE during development (Fujimoto et al. 2011; Rice et al. 2007; Tseng et al. 2008).

Disruption of circulating hormone levels can have markedly different effects, depending on the stage of development, and even transient disruptions can produce permanent effects, such as mental retardation, impaired motor skills, and hearing and speech impediments (Boyages 2000; Fisher and Brown 2000). Several factors might contribute to a high vulnerability of the fetus and neonate to PBDEs. Relatively brief periods of thyroid hormone insufficiency (e.g., 14 days) can produce measurable neurological deficits in newborn infants (van Vliet 1999). Furthermore, unlike the adult thyroid gland, which contains a relatively large store of T_4 that is sufficient to support circulating levels of hormone for several months, the neonatal thyroid contains only enough hormone to support circulating levels of hormone for ≥1 day (van den Hove et al. 1999; Vulsma et al. 1989). Thus, even acute exposures to a dose of lower-brominated PBDEs sufficient to suppress thyroid hormone production could potentially result in thyroid insufficiency in the neonate. The absorbed dose of lower-brominated PBDEs per unit of body mass is also likely to be higher in infants compared to adults exposed to similar levels of PBDEs because of higher intakes per unit of body mass and exposure from breast milk. It should be noted that screening of all newborn children for hypothyroidism is already a widely accepted and legislatively mandated practice (LaFranchi 1999; Landenson et al. 2000). Newborns are tested for thyroid hormone levels within the first few days of life in the United States and most other developed countries, and treatment is started immediately if indicated (LaFranchi 1999; Landenson et al. 2000).

The human relevance of the thyroid effects of lower-brominated BDEs in animals is unclear. Humans are generally regarded as being less sensitive than rats to effects of PBDEs on circulating thyroid hormones. This is thought to be related to a smaller and more rapid turnover of the hormone pool in the rat thyroid, and to a more rapid clearance of secreted hormone in the rat; the latter being, in part, related to the absence of TBG in rats (Capen 1997). TTR is the major thyroid hormone binding protein in rats, whereas TBG is the main binding protein in man and most other mammals. However, although TTR is a minor thyroid hormone binding protein in humans, it is the principal protein involved in T_4 transport to the brain in both rats and man (Blay et al. 1993; Sinjari et al. 1998). TTR does not transport T_4 from the bloodstream to the brain, but rather is the main T_4 binding protein in cerebral spinal fluid (CSF) in rats and humans. In the rat, T_4 is transported to the brain primarily through the blood-brain barrier, and not via the choroid plexus and CSF (Blay et al. 1993). Also, the mechanism by which lower-brominated BDEs cause decreased serum T_4 might involve hepatic microsomal enzyme induction and consequent increased metabolic formation of hydroxy signaling-metabolites, but humans are not particularly sensitive to this effect.

Based on limited findings from human and animal studies, another target of concern in infants and children exposed to PBDEs is the developing reproductive system. Main et al. (2007) found a significant positive relationship between levels of PBDE in breast milk and congenital cryptorchidism (undescended testes) in male offspring. The study compared levels of 14 PBDE congeners in breast milk of mothers of 62 Danish and Finnish boys with cryptorchidism to mothers of 68 controls from the same population. Significant elevation of concentrations in cases versus controls were seen for the sum of all 14 congeners, for the sum of the 7 most prevalent congeners found in all mothers (BDE 28, BDE 47, BDE 66, BDE 99, BDE 100, BDE 153, BDE 154), and for 5 of the 7 most prevalent congeners. No significant associations were observed between PBDE concentrations in children's adipose tissue and cryptorchidism (Koskenniemi et al. 2015). Other studies of reproductive development found no relationship between concentrations of individual PBDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153) in mid-pregnancy serum samples from California mothers and hypospadias in their male offspring (Carmichael et al. 2010) and no relationship between current serum levels of PBDE and various measures of sexual maturation (e.g., age at thelarche, current breast development, age at menarche) in a small cohort of teen-aged Dutch children (Leijs et al. 2008). However, serum PBDE levels in 6–8-year-old females were significantly associated with delayed onset of puberty in a longitudinal cohort of U.S. girls (Windham et al. 2015a). A study of 55 Dutch boys found significant positive associations between maternal serum levels of BDE 154 collected on week 35 of pregnancy (but not other congeners measured, including BDE 47 and BDE 153, that occurred at higher levels) and serum levels of the sex hormones, E2, free E2, and inhibin B (but not testosterone, LH, FSH, or sex hormone binding globulin) in the baby boys at 3 months of age and testes volume in the boys at 18 months of age, but no effect on penile length at either age (Meijer et al. 2012). Additionally, Warembourg et al. (2016) reported an inverse association between cord serum BDE 209 and total testosterone (but not free testosterone, E2, aromatase index, sex hormone binding globulin, or Anti-Müllerian hormone) in 141 French boys.

In animal studies, reproductive effects were observed in adult F1 offspring exposed to a single dose of pentaBDE at 0.06 mg/kg on GD 6, including reductions in testicular weight, sperm/spermatid number, and daily sperm production in males and a decreased number of secondary follicles and ultrastructural changes in the ovaries in females (although F1 fertility when mated to an unexposed animal was not impaired) (Kuriyama et al. 2005; Talsness et al. 2005, 2008). In animals exposed pre- or perinatally to decaBDE, one study reported reproductive effects in adult male offspring exposed to decaBDE doses of 10–1,500 mg/kg/day from GD 0 to 17, including testicular lesions, decreased AGD, and altered sperm parameters (Tseng et al. 2013); however, no exposure-related changes in AGD, onset of puberty, or reproductive organ weight and histology were reported in offspring exposed to decaBDE at doses up to

1,000 mg/kg/day during gestation and lactation (Biesemeier et al. 2011; Fujimoto et al. 2011) or doses up to 20 mg/kg/day from PND 2 to 15 (Rice et al. 2007). In females, no exposure-related changes in reproductive development were reported in offspring exposed to decaBDE at doses up to 1,000 mg/kg/day during gestation and lactation (Biesemeier et al. 2011; Fujimoto et al. 2011) or doses up to 20 mg/kg/day from PND 2 to 15 (Rice et al. 2007). In *in vitro* assays, lower-brominated PBDEs have been shown to be mildly estrogenic, anti-androgenic, anti-progestagenic, and anti-glucocorticogenic and higher-brominated PBDEs have been shown to be mildly anti-estrogenic (Hamers et al. 2006; Kojima et al. 2009; Meerts et al. 2001; Mercado-Feliciano and Bigsby 2008a, 2008b; Stoker et al. 2005). PBDEs have also been shown to alter expression and activity of steroidogenic enzymes *in vitro* (Canton et al. 2005, 2008; He et al. 2008a; Karpeta et al. 2011, 2013; Wang et al. 2011c; Zhao et al. 2011).

The developing immune system may also be a target of concern in infants and children exposed to PBDEs. In a one-generation study in rats, F0 rats were exposed to pentaBDE at 0, 0.5, 5, or 25 mg/kg/day via gavage for 70 days prior to mating, through mating, gestation, and lactation (PND 21), and PND 43 F1 rats were assessed for serum immunoglobin levels, B and T lymphocyte quantification in the spleen, spleen cell proliferation *in vitro*, and immune organ weight and histology and PND 56 rats were assessed for immune function in the KLH antigen immune challenge (Bondy et al. 2013). Observed effects at PND 43 included reduced serum IgE and IgG1 levels in females; a significant, dose-related reduction in the proportion of B cells and a significant concomitant increase in the proportion of T cells in the spleen in males and females; increased proliferation of unstimulated spleen cells harvested from males and females; and histopathological changes in the thymic cortex in males. No exposure-related changes were observed in immune function in PND 56 rats (Bondy et al. 2013). Two studies examined immune function in PND 28 mice was assessed using the RSV intranasal infection test following exposure to decaBDE from GD 10 to PND 21 at doses ranging from 3.3 to 3,000 mg/kg/day (Watanabe et al. 2008, 2010b). At doses ≥260 mg/kg/day, RSV pulmonary viral titers were elevated at 1–5 days post-infection, and exposure to 3,100 mg/kg/day exacerbated the histopathological changes in the lung caused by RSV infection. In the only developmental immune study in humans, a reduced risk of atopic dermatitis was found in Japanese infants (diagnosed at 7 months of age by questionnaire) with higher PBDE concentrations in umbilical cord blood (Ochiai et al. 2014).

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to PBDEs are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by PBDEs are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to PBDEs

PBDEs are persistent environmental contaminants that accumulate in adipose tissue, serum, and breast milk serum of the general population (see Section 3.4, Toxicokinetics). Therefore, PBDE concentrations in these tissues are indicators of general exposure for PBDEs, and PBDE concentrations in maternal adipose tissue, serum, breast milk, and cord serum are useful as markers of maternal body burdens as well as lactational and *in utero* exposures (Ahn et al. 2009; CDC 2015; Huwe et al. 2008; Kim et al. 2012a; Marchitti et al. 2013; Mazdai et al. 2003; Meironyté Guvenius et al. 2001). The National Report on Human Exposure to Environmental Chemicals (CDC 2015) has methods for many PBDE congeners to identify exposure and provides reference values representative of U.S. population for comparison with exposures to potentially more exposed populations; it is continually updated on-line (see http://www.cdc.gov/exposurereport/ to look for the most recent U.S. human PBDE exposure information). Studies have also proposed that PBDE concentrations in hair are also useful markers of exposure (Aleksa et al. 2012a, 2012b; Kucharska et al. 2015; Liu et al. 2016; Malarvannan et al. 2013; Poon et al. 2014), although Zheng et al. (2011) reported that PBDE concentrations in hair were not correlated with PBDE concentrations in indoor dust from urban, e-waste, and rural areas in South China. It is not clear if the lack of correlation between PBDE concentrations in indoor dust and hair reported by Zheng et al. (2011) indicates that hair is a poor monitor of exposure or if exposure via dust was not significant for the Chinese subjects involved in the study. Other potential exposure sources, including outdoor dust for individuals living near an e-waste area or ingestion of contaminated food, were not controlled for in this study. Urinary bromophenols have also been suggested as potential biomarkers of human exposure to PBDEs (Feng et al. 2016a; Ho et al. 2015).

Estimates of PBDE serum concentrations among electronics-dismantling workers before and after exposure-free vacation (median duration 28 days, range 21–35 days) indicate that the higher-brominated congeners have shorter half-lives than lower-brominated congeners (Sjödin et al. 1999b). The medians and ranges of percentage decreases in serum concentrations, based on 5–11 measurements per congener, were 14 (range 3.5–39), 14 (2.1–38), 14 (6.7–42), 30 (7.9–52), and 66 (47–100) for BDE 47, BDE 153, BDE 154, BDE 183, and BDE 209, respectively. Although actual half-lives were not calculated, the data suggest that the half-lives of the lower-brominated congeners were <1 year. Similar findings were found in another study of eight PBDE-exposed workers (four electronics dismantlers and four workers in a factory making flame-retarded rubber), where calculated apparent half-lives were: 15 days for BDE 209; 28, 39, and 18 days for nonabrominated congeners BDE 208, BDE 207, and BDE 206; 37, 72, 85, and

91 days for BDE 203 and three other octabrominated congeners of uncertain chemical structure; and 94 days for BDE 183 (Thuresson et al. 2006).

A study in Sweden examined the feasibility of using feces as a noninvasive matrix to determine serum concentrations of PBDEs in toddlers for biomonitoring purposes (Sahlström et al. 2015). The cohort consisted of 22 healthy toddlers 11–15 months of age. Twelve (tri- to decabrominated) BDEs were analyzed, but only nine could be quantified; not detected were BDE 28, BDE 99, and BDE 100. Median concentrations of BDEs in feces ranged from 18 ng/g lipid for BDE 209 to 0.055 ng/g lipid for BDE 196. Concentrations of tetra- to octaBDE in serum were significantly higher than in feces; the highest difference was for BDE 153. BDE 209 was significantly more concentrated in feces than in serum. Significant correlations in concentrations were found for all BDEs detected, except BDE 197 and BDE 203.

Congener patterns in humans may provide information on the nature or pathway of PBDE exposures (Hooper and McDonald 2000). Low tetra:deca congener ratios are suggestive of direct, recent, or occupational exposures to the parent PBDE mixture. Higher ratios may indicate an environmental pathway where exposures result from PBDEs that have leached from the parent mixtures and have been degraded in the environment, although they may also reflect metabolic debromination following exposure to parent PBDE mixtures.

3.8.2 Biomarkers Used to Characterize Effects Caused by PBDEs

Biomarkers of effects for PBDEs are likely to be common to the general class of halogenated aromatic hydrocarbons, rather than specific for PBDEs, because PBBs, PCBs, and other structurally similar chemicals cause generally similar effects.

As discussed in Chapter 2, the thyroid, nervous system, and reproductive system are critical targets of exposure to lower-brominated PBDEs in animals, and evidence from human studies also suggests that these systems may be a target of human toxicity. Critical effects used for derivation of the MRLs for lower-brominated PBDEs include: endocrine effects in rats (reduced serum T_4) for the intermediate inhalation MRL (Great Lakes Chemical Corporation 2000); endocrine effects in F0 rat dams (reduced serum T_4) and reproductive and neurobehavioral effects in F1 adult offspring (impaired spermatogenesis, ultrastructural changes in ovaries, increased resorptions in F1 females mated to unexposed males, and increased spontaneous motor activity) for the acute oral MRL (Kuriyama et al. 2005, 2007; Talsness et al.

2005); and reproductive effects in male rats (decreased serum testosterone) for the intermediate oral MRL (Zhang et al. 2013b). There are several potential biomarkers for these effects, such as alterations in serum thyroid or reproductive hormone levels or changes in neurobehavioral screens; however, none of these effects are specific to PBDE exposure.

Similarly, the nervous system and pancreas are critical targets of exposure to decaBDE in animals. There are two human studies suggesting delayed cognitive development in children exposed to decaBDE (Chao et al. 2011; Gascon et al. 2012) and limited evidence for an association between BDE 153 and diabetes (BDE 209 was not assessed) in humans (Airaksinen et al. 2011; Lee et al. 2011; Lim et al. 2008; Smarr et al. 2016). Critical effects used for derivation of the MRLs for decaBDE include altered neurobehavior in juvenile mice following neonatal exposure for the acute oral MRL (Johansson et al. 2008; Viberg et al. 2003b) and altered insulin homeostasis (elevated serum glucose levels) in rats for the intermediate oral MRL (Zhang et al. 2013a). Again, there are potential biomarkers for these effects, such as alterations in serum glucose levels or changes in neurobehavioral screens; however, none of these effects are specific to decaBDE exposure.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Only one study was located that provided information regarding interactions between PBDE and other chemicals in humans. Fitzgerald et al. (2012) examined the association between exposure to PBDEs and neuropsychological function among 144 adult men and women residents of upper Hudson River communities and also studied the possible interactive effects of exposure to PCBs. A series of 34 tests to assess cognitive and motor function, affective state, and olfactory function were conducted. Serum samples were collected and analyzed for concentrations of nine of the most commonly detected PBDE congeners in human serum (ΣPBDE) and 30 PCB congeners that usually constitute 95% of the congeners found in human serum (ΣPCB). After adjustment for relevant confounders, the results of multiple linear regression analyses showed no significant associations between ΣPBDE and scores on the neuropsychological tests. However, in subjects with a ΣPCB concentration above the median of 467 ppb (on a lipid basis), an increase in ΣPBDE concentration from the 25^{th} to the 75^{th} percentile was significantly associated with decreases between 7 and 12% in scores of some tests of memory and learning. Tests also showed that the interaction was greater than additive. No specific mechanism was proposed for the interaction between PBDEs and PCBs.

A few animal studies have examined the effects of the interaction of PBDEs and other chemicals on thyroid-related parameters. For example, Hallgren and Darnerud (2002) reported that daily oral administration of 18 mg/kg/day BDE 47 and 4 mg/kg/day Aroclor 1254 to female rats for 14 days resulted in a significant decrease in plasma free T_4 that suggested additive effects compared to administration of either compound alone. The reduction in free T_4 coincided with a decrease in *ex vivo* binding of ^{125}I-T_4 to the hormone transporter TTR and induction of the microsomal enzymes EROD and MROD, and led the investigators to suggest that the reduced free T_4 could be explained by alterations in serum binding to TTR caused by metabolites of Aroclor 1254 or BDE 47. In the same study, co-administration of BDE 47 and a technical mixture of chlorinated paraffins resulted in a reduction in plasma free T_4 and induction of EROD that were greater than the effects of the single compounds, indicating synergistic effects. Consistent with the findings of Hallgren and Darnerud (2002), Miller et al. (2012) reported that oral co-administration of equimolar doses of the commercial PBDE mixture DE-71 and a mixture of various Aroclors to pregnant rats from GD 6 until PND 21 significantly reduced circulating levels of total T_4 in male and female offspring from PND 7 to 21 in a manner that indicated additive effects. The reduction in total T_4 was dose-dependent over a wide range of doses, 3–40 µmol/kg/day. The mixture of Aroclors resembled the PCB congener pattern found in contaminated fish consumed by residents near the Fox River in Wisconsin.

Wang et al. (2011a) found little evidence of interactions between perfluorooctane sulfonate (PFOS) and BDE 47 on postnatal levels of total T_3 and T_4 in serum from rats (dams and their offspring) administered the chemicals in the diet on GD 1 through PND 14. The investigators also examined the transcriptional and translational expression of several thyroid hormone-mediated genes in neonates' brains and found that PFOS and BDE 47 had interactive effects on only levels of brain-derived neurotrophic factor (*BDNF*); the chemicals showed a synergistic effect on PND 1 in the cortex and an antagonistic effect on PND 14 in the hippocampus. A possible mechanism for the interaction was not discussed. He et al. (2011) also found lack of interactive effects between BDE 47 and 2,2',4,4'-tetrachlorobiphenyl (PCB 153) on serum T_4 levels in rats. In this study, 10-day-old pups received a dose of up to 10 mg/kg BDE 47 and/or 5 mg/kg PCB 153. At 2 months of age, there was no evidence of any interaction between the chemicals on serum levels of T_4. However, performance on a Morris water maze was significantly impaired in the group dosed with 5 mg/kg BDE 47 and PCB 153 and in the group dosed with 10 mg/kg BDE 47 and PCB 153 relative to the groups dosed with either chemical alone. The effect of the combined action of BDE 47 and PCB 153 on performance on a Morris maze had been reported also in an earlier study (He et al. 2009).

Two studies by Eriksson and coworkers (Eriksson et al. 2006; Fischer et al. 2008), examined the interactive effects of BDE 99 and 2,2',5,5'-tetrachlorobiphenyl (PCB 52) or BDE 99 and methyl mercury on neurodevelopmental parameters in mice. In the first study, combined administration of equimolar amounts of BDE 99 and PCB 52 (1.4 µmol/kg each; 0.8 mg/kg BDE 99, 0.4 mg/kg PCB 52) to 10-day-old male mice resulted in reductions in spontaneous activity and habituation capability at 4 and 6 months of age that were significantly more pronounced than those obtained with a single much higher dose (14 µmol/kg) of PCB 52. This led the investigators to suggest that the interaction was greater than additive and that different mechanisms may be involved and/or different regions of the brain are differently affected. Using the same protocol, Fischer et al. (2008) reported that coexposure to BDE 99 and methyl mercury exacerbated neurobehavioral defects manifested as alterations in spontaneous behavior, lack of habituation, and impaired learning/memory in male mice tested during the first 6 months of life. BDE 99 and methyl mercury also interacted to promote a significant reduction in the density of cholinergic nicotinic receptors in the hippocampus, suggesting that the neurobehavioral alterations may involve the cholinergic system. The interactive properties of BDE 99 and methyl mercury were also assessed in a study in rats (Zhao et al. 2014). Combined administration of these chemicals to pregnant rats during gestation and up to PND 21 resulted in enhanced methylmercury-induced neurotoxicity in the pups compared to treatment with methylmercury alone. Specifically, tests showed delayed appearance of negative geotaxis reflexes, impaired motor coordination, and induction of oxidative stress in the cerebellum from pups. While retention of mercury was not affected by co-exposure to BDE 99, combined exposure to methylmercury and BDE 99 seemed to increase the concentrations of BDE 99 in selected brain regions from pups. The investigators concluded that combined exposure to methylmercury and BDE 99 produced oxidative stress due to inhibition of antioxidant enzymes and production of free radicals. In a study of the mechanism(s) of neurotoxicity of BDE 47, He et al. (2009) reported that the combined administration of BDE 47 and PCB 153 to 10-day-old rats induced ultrastructural alterations in neurons in the hippocampal CA1 region, assessed at the age of 2 months, in a manner that suggested a synergistic mode of action. These alterations were associated with changes in expression of mRNA and proteins involved in three apoptosis pathways. For the most part, the changes in expression levels of the various factors due to the combined action of BDE 47 and PCB 153 were consistent with an additive interaction between the two chemicals.

A study in rats reported that gestational and lactational exposure to 5.7 or 11.4 mg/kg/day of the commercial PBDE mixture DE-71 did not alter cochlear function in adult offspring and neither did exposure to 3 mg/kg/day of an environmental PCB mixture (equimolar to 5.7 mg/kg/day of the PBDE mixture) (Poon et al. 2011). Exposure to 6 mg/kg/day of the PCB mixture did impair cochlear function.

However, exposure of the rats to the combined two low doses of the chemicals, neither of which alone affected cochlear function, resulted in a deficit in cochlear function in the offspring similar to that in the high-dose PCB group, suggesting an additive effect of the mixture on the outcome measured. The mechanism of the interaction was not elucidated, but it did not appear to be directly related to reductions in serum T_4. The PCB mixture used in this study was the Fox River mixture (see above Miller et al. 2012).

It should be noted that the alterations in health outcomes in humans or animals summarized in Section 3.2, Discussion of Health Effects by Route of Exposure, that were caused by exposure to commercial or environmental PBDE mixtures, are in fact the result of interactions between the individual components of the particular PBDE mixture.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to PBDEs than will most persons exposed to the same level of PBDEs in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of PBDEs, or compromised function of organs affected by PBDEs. Populations who are at greater risk due to their unusually high exposure to PBDEs are discussed in Section 6.7, Populations with Potentially High Exposures.

Information was located on a small part of the U.S. population that might be unusually susceptible to PBDEs. As indicated in Section 3.4.4.2, the detection of PBDEs in human breast milk samples suggests that breast milk represents an elimination route of absorbed PBDEs in women. Both lower and higher-brominated congeners have been detected in breast milk (Antignac et al. 2008, 2009; Malarvannan et al. 2013; Park et al. 2011; Schecter et al. 2010). Therefore, women with high body burdens of PBDEs who breastfeed may be placing their infants at a higher risk of potential health effects, although it is unclear the degree to which PBDEs are cleared from the body during breast feeding (Hooper et al. 2007; Jakobsson et al. 2012; LaKind et al. 2009; Thomsen et al. 2010). In general, however, any risks from exposures in mother's milk are outweighed by the benefits of breastfeeding.

Pregnant women and developing infants and fetuses should be viewed as possibly sensitive populations for exposure to lower-brominated PBDEs, as they are for other thyroid hormone disrupting chemicals (Glinoer 1990; McDonald 2002; Morreale de Escobar et al. 2000). The condition of pregnancy normally

puts a significant strain on the maternal thyroid system, which can be exacerbated by iodine deficiency; according to data from 1988 to 1994, iodine deficiency is prevalent in approximately 12% of the general population and 15% of women of child-bearing age in the United States (Hollowell et al. 1998). Thyroid hormones are essential for normal development of the nervous system, lung, skeletal muscle, and possibly other organ systems, and the fetus is dependent on maternal thyroid hormones at least until the fetal thyroid begins to produce T_4 and T_3, which occurs in humans at approximately 16–20 weeks of gestation (Zoeller and Crofton 2000). As discussed in Section 3.2.2.2 (Endocrine Effects subsection), human studies have found inconsistent associations between PBDE levels in maternal/infant serum, cord blood, or breast milk and thyroid hormone levels in pregnant women and infants. However, decreased maternal, fetal, and neonatal serum T_4 levels have been consistently reported in animals exposed to lower-brominated PBDEs (but not decaBDE). Therefore, it is unclear whether or not exposure to lower brominate PBDEs will cause thyroid hormone disruption in humans, and mechanistic differences may account for the observed interspecies differences (as discussed in Section 3.5.3, Animal-to-Human Extrapolations).

Developing fetuses, infants, and children should also be regarded as a possibly sensitive population with regards to neurodevelopmental effects. As discussed in Section 3.7 (Children's Susceptibility), numerous studies have reported results suggestive of an effect of PBDE on neurodevelopment in children. PBDE levels in cord blood, maternal or infant serum, and/or breast milk have been correlated with cognitive deficits (including impaired verbal memory and comprehension), adaptive behavior deficits, increased impulsivity and impaired attention, poor social competence, and impaired fine motor coordination in infants and children. Additionally, pre- and peri-natal studies in animals consistently report neurodevelopmental effects following exposure to lower-brominated PBDEs and decaBDE (at higher doses). The susceptibility of the developing nervous system may be due to neuroendocrine effects, as discussed in Section 3.6 (Toxicities Mediated Through The Neuroendocrine Axis). For example, neuronal migration and differentiation of fetal hNPCs were significantly impaired following *in vitro* exposure to tetraBDE (Schreiber et al. 2010), and decaBDE was shown to be a thyroid hormone receptor antagonist that significantly inhibited T_4-induced dendritic arborization in cultured rat cerebellar Purkinje cells (Ibhazehiebo et al. 2011).

People with exposure to anti-thyroid drugs (e.g., lithium), thyroid disease, or otherwise compromised thyroid function might have a more pronounced response to PBDEs because of their underlying limitations in thyroid hormone production. Similarly, people with compromised function of other organs,

such as those with liver or kidney diseases (e.g., liver cirrhosis or hepatitis), could be considered more susceptible to health effects of PBDEs.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to PBDEs. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to PBDEs. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. No texts were located that provide specific information about treatment following exposures to PBDEs; however, recommendations based on experiences with PCBs are relevant. The following texts provide specific information about treatment following exposures to PCBs:

Caravati EM, Mcguigan MA, Whyte IM, et al. 2004. Polyhalogenated biphenyls. In: Dart RC, ed. Medical toxicology. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 1342-1343.

Leikin JB, Pauloucek FP. 2008. Polychlorinated biphenyls. In: Leikin JB, Pauloucek FP, eds. Poisoning and toxicology handbook. 4th ed. Boca Raton, FL: CRC Press, Taylor & Francis Group, 840.

The treatment methods discussed below are general methods that would apply to any persistent, lipophilic chemical, and have not been tested for efficacy, indicating that they might not be effective in reducing the toxic effects of PBDEs. There is no indication of hazards associated with the treatments. The methods are particularly appropriate for trying under conditions of acute exposure, but PBDEs are not acutely toxic chemicals. Scenarios where life-threatening acute exposure would occur are unlikely, although accidental or intentional ingestion of the commercial products is a conceivable concern. The relevance of the methods to common background environmental exposures to these chemicals is unclear, and it is questionable whether current exposure and tissue levels in the general population are a health concern.

3.11.1 Reducing Peak Absorption Following Exposure

Ingested PBDEs are absorbed by the gastrointestinal tract of humans and animals (see Section 3.4). Although there are no specific recommendations for clinical treatment of acute intoxication from ingested PBDEs, recommendations based on experiences with PCBs are relevant. Treatments for acute poisonings from PCBs and related substances include the induction of emesis or gastric lavage and stomach pumping to decrease gastrointestinal absorption of the chemicals (Lemesh 1992). These procedures would not be beneficial if performed too long after exposure occurred. Administration of activated charcoal as a slurry,

either aqueous or mixed with a saline cathartic or sorbitol, is frequently recommended to decrease the gastrointestinal absorption of PCBs, but the value of this treatment for reducing absorption of PCBs, PBBs, and PBDEs is unknown (HSDB 2012). Repetitive administration of activated charcoal might be useful in preventing reabsorption of metabolites. Rice bran fiber decreased absorption of PCBs in the gastrointestinal tract and had a stimulatory effect on fecal excretion of PCBs in rats (Takenaka and Takahashi 1991), but it is unclear if rice bran would be of benefit in poisoned humans.

The detection of PBDEs in the serum and fat of people who were occupationally exposed to these chemicals indicates that PBDEs can be absorbed by the lungs, skin, and/or orally by hand-to-mouth contact. Although no specific methods to reduce absorption of dermally applied or inhaled PBDEs were located, multiple washings of contaminated skin with soap and water immediately following exposure have been suggested to reduce the dermal absorption of PCBs (HSDB 2012). Studies with monkeys showed that soap and water was as effective as or better than such solvents as ethanol, mineral oil, or trichlorobenzene in removing PCBs from skin (Wester et al. 1990). Personal protective equipment (e.g., long sleeves, gloves, safety glasses, respiratory protection) and industrial hygiene programs generally help to limit occupational exposures.

3.11.2 Reducing Body Burden

As discussed in Section 3.4, while decaBDE is absorbed to a lesser degree than lower-brominated PBDEs, all PBDE congeners can accumulate in lipid-rich tissues. However, lower-brominated PBDEs are more likely to accumulate as they are more slowly metabolized and eliminated from the body. No studies evaluating methods to reduce body burden of PBDEs were located.

Several methods to enhance the elimination of PBBs from the body have been examined in animals and may be applicable to PBDEs. Methods for increasing the elimination of these chemicals include the restriction of caloric intake (to reduce total body fat), and the administration of various agents that interact with bile acids including activated charcoal, mineral oil, and bile-binding resins such as cholestyramine (Kimbrough et al. 1980; McConnell et al. 1980; Polin and Leavitt 1984; Polin et al. 1985, 1991; Rozman et al. 1982). It should be mentioned, however, that based on the pharmacokinetic considerations discussed in Section 3.8.1, a rapid breakdown of fat, as might occur in dieting, might lead to a transient increase in PBDE levels in serum and other body tissues, possibly posing a significant re-exposure problem. Although some of the studies observed no enhanced elimination (Kimbrough et al. 1980; McConnell et al. 1980), others identified treatments that were effective in enhancing the biliary and

intestinal elimination of PBB residues (Polin et al. 1991; Rozman et al. 1982). Polin et al. (1991) found that dietary intervention to reduce PBBs was dose dependent; treatment with 10% mineral oil and a 45% reduction in food intake resulted in a 69 and 23% reduction in body burden in rats fed PBBs at dietary concentrations of 0.1 and 100 ppm, respectively (Polin et al. 1991). A combination of mineral oil, colestipol, and dietary restriction was successful in reducing the PBB body burdens in chickens (Polin and Leavitt 1984; Polin et al. 1985), while each treatment alone had no effect in reducing PBB body burden. A 3-week treatment regimen that included dietary supplements of polyunsaturated oil, vitamins, and minerals, and heat stress has been applied in a pilot study to seven human subjects that were known to have been exposed to PBBs; following treatment, statistically significant reductions were measured in PBB concentrations in fat (Schnare et al. 1984). Although the lack of a separate control group complicates interpretation of the results of this study (each subject served as his/her own control), this treatment was developed for the purpose of reducing body burdens of fat-soluble psychoactive drugs (Schnare et al. 1984).

Additionally, a few human studies that have evaluated methods to reduce body burden of PCBs, another class of lipophilic compounds, may be applicable to PBDEs. A liquid diet was used for 16 individuals who developed symptoms following exposure to PCBs and polychlorinated dibenzofurans (Imamura and Tung 1984). Symptoms were reduced several months after the fasting period. This study is limited in that a control group was not used, and body burdens were not measured. Based on information for PCBs, mobilization of PBDEs from adipose tissue is not recommended in individuals with hepatic or renal disease (Lemesh 1992). More recently, 14 individuals administered 15 g/day of dietary olestra (a non-absorbable lipid in potato crisps) for 1 year showed a steady decline in serum lipid concentrations of PCBs (Jandacek et al. 2014). At the end of 1 year, PCB concentrations were significantly decreased by ~8% compared with pre-trial values. However, PCB concentrations were not significantly decreased compared with 14 concurrent controls administered 15 g/day of dietary olive oil (which showed a nonsignificant ~4% decrease in PCB concentration compared with pre-trial values). Further studies need to be conducted with larger study groups to determine the efficacy of olestra for reducing body burden of PCBs and/or other lipophilic compounds.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

There are no known methods for interfering with the mechanism of action of PBDEs. Although the mechanism of action of PBDEs is not completely understood, PBDEs share some toxicological properties with other structurally similar polyhalogenated aromatic compounds, particularly PBBs, PCBs, CDDs,

and CDFs (ATSDR 1994, 1998, 2000). Experimental evidence indicates that some PBB congeners exert toxic actions by a process involving several steps (Safe 1984). This process begins with the binding of particular congeners to the AhR and leads to enhancement of the CYP1A1 gene expression (see Section 3.5). The toxicity of specific congeners is related to their ability to assume a coplanar configuration for binding to AhR (Hardy 2002a). It may be inferred that interfering with the initial step, binding to the receptor, or with any of the subsequent steps, would possibly prevent the expression of the toxic effects. Several compounds have been identified that partially antagonize one or more AhR-mediated responses (Bannister et al. 1989); their use, however, has been limited to experimental studies in animals. These compounds were successful antagonists when given before or at the same time as the potent AhR activator TCDD (Bannister et al. 1989). In contrast to PBBs, PCBs, and related compounds, AhR binding affinity of PBDE congeners is not correlated with planarity (Chen et al. 2001). This may be due, in part, to increased distance between the phenyl rings relative to PBBs and PCBs. It has also been speculated that the large size of the bromine atoms of PBDEs relative to chlorine atoms of PCBs may distort the AhR binding site so that coplanar configuration is not required (Chen et al. 2001). Because AhR binding by PBDEs apparently differs in some respects from AhR binding by PBBs and other related compounds, AhR antagonists identified via experiments with PBBs, TCDD, and related compounds might not effectively antagonize AhR binding and effects of PBDEs.

PBDEs may also cause toxicity by other mechanisms of action. PBDE-induced decreases in thyroid T_4 hormone, which can affect neurobehavioral development, are likely to involve multiple mechanisms (see Section 3.5.3). These include induction of hepatic microsomal enzymes, particularly UDPGT, which can increase the rate of T_4 conjugation and excretion, and metabolic formation of hydroxy-metabolites of PBDEs. PBDEs and their hydroxy metabolites can bind with high affinity to thyroid transport proteins because they are structurally similar to T_4 hormone (i.e., are also hydroxy-halogenated diphenyl ethers) (see Section 3.5.2). Effects of PBDEs on thyroid status via induction of hepatic enzymes, however, are unlikely to occur in humans, and the impact of hydroxy-metabolites on serum T_4 needs further clarification. Effects of PBDEs on the function and development of the nervous system could also involve disruption of calcium homeostatic mechanisms and intracellular signalling events (Chen et al. 2010; Dingemans et al. 2008, 2010a, 2010b; Fan et al. 2010; Kodavanti 2003; Kodavanti and Derr-Yellin 2001, 2002; Smolnikar et al. 2001; Wiegand et al. 2001), altered cholinergic or dopaminergic functions (Ankarberg et al. 2001; Bradner et al. 2013; Dreiem et al. 2010; Fischer et al. 2008; Mariussen and Fonnum 2002, 2003; Mariussen et al. 2003; Slotkin et al. 2013; Viberg and Eriksson 2011; Viberg et al. 2002, 2003a, 2004b, 2005), and/or free radical-induced neuronal death (Chen et al. 2010; He et al. 2008b;

Huang et al. 2010; Reistad et al. 2002). Clinical interventions designed to interfere with the aforementioned mechanisms have yet to be developed.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of PBDEs is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of PBDEs.

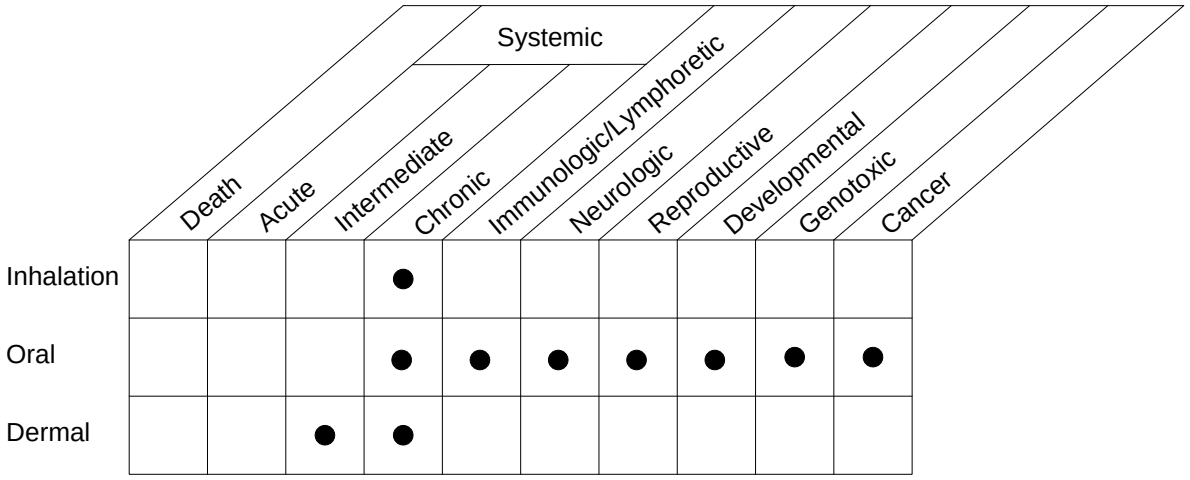
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of PBDEs

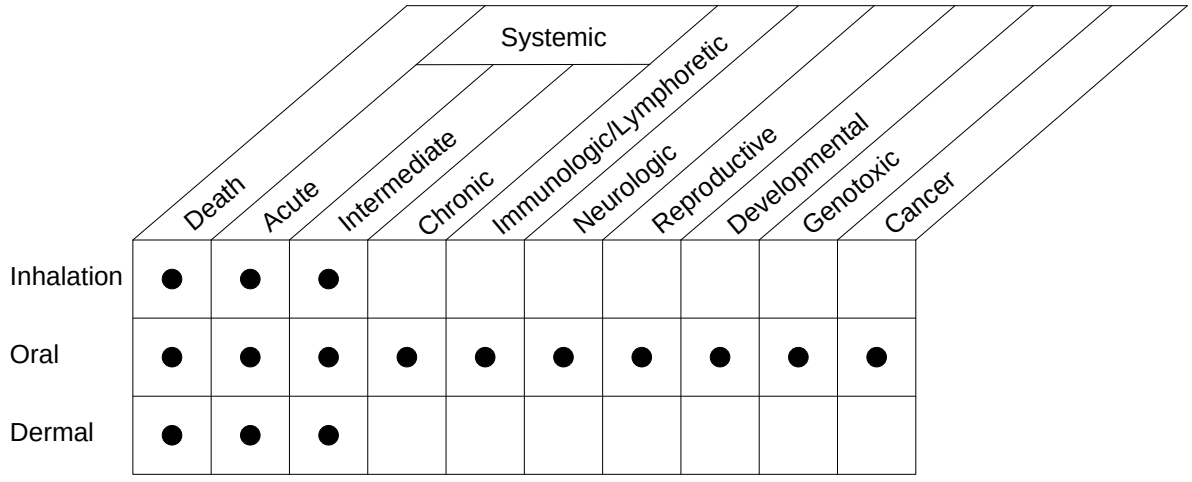
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to PBDEs are summarized in Figure 3-8. The purpose of this figure is to illustrate the existing information concerning the health effects of PBDEs. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Studies on the systemic and carcinogenic effects of PBDEs in humans are based primarily on tissue PBDE levels without knowledge of route of exposure; in most cases exposure was attributed to the oral route (Figure 3-8). Information on health effects of PBDEs in animals is available for all effect categories, but is mainly limited to oral exposure studies in animals.

Figure 3-8. Existing Information on Health Effects of Polybrominated Diphenyl Ethers (PBDEs)



Human



Animal

● Existing Studies

3.12.2 Identification of Data Needs

Acute-Duration Exposure. Acute-duration studies of lower-brominated PBDEs have documented effects mainly on the developing nervous system, the developing reproductive system, the thyroid, and the liver of orally exposed rats and mice (Bruchajzer 2011; Bruchajzer et al. 2010, 2011; Darnerud and Sinjari 1996; Dingemans et al. 2007; Dufault et al. 2005; Eriksson et al. 2001, 2002b, 2006; Fischer et al. 2008; Fowles et al. 1994; Gee and Moser 2008; Hallgren and Darnerud 2002; Hallgren et al. 2001, 2015; He et al. 2009, 2011; Hoppe and Carey 2007; Kuriyama et al. 2005, 2007; Richardson et al. 2008; Sand et al. 2004; Stoker et al. 2004; Talsness et al. 2005, 2008; Viberg et al. 2002, 2003a, 2004a, 2004b, 2005, 2006; Zhou et al. 2001, 2002). The most sensitive effects were observed in F0 and F1 rats exposed to ≥0.06 mg/kg of pentaBDE on GD 6, including decreased maternal serum T_4 in dams, decreased number of spermatids and sperm and daily sperm production in F1 males, decreased relative epididymis weight in F1 males, altered neurobehavior in F1 males, ultrastructural changes in ovaries of F1 females, and increased resorptions in F1 females mated to unexposed males (Kuriyama et al. 2005, 2007; Talsness et al. 2005). Collectively, these end points were selected as a basis for an acute MRL for lower-brominated PBDEs. While hepatic effects were consistently observed, they occurred at much higher doses (≥8 mg/kg/day). Two studies in mice indicate that immunosuppression is a potentially critical health end point for acute exposure to lower-brominated PBDEs; additional studies may provide more support for this end point (Darnerud and Thuvander 1998; Fowles et al. 1994; see discussions of data needs for Immunotoxicity).

Several acute-duration studies of decaBDE have also documented effects on the developing nervous system in rats (Chen et al. 2014; Viberg et al. 2007) and mice (Buratovic et al. 2014; Johansson et al. 2008; Rice et al. 2007, 2009; Viberg et al. 2003b). The most sensitive neurobehavioral effects, decreased open field activity and impaired habituation, were observed in 2–6-month-old male mice that were exposed once to decaBDE at doses ≥2.22 mg/kg on PND 3 (Buratovic et al. 2014; Johansson et al. 2008; Viberg et al. 2003b). This effect was not observed at 1.34 mg/kg (Buratovic et al. 2014; Johansson et al. 2008). These neurobehavioral end points were selected as the basis for an acute MRL for decaBDE. The remaining acute database for decaBDE provides only limited data regarding hepatic, endocrine, body weight, and developmental effects in rats and mice (Bruchajzer et al. 2010; Carlson 1980b; Chi et al. 2011; IRDC 1974; NTP 1986; Sakamoto et al. 2013; Zhou et al. 2001). Additional studies, specifically neurodevelopmental studies in species other than mice and immunotoxicity screens, would help to clearly

establish the most sensitive target and species for acute exposure, as well as which animal toxicity data are the most relevant to humans and useful for assessing acute health risks of decaBDE.

The inhalation database for acute-duration exposure to lower-brominated PBDEs is essentially limited to a single 14-day unpublished industry-sponsored studies of octaBDE in rats (Great Lakes Chemical Corporation 1978). A liver effect level was identified in this study, but MRL estimation is precluded by small animal groups and incomplete assessment of other toxicological end points, particularly a lack of information on thyroid hormone levels. Additional dose-response studies may provide an adequate basis for derivation of an acute inhalation MRL for lower-brominated PBDEs. Acute-duration inhalation exposure toxicity studies of decaBDE were not located.

Intermediate-Duration Exposure. Available intermediate-duration oral studies in animals indicate that the male reproductive system, the developing nervous system, the thyroid, and the liver are the main systemic targets of repeated exposures to lower-brominated PBDEs. The most sensitive end points were reproductive effects in male rats exposed to tetraBDE for 8 weeks, which identified a minimal LOAEL of 0.001 mg/kg/day for a 34% decrease in serum testosterone levels (Zhang et al. 2013b). Histological changes in the testes were also observed at ≥0.03 mg/kg/day in the study by Zhang et al. (2013b) and a similar study in rats by Huang et al. (2015). The minimal LOAEL for decreased serum testosterone was selected as the basis for an intermediate-duration MRL for lower-brominated PBDEs. A study in mice supports that the male reproductive system is a target for tetraBDE toxicity, reporting histological changes in the testes at tetraBDE doses ≥0.045 mg/kg/day for 30 days (Wang et al. 2013). As observed with acute exposure, altered neurobehavior was consistently observed in animals exposed to lower-brominated PBDEs for intermediate durations during development, with effects occurring at or above the doses of observed male reproductive effects (≥2 and ≥0.03 mg/kg/day in rats and mice, respectively) (Blanco et al. 2013; Bowers et al. 2015; Branchi et al. 2005; Cheng et al. 2009; Koenig et al. 2012; Ta et al. 2011; Woods et al. 2012). Liver effects, including increased liver weight, hypertrophy, and histopathological changes, were observed following exposure to lower-brominated PBDEs at doses as low as 0.45 mg/kg/day (Becker et al. 2012; Bondy et al. 2011, 2013; Bruchajzer 2011; Bruchajzer et al. 2012; Dunnick et al. 2012; IRDC 1976, 1977; Maranghi et al. 2013; Oberg et al. 2010; WIL Research Laboratories 1984; Zhang et al. 2014, 2015a, 2015b).

The thyroid is also a critical target in both adult and developing animals, with consistent observations of reduced serum T_4 levels at doses as low as 2.85 mg/kg/day and enlargement and histological alterations to the thyroid at doses as low as 0.45 mg/kg/day (Bansal et al. 2014; Becker et al. 2012; Bondy et al. 2011,

2013; Bowers et al. 2015; Driscoll et al. 2009; Dunnick et al. 2012; Ellis-Hutchings et al. 2006; Ernest et al. 2012; Hoppe and Carey 2007; IRDC 1977; Kodavanti et al. 2010; Maranghi et al. 2013; Miller et al. 2012; Poon et al. 2011; Skarman et al. 2005; Stoker et al. 2004, 2005; Szabo et al. 2009; Wang et al. 2011a; WIL Research Laboratories 1984; Zhou et al. 2002). Essentially all of the available data on thyroid effects of lower-brominated PBDEs have been obtained from oral studies in rats. It is speculated that the extent that PBDEs affect circulating levels of thyroid T_4 or T_3 might vary with species, and rats are often regarded as more sensitive than humans. Studies designed to elucidate the mechanism(s) of action for thyroid and other effects of lower-brominated PBDEs would help to better understand how the animal toxicity data can best be used to identify target end points and assess health risks in humans.

Available intermediate-duration oral studies provide limited evidence for effects in the pancreas, nervous system, liver, immune system, reproductive system, and several organ systems in developing animals following repeated exposures to decaBDE. The most sensitive end points were pancreatic effects from a study in male rats exposed to decaBDE for 8 weeks, which identified a minimal LOAEL of 0.05 mg/kg/day based on a 12% increase in serum glucose levels (Zhang et al. 2013a). The increase in serum glucose is considered to be part of a spectrum of effects indicative of altered insulin homeostasis and toxicity to the pancreas, including decreased serum insulin and morphological changes in pancreatic islet cells, following decaBDE exposure to doses ≥1 mg/kg/day (Zhang et al. 2013a). This minimal LOAEL for elevated serum glucose levels was used as a basis for the intermediate-duration oral MRL for decaBDE. Other effects observed following intermediate-duration exposure to decaBDE were observed at higher doses. In animals exposed during development, adverse effects included histopathological changes in the liver, kidney, and testes of rats exposed to ≥2 mg/kg/day (Fujimoto et al. 2011; Tseng et al. 2008, 2013); altered hippocampal electrophysiology in rats exposed to 20.1 mg/kg/day (Xing et al. 2009); and impaired immunity in mice exposed to 2,900 mg/kg/day (Watanabe et al. 2010b). In adult animals, observed effects following intermediate-duration decaBDE exposure included decreased anxiety in mice at doses 20 mg/kg/day (Heredia et al. 2012); histopathological changes in the ovaries, liver, spleen, and thymus and altered immune end points (T-cell distribution, lymphocyte proliferation, serum immunoglobulins) in rats exposed to 300 mg/kg/day (Liu et al. 2012); altered CD4 T-cell function in mice exposed to 800 mg/kg/day (Feng et al. 2016b); reduced serum testosterone, reduced sperm count and viability, and degenerative changes in the seminiferous tubules in mice exposed to 950 mg/kg/day (Sarkar et al. 2015); and histopathological changes in the liver in mice exposed to 9,400 mg/kg/day (Lee et al. 2010; Liu et al. 2012; Sakamoto et al. 2013). Since the majority of the observed effects lack supporting evidence from other studies, and many were single-dose studies, additional intermediate-duration studies evaluating these end points following decaBDE exposure at multiple doses would help to better

understand how the animal toxicity data can best be used to identify target end points and assess health risks in humans. Of particular interest would be additional studies evaluating altered insulin homeostasis, the critical effect that serves as the basis for the current intermediate-duration oral MRL for decaBDE.

The inhalation database for intermediate-duration exposure to PBDEs consists of one well-conducted 13-week unpublished industry study of octaBDE in rats (Great Lakes Chemical Corporation 2000). Hepatic, nasal, lung, thyroid, and ovarian effects were observed, and a NOAEL for changes in thyroid hormone levels was used as the basis for estimation of an intermediate-duration inhalation MRL. Intermediate-duration inhalation exposure toxicity studies of decaBDE were not located.

Chronic-Duration Exposure and Cancer. One chronic study of high purity decaBDE has been conducted. In this study, a commercial decaBDE product (94–97% pure) was fed to rats and mice for 103 weeks (NTP 1986). Comprehensive gross and histological examinations were performed on all animals, but no hematology, clinical chemistry, or urine indices, or thyroid hormone levels, were evaluated. The lowest tested dose in the study, 1,120 mg/kg/day in male rats, was a LOAEL for a liver lesion (neoplastic nodules) that is precancerous and associated with thrombosis in the same tissue, precluding estimation of an MRL. Additional chronic dose-response information would provide information on the NOAEL/LOAEL threshold and an appropriate basis for derivation of a chronic MRL for decaBDE. Neoplastic effects in this study included increased incidences of neoplastic nodules in the liver in the male and female rats and hepatocellular adenoma or carcinoma (combined) in the male mice. Slightly elevated incidences of thyroid gland follicular cell tumors were additionally observed in exposed male mice, although the increases were equivocal.

In the only other chronic oral study available, rats were fed a 77.4% pure commercial decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaBDE) for approximately 2 years (Kociba et al. 1975; Norris et al. 1975a). Evaluations that included clinical signs, body weight, food consumption, hematology, clinical chemistry, urine indices, and comprehensive histological examinations showed no exposure-related effects. The highest tested dose (1 mg/kg/day) was a NOAEL, but this effect level is not an appropriate basis for MRL estimation due to insufficient sensitivity of the study. In particular, a chronic oral MRL based on this study would be higher than the intermediate MRL. No exposure-related neoplastic changes were found, but the power of this study to detect carcinogenic effects is limited by the low dose levels. Considering the limitations of the available data, well-designed chronic toxicity studies of lower-brominated PBDEs may provide adequate bases for MRL derivation and cancer assessment for lower-brominated PBDEs. Evaluations that include the thyroid and neurobehavioral end points would be

particularly informative because acute and intermediate-duration oral studies indicate that the thyroid and developing central nervous system are particularly sensitive targets for lower-brominated PBDEs.

Genotoxicity. Limited information was located regarding the genotoxicity of PBDEs in humans. A study of Chinese workers exposed to PBDEs suggested an association between levels of total PBDEs in blood and frequency of micronuclei in peripheral lymphocytes, but no causality was demonstrated (Yuan et al. 2008). Moreover, other pollutants could have played a role. A study of the general population from Korea did not find an association between serum levels of 2,2',4,4'-tetraBDE and 2,2',4,4',5-pentaBDE and chromosome telomere length in peripheral lymphocytes (Shin et al. 2010). Cytogenetic examination of additional populations previously or currently exposed to PBDEs would provide valuable information. A study in mice reported that maternal exposure to decaBDE during gestation resulted in sperm DNA damage in male offspring examined at 71 days of age (Tseng et al. 2013). Additional studies trying to replicate these results would be helpful. Testing the offspring for fertility would also be informative. DecaBDE and 2,2',4,4',5-pentaBDE have been tested in prokaryotic organisms and both yielded negative results in gene mutation tests (Evandri et al. 2003; NTP 1986). Relatively few PBDE congeners have been examined for genetic effects in mammalian cells. DNA damage and increased recombination activity have been reported (He et al. 2008a, 2008b; Helladay et al. 1999; Ji et al. 2011; Pellacani et al. 2012). Further studies with the PBDE congeners that are most frequently found in the environment and in human blood and tissues would be valuable. Studies designed to explore possible mechanisms of genotoxicity of PBDEs and also of metabolites would also be valuable.

Reproductive Toxicity. Reports of five one-generation studies in rats and mice were located. No exposure-related changes were observed in reproductive end points (number of pregnancies, gestation length, number, size, or sex ratio of litters) at pentaBDE doses up to 25 mg/kg/day or tetraBDE doses up to 1 mg/kg/day (Bondy et al. 2011, 2013; Koenig et al. 2012; Poon et al. 2011; Ta et al. 2011; Woods et al. 2012). No fertility impairments were observed in F1 males or females that were exposed once to pentaBDE on GD 6 at doses up to 0.3 mg/kg/day, when mated to unexposed animals (Kuriyama et al. 2005; Talsness et al. 2005, 2008). However, in a one-generation study in mink, females exposed to pentaBDE at doses ≥0.25 mg/kg/day from pre-mating day 28 through PNW 6 did not whelp (Bull et al. 2007; Zhang et al. 2009). It is not clear in the study reported by Bull et al. (2007) whether mink exposed to 0.25 mg/kg/day never became pregnant or had complete litter loss. However, Zhang et al. (2009) reported that female mink exposed to 0.31 mg/kg/day had no exposure-related changes in mating success; rather, sows showed complete litter loss with 70% showing clear postimplantation loss. Despite a lack of effects from most one-generation studies, reduced serum testosterone and testicular damage (increased

multinucleated giant cells, germ cell loss, and apoptotic cells) was reported in adult male rats and mice exposed to tetraBDE doses as low as 0.001 and 0.045 mg/kg/day, respectively (Huang et al. 2015; Wang et al. 2013; Zhang et al. 2013b). Reduced serum testosterone in rats exposed to ≥0.001 mg/kg/day for 8 weeks was selected as the critical effect for the intermediate-duration MRL for lower-brominated PBDEs; therefore, additional studies with other congeners evaluating testicular damage may better characterize the reproductive toxic potential of PBDEs and assure the adequacy of the intermediate oral MRL. Also, studies in other species (e.g., rabbits) may be warranted to investigate species-specific reproductive effects and two-generation studies designed to assess effects on fertility in both sexes would better characterize the potential for reproductive toxicity as a result of exposure to lower-brominated PBDEs.

For decaBDE, information on the reproductive toxicity is limited to a single one-generation oral study in rats that found no exposure-related functional effects following exposure to an impure decaBDE mixture containing lower-brominated PBDEs (77% decaBDE, 22% nonaBDE, 0.8% octaBDE) at dietary doses up to 100 mg/kg/day for 60 days prior to mating through PND 21 (Dow Chemical Co. 1975; Norris et al. 1975a). Studies that evaluated reproductive organ histology following exposure to decaBDE generally did not report detectable effects. As suggested for lower-brominated PBDEs, studies in other species may be warranted to investigate species-specific reproductive effects and two-generation studies designed to assess effects on fertility in both sexes would better characterize the potential for reproductive toxicity as a result of exposure to decaBDE.

Developmental Toxicity. Numerous oral developmental toxicity studies have shown no evidence of teratogenicity in rats or rabbits exposed to lower-brominated PBDEs or decaBDE, although fetotoxic effects, including skeletal ossification variations at maternally toxic doses, have occurred with exposures to lower-brominated mixtures (Argus Research Laboratories 1985a, 1985b; Biesemeier et al. 2011; Blanco et al. 2012; Breslin et al. 1989; Chi et al. 2011; Dow Chemical Co. 1975, 1985; Ellis-Hutchings et al. 2009; Hardy et al. 2002; Life Science Research Israel Ltd. 1987; Norris et al. 1975a; WIL Research Laboratories 1986). The available evidence adequately shows that teratogenicity and fetal toxicity is not a critical effect of concern for either lower-brominated PBDEs or decaBDE. However, there is evidence that the developing nervous and endocrine systems, and potentially the developing reproductive and immune systems are sensitive targets of particular PBDE congeners. Numerous studies show that developmental exposure can lead to neurological changes at later life stages, as rats and mice exposed pre- or peri-natally to lower-brominated PBDEs or decaBDE show neurobehavioral alterations following exposure to lower-brominated PBDEs and decaBDE at doses ≥0.06 and ≥2.22 mg/kg/day, respectively, as

well as several other neurological effects at higher doses, including delayed ontogeny of reflexes, ultrastructural changes, altered nicotinic receptor density, altered electrophysiology, and altered gene and protein expression levels (Biesemeier et al. 2011; Blanco et al. 2013; Branchi et al. 2001, 2002, 2005; Cheng et al. 2009; Eriksson et al. 2001, 2002b, 2006; Fischer et al. 2008; Fujimoto et al. 2011; Gee and Moser 2008; He et al. 2009, 2011; Johansson et al. 2008; Koenig et al. 2012; Kuriyama et al. 2004, 2005; Rice et al. 2007; Sand et al. 2004; Ta et al. 2011; Viberg et al. 2002, 2003a, 2003b, 2004a, 2004b, 2005, 2006, 2007; Woods et al. 2012; Xing et al. 2009). Developing animals exposed to lower-brominated PBDEs, but not decaBDE, also consistently showed decreased serum T_4 levels, although these effects may be transient (Blanco et al. 2013; Bondy et al. 2011, 2013; Ellis-Hutchings et al. 2006; Kodavanti et al. 2010; Kuriyama et al. 2007; Miller et al. 2012; Poon et al. 2011; Shah et al. 2011; Skarman et al. 2005; Szabo et al. 2009; Wang et al. 2011a; Zhang et al. 2009; Zhou et al. 2002). There is limited evidence that developmental exposure to lower-brominated PBDEs may effect reproductive system development (Kuriyama et al. 2005; Talsness et al. 2005, 2008) and that both lower-brominated PBDEs and decaBDE may effect immune system development (Bondy et al. 2013; Watanabe et al. 2008, 2010b). Additional studies evaluating reproductive and immune system development following exposure to PBDEs would better characterize the developmental toxic potential of PBDEs on these systems.

Immunotoxicity. Information regarding the immunosuppressive potential of PBDEs is limited. Immune function assays in adult animals are limited to lower-brominated PBDEs. Acute-duration oral studies in animals exposed to pentaBDE reported that plaque-forming splenic cell antibody response to injected sheep red blood cells was significantly reduced in mice exposed to 72 mg/kg/day pentaBDE for 14 days (Fowles et al. 1994) and *in vitro* production of IgG immunoglobulin from pokeweed mitogen-stimulated splenocytes was reduced in mice, but not in rats, exposed to 36 mg/kg/day pentaBDE for 14 days (Darnerud and Thuvander 1998). The only intermediate-duration immune function assay reported no exposure-related immune effects in the KLH antibody production assay or the PHA skin challenge in mink exposed to pentaBDE at doses up to 0.78 mg/kg/day for 9 weeks (Martin et al. 2007). The majority of studies report no histological changes in immune tissues with acute- or intermediate-duration exposure to PBDEs. However, as discussed in the Developmental section above, exposure to lower-brominated PBDEs or decaBDE during development may lead to impaired immune function (Bondy et al. 2013; Watanabe et al. 2008, 2010b). Additional oral studies using a battery of immunological tests and a lower range of doses for several congeners, including decaBDE, would serve to better characterize the immunotoxic potential of PBDEs.

Neurotoxicity. While a large body of evidence indicates that the developing nervous system is a target for PBDE toxicity, a limited amount of information is available on neurological effects of PBDEs in adult animals. In a comprehensive neurotoxicity screen, no adverse effects were observed in rats exposed once to pentaBDE at doses up to 1.2 mg/kg/day (Belles et al. 2010). In a neurobehavioral study, no exposure-related changes were observed in open-field behavior, anxiety-like behavior, or learning and memory in male rats exposed to low doses of pentaBDE at doses up to 0.015 mg/kg/day for 90 days (Daubie et al. 2011). In a higher dose study, impaired attention and inhibitory control were observed in male mice exposed to pentaBDE at 26.2 mg/kg/day for 125 days; no exposure-related changes were observed at 17.5 mg/kg/day (Driscoll et al. 2009). Impaired learning and memory were observed in male rats exposed to tetraBDE at doses ≥0.1 for 30 days (Yan et al. 2012).

For decaBDE, no studies evaluating neurological end points in adult rats or mice following acute exposure were identified. Decreased anxiety behavior was observed in male mice exposed to decaBDE for 15 days; however, no exposure-related changes were observed in a functional observation battery or learning and memory (Heredia et al. 2012). In another study, no changes were observed in open-field behavior of male rats exposed to decaBDE at doses up to 50 mg/kg/day 90 days (Wang et al. 2011b). No overt signs of neurotoxicity were observed in rats and mice exposed to decaBDE in estimated dietary doses as high as 16,000–19,000 mg/kg/day for 14 days, 8,000–9,000 mg/kg/day for 13 weeks, or 2,550–7,780 mg/kg/day for 103 weeks (NTP 1986). Although the high doses and extended exposure durations provided opportunities for the induction and/or development of clinical signs, the study is limited by lack of testing for subtle behavioral changes and neurodevelopmental effects. Additional comprehensive neurotoxicity batteries in adult animals exposed orally to repeat doses of PBDEs would better characterize the potential for PBDEs to cause neurotoxic effects in adults.

Epidemiological and Human Dosimetry Studies. There are numerous epidemiological studies evaluating potential associations between tissue PBDE concentrations and adverse health effects; however, none of the studies provided quantitative exposure information. Therefore, the available epidemiological studies are not useful for quantitative risk assessment. Available studies indicate that PBDE exposure may lead to neurodevelopmental effects (Adgent et al. 2014; Chao et al. 2011; Chen et al. 2014; Chevrier et al. 2016; Cowell et al. 2015; Ding et al. 2015; Eskenazi et al. 2013; Gascon et al. 2012; Herbstman et al. 2010; Hoffman et al. 2012; Roze et al. 2009; Sagiv et al. 2015; Shy et al. 2011; Vuong et al. 2016a). Evidence for associations between PBDE exposure and other effects in humans are inconsistent between studies, including observations of altered thyroid hormone levels (Abdelouahab et al. 2011, 2013; Bloom et al. 2008, 2014; Chevrier et al. 2010; Dallaire et al. 2009; Eggesbo et al. 2011;

Eguchi et al. 2015; Hagmar et al. 2001; Herbstman et al. 2008; Huang et al. 2014; Julander et al. 2005; Kicinski et al. 2012; Kim et al. 2011a, 2011d, 2012a, 2012b, 2013a; Leijs et al. 2012; Lignell et al. 2016; Makey et al. 2016; Mazdai et al. 2003; Shy et al. 2012; Stapleton et al. 2011; Turyk et al. 2008; Yuan et al. 2008; Wan et al. 2010; Wang et al. 2010; Xu et al. 2014a, 2014b, 2015a), male reproductive effects (Abdelouahab et al. 2011; Akutsu et al. 2008; Hagmar et al. 2001; Johnson et al. 2013; Meeker et al. 2009; Mumford et al. 2015; Turyk et al. 2008), female reproductive effects (Buck Louis et al. 2013; Chao et al. 2007, 2010; Chen et al.2011; Harley et al. 2010; Johnson et al. 2012; Karmaus et al. 2011; Wainman et al. 2016), non-neurological developmental effects (Agay-Shay et al. 2015; Carmichael et al. 2010; Chao et al. 2007; Chen et al. 2015; Erkin-Cakmak et al. 2015; Foster et al. 2011; Harley et al. 2011; Kim et al. 2011d, 2012a, 2012b, 2015; Koskenniemi et al. 2015; Leijs et al. 2008; Lopez-Espinosa et al. 2015; Ma et al. 2012a; Main et al. 2007; Mazdai et al. 2003; Meijer et al. 2012; Miranda et al. 2015; Müller et al. 2016; Ochiai et al. 2014; Peltier et al. 2015; Ren et al. 2011; Robledo et al. 2015a; Serme-Gbedo et al. 2016; Tan et al. 2009; Vuong et al. 2016b; Warembourg et al. 2016; Windham et al. 2015a; Wu et al. 2010; Xu et al. 2015b), and risk for diabetes (Airaksinen et al. 2011; Lee et al. 2011; Lim et al. 2008; Smarr et al. 2016; Turyk et al. 2015). In other epidemiological studies, serum PBDE levels were not significantly associated with carotid atherosclerosis, risk of stroke, bone mineral density, immune function, non-Hodgkin's lymphoma (Fernlof et al. 1997; Hardell et al. 1998; Kumar et al. 2014a, 2014b; Lee et al. 2012; Lind et al. 2012; Lindstrom et al. 1998;Weiss et al. 2006), breast cancer (Holmes et al. 2014; Hurley et al. 2011), thyroid cancer (Aschebrook-Kilfoy et al. 2015), prostate cancer (Pi et al. 2016), reproductive effects in men (Toft et al. 2014), Polycystic Ovary Syndrome (Vagi et al. 2014), or uterine fibroids (Trabert et al. 2015).

Epidemiological studies with quantitative estimates of exposure would be useful for quantitative risk assessment. Considering the possibility that PBDEs can be transferred to the fetus across the placenta and that greater amounts might be transferred to nursing infants via breast milk, as well as evidence that perinatal exposure to PCBs and other similar chemicals may induce subtle neurological damage and immunological and thyroid effects in children, transgenerational studies would be particularly informative. Additional studies evaluating the potential link between pre- and peri-natal PBDE exposure and childhood behavior disorders, such as Autism Spectrum Disorder (ASD) and ADHD, have been specifically requested by the scientific community based upon potential links between these disorders and endocrine disruption (de Cock et al. 2012; Messer 2010). However, limitations that are likely to constrain epidemiological investigations, such as unmeasured PBDE exposure concentrations and lack of controls for confounding co-exposures, may be difficult to address. Studies that assess PBDE concentrations in serum or breast milk along with concentrations of other persistent organic pollutants (POPs), such as

PCBs, PCDDs, and /or PCDFs, would be useful to evaluate responses that may be co-dependent on other persistent lipophilic agents.

Biomarkers of Exposure and Effect.

Exposure. PBDEs accumulate in adipose tissue, serum, and breast milk of the general population due to their lipophilic characteristics. Concentrations of PBDEs in breast milk are useful, non-invasive markers of maternal body burdens and of *in utero* and lactational exposures, but body burden assessments are limited by a lack of time-trend data for PBDEs in the milk of U.S. populations (Hooper and McDonald 2000). Breast milk monitoring programs would provide time-trend data that would verify whether regulatory action to limit the use of PBDEs is reversing the previous trend of an exponential increase in PBDE concentrations in breast milk (Norén and Meironyté 1998, 2000). Studies on the predictive value of concentrations of PBDEs in serum and adipose tissue could provide useful information for detection and monitoring of exposure. It should be noted, however, that solubilities in adipose and breast milk are likely to vary with the congener. For example, decaBDE is much less soluble in adipose than pentaBDE. These differences must be considered when designing studies evaluating PBDE exposure. Recent studies have indicated that PBDE concentrations in hair may also be useful to estimate exposure levels (Aleksa et al. 2012a, 2012b; Malarvannan et al. 2013), although Zheng et al. (2011) reported that PBDE concentrations in hair were not correlated with PBDE concentrations in indoor dust from urban, e-waste, and rural areas in South China. Additional studies would be useful to validate this approach.

A potential biomarker of exposure to PBDEs relates to their effect on the thyroid gland. Thyroid changes in rats and mice include reduced serum T_4 levels, with no changes in serum TSH (Darnerud and Sinjari 1996; Fowles et al. 1994; Hallgren and Darnerud 1998; WIL Research Laboratories 1984; Zhou et al. 2001, 2002). However, using thyroid changes as a biomarker may not be reliable, as thyroid changes are not specific to exposure to PBDEs and the effects associated with the thyroid in non-clinical studies are likely specific to the rodent and may or may not be directly relevant to the human. Additional studies could characterize thyroid effects of PBDEs in humans and develop specific correlations between levels and duration of exposure and alterations in serum levels of T_4.

Effect. Biomarkers that could be used to characterize health effects caused specifically by exposure to PBDEs have not been identified. Additional information on the mechanisms of toxicity may suggest a useful biomarker of effect; however, at this time, there is little to suggest that such biomarkers exist.

Absorption, Distribution, Metabolism, and Excretion. No information was located regarding the toxicokinetics of PBDEs in humans following controlled oral exposure, although information of toxicokinetics has been gathered based on levels of PBDEs and metabolites in tissues of environmentally exposed individuals and numerous animal studies and *in vitro* studies.

Absorption studies in animals indicate that decaBDE is absorbed to a lesser degree than lower-brominated PBDEs. The most recent and best available estimates of oral absorption efficiencies indicate a range of 70–85% for the tetraBDE (BDE 47), pentaDE (BDE 99, BDE 100), and hexaBDE (BDE 153, BDE 154), and 10–26% for decaBDE (BDE 209) (Chen et al. 2006; Hakk et al. 2002a, 2002b, 2009; Klasson Wehler et al. 2001; Morck and Klasson Wehler 2001; Morck et al. 2003; Örn and Klasson-Wehler 1998; Riu et al. 2008; Sandholm et al. 2003; Sanders et al. 2006a, 2006b; Staskal et al. 2005). Quantitative absorption studies in humans could corroborate the conclusions on oral uptake in animals that are based on elimination and excretion data.

Distribution studies in animals indicate that lower-brominated BDE congeners, following absorption and an initial wide distribution, are preferentially accumulated in adipose tissues (Chen et al. 2006; El Dareer et al. 1987; Hakk et al. 2002a, 2009; Morck and Klasson-Wehler 2001; Morck et al. 2003; Norris et al. 1975a; NTP 1986; Örn and Klasson-Wehler 1998; Riu et al. 2008; Sanders et al. 2006a, 2006b; Staskal et al. 2005, 2006a). In comparison, decaBDE is more readily distributed to highly perfused tissues and less readily distributed to adipose tissues. Evidence for the transfer of PBDEs from pregnant mothers to the developing fetus and for the transfer of PBDEs from maternal blood to breast milk and then to nursing infants comes from a number of studies of PBDE concentrations in maternal and cord serum samples and breast milk samples from groups of non-occupationally exposed women. In general, the tetra- and penta-brominated PBDEs have been the predominant congeners detected in maternal and cord serum samples and breast milk samples, but some recent studies assaying for a wider range of PBDE congeners have found evidence for distribution of hepta-, octa-, or decaBDEs into cord serum and breast milk (Antignac et al. 2009, 2008; Chen et al. 2013; Daniels et al. 2010; Hites 2004; Kawashiro et al. 2008; Li et al. 2013a; Malarvannan et al. 2013; Mazdai et al. 2003; Meijer et al. 2008; Park et al. 2011; Qiu et al. 2009; Schecter et al. 2010, 2006; Vizcaino et al. 2011; Wan et al. 2010). Maternal transfer of both lower-brominated PBDEs and decaBDE has also been shown in animal studies (Cai et al. 2011; Zhang et al. 2011). The available studies appear adequate to characterize distribution of PBDEs.

Current evidence indicates that CYP2B6-mediated metabolism of BDE 47 and BDE 99 produces multiple hydroxylated metabolites via hydroxylation and ether bond cleavage, based on *in vitro* studies with

human liver microsomes or hepatocytes and human recombinant CYPs (Erratico et al. 2012, 2013; Feo et al. 2013). The major metabolites of BDE 47 and BDE 99 formed by human liver microsomes were not the same as those identified using rat liver microsomes (Erratico et al. 2013, 2012, 2011). Different classes of CYP enzymes appear to be involved in *in vitro* rat liver metabolism of BDE 47 and BDE 99: CYP1A1, CYP2A2, and CYP3A1 for BDE 47 and CYP1A1, CYP2A2, CYP2B1, and CYP3A1 for BDE 99 (Erratico et al. 2011). Production of hydroxylated metabolites of BDE 153 (Lupton et al. 2009) and BDE 209 (Stapleton et al. 2009) has not been demonstrated with human liver microsomes or hepatocytes. It is uncertain if these latter findings are reflective of a limited *in vivo* capacity of humans to metabolize these BDE congeners or because the proper *in vitro* conditions for metabolizing these congeners were not provided. Currently, studies of metabolism of BDE 47 and BDE 99 using *in vitro* human and rat systems have found evidence of metabolic oxidative debromination only with BDE 47 in human liver microsomes. In contrast, a number of *in vivo* studies have found evidence for oxidative debromination by analysis of feces collected from rats exposed to BDE 47 (Marsh et al. 2006), BDE 99 (Hakk et al. 2002a), BDE 100 (Hakk et al. 2006), BDE 154 (Hakk et al. 2009), and BDE 209 (Morck et al. 2003; Sandholm et al. 2003). Additional metabolism studies would help to characterize the enzymes involved as well as the transformation of some congeners to biologically active hydroxylated BDEs. There are still data gaps in the toxicokinetics of decaBDE, including an incomplete understanding of the debromination of decaBDE to lower-brominated BDEs.

The detection of PBDEs in human breast milk samples suggest that breast milk represents an elimination route of absorbed PBDEs in women (see Jakobsson et al. 2012 and Frederiksen et al. 2009 for reviews of PBDE levels in breast milk); however, studies do not provide a clear account of the degree to which PBDEs are cleared from the body during breastfeeding (Hooper et al. 2007; Jakobsson et al. 2012; LaKind et al. 2009; Thomsen et al. 2010). Rat studies indicate that ingested PBDEs are principally excreted in the feces with <2% of administered radioactivity excreted in the urine within 3 days of dose administration (Chen et al. 2006; Hakk et al. 2002a, 2006; Morck et al. 2003; Norris et al. 1973, 1975b; Orn and Klasson-Wehler 1998; Sanders et al. 2006a; Riu et al. 2008). However, a different elimination pattern has been observed in mice, especially with BDE 47. In mice given single oral doses of ^{14}C-labeled PBDE congeners, fecal and urinary elimination were principal routes of elimination for BDE 47 (Orn and Klasson-Wehler 1998; Sanders et al. 2006a), whereas fecal elimination appeared to be more important than urinary elimination with BDE 99 (Chen et al. 2006) and BDE 153 (Sanders et al. 2006b). Complementary studies with female C57BL/6J given single intravenous 1-mg/kg doses of ^{14}C-labeled BDE 47, BDE 99, BDE 100, or BDE 153 also indicate that the importance of urinary excretion in mice is congener specific (Staskal et al. 2006b). Quantitative studies in humans could

determine which excretion route is more relevant for humans (feces or urine) for different congeners based on elimination and excretion data. Additionally, further studies regarding the degree of elimination in breast milk during breast feeding could better characterize exposure risks for breastfeeding infants.

Comparative Toxicokinetics. Insufficient data are available to determine whether there are qualitative differences in the toxicokinetic disposition of PBDEs between humans and animals and among animal species. However, elimination studies in rats and mice (discussed above) highlight that toxicokinetics may differ between species. Differences are likely to be dependent on the specific congener or mixture studied, and pharmacokinetic modeling studies could help to determine the validity of extrapolating data. Most of the available toxicokinetic studies of PBDEs have been performed in rats, and studies in other species could help to ascertain the most relevant animal model.

Methods for Reducing Toxic Effects. The mechanism by which PBDEs enter the blood stream is not known, there are no established methods for reducing body burden of PBDEs, and the mechanisms of toxic action of PBDEs are incompletely understood. A more complete characterization of the cytosolic AhR protein and understanding of physiological effects of receptor blockage would be useful for the possible identification of blockers of AhR-mediated toxic effects. Further studies aimed at elucidating the nonreceptor-mediated mechanism of action of some PBDEs would also be valuable.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

Body burden data, as well as intake modeling, suggest that infants and toddlers have higher exposures to PBDEs as compared to older children or adults (EPA 2010; Lorber 2008; Trudel et al. 2011; Wong et al. 2013). Several epidemiological studies have reported results suggestive of an effect of PBDE on neurodevelopment in children (Adgent et al. 2014; Chao et al. 2011; Chen et al. 2014; Chevrier et al. 2016; Cowell et al. 2015; Ding et al. 2015; Eskenazi et al. 2013; Gascon et al. 2012; Herbstman et al. 2010; Hoffman et al. 2012; Roze et al. 2009; Sagiv et al. 2015; Shy et al. 2011; Vuong et al. 2016a), and these findings are supported by developmental studies in animals (Biesemeier et al. 2011; Blanco et al. 2013; Branchi et al. 2001, 2002, 2005; Cheng et al. 2009; Eriksson et al. 2001, 2002, 2006; Fischer et al. 2008; Fujimoto et al. 2011; Gee and Moser 2008; He et al. 2009, 2011; Johansson et al. 2008; Koenig et al. 2012; Kuriyama et al. 2004, 2005; Rice et al. 2007; Sand et al. 2004; Ta et al. 2011; Viberg et al. 2002, 2003a, 2003b, 2004a, 2004b, 2005, 2006, 2007; Woods et al. 2012; Xing et al. 2009). Epidemiological

studies in infants are inconclusive regarding thyroid effects and exposure to PBDEs (Abdelouahab et al. 2013; Herbstman et al. 2010; Kim et al. 2011a, 2012a; Lin et al. 2011; Mazdai et al. 2003; Shy et al. 2012); however, numerous animal studies indicated that developmental exposure to lower-brominated PBDEs result in thyroid hormone alterations (Blanco et al. 2013; Bondy et al. 2011, 2013; Ellis-Hutchings et al. 2006; Kodavanti et al. 2010; Kuriyama et al. 2007; Miller et al. 2012; Poon et al. 2011; Shah et al. 2011; Skarman et al. 2005; Szabo et al. 2009; Wang et al. 2011a; Zhang et al. 2009; Zhou et al. 2002). Additional studies would better characterize the potential susceptibility of children to the effects of PBDEs on the thyroid and neurodevelopment, particularly considering the possibility that these effects are related to the dependence of central nervous system development on thyroid hormones.

Data from two human epidemiological studies suggest that PBDE exposure may alter reproductive system development in boys (Main et al. 2007; Meijer et al. 2012). In contrast, other studies found no associations between serum or adipose tissue PBDE concentrations and reproductive development (Carmichael et al. 2010; Koskenniemi et al. 2015; Leijs et al. 2008). These findings, along with limited evidence of reproductive effects in animals exposed to lower-brominated PBDEs during development (Kuriyama et al. 2005; Talsness et al. 2005, 2008), indicate that additional studies of reproductive development in young animals would help to more fully assess children's susceptibility to PBDEs.

No information is available regarding the immunosuppressive potential of PBDEs in children, but serum levels of BDE 28 and 209 were found associated with an increased risk of asthma in a study of 3–6-year-old Chinese children (Meng et al. 2016). Limited evidence in young animals exposed to PBDEs (Bondy et al. 2013; Watanabe et al. 2008, 2010b) indicates that additional studies of immune competence in developing animals would also help to more fully assess children's susceptibility to PBDEs.

A recent small study of Swedish toddlers (n=22) found good correlations between concentrations of some PBDEs in serum and in feces, suggesting that fecal analysis, a noninvasive test, might be a good alternative for biomonitoring PBDEs in toddlers (Sahlström et al. 2015). Larger studies would be valuable to improve the predictive power of the statistical analyses performed in this study. In addition, it would be helpful to determine whether the method is applicable to infants and children of different ages.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Fifty-six ongoing research efforts have been identified that may provide data related to the toxic actions of PBDEs in humans (RePORTER 2014, 2016). These projects are summarized in Table 3-7.

Animal Studies. Dr. Charles Herbert of the Southern Research Institute is conducting chronic studies of the commercial pentaBDE mixture DE-71 in rats and mice under contract with NTP and Dr. Lu Taylor of the Battelle Memorial Institute is conducting a perinatal study BDE 47 in CB6F1-Tg(HRAS2) transgenic mice under contract with NTP.

Several animal studies are investigating neurobehavioral changes in animals exposed to PBDEs. Four ongoing studies are investigating behavioral and biochemical end points relevant to ADHD (Drs. Susan Schantz and Paula Eubig, University of Illinois Urbana-Champaign; Dr. Richard Seegal, Wadsworth Center). To assess the role of the immune system and its interaction with environmental contaminants in autism and other neurodevelopmental disorders, Dr. Isaac Pessah of the University of California at Davis is investigating the effects of perinatal PBDE exposure on brain development, complex social behaviors, and immune system function in mouse strains with low (C57BL/6J) or high (SJL mice) susceptibility to autoimmunity. Using an established mouse model, Dr. Margarita Behrens, from the Salk Institute for Biological Sciences, will examine the effects of exposure to PBDE on autism spectrum disorder. Dr. Helen Sable of the University of Memphis is examining if developmental PBDE exposure in rats alter dopamine receptor expression and enhance behavioral sensitization following psychostimulant exposure (as seen with PCBs).

Dr. Deena Small of the University of New England is examining bone growth and remodeling in mice exposed to pentaBDE from PND 1 to 60. In addition, Dr. Small will use cell culture-based assays that measure gene expression, enzyme activity and calcium deposition in cultured bone cell lines exposed to the pentaBDEs.

Toxicokinetic Studies. Dr. Tracey Woodruff of the University of California, San Francisco is measuring concentrations of PBDEs and OH-PBDEs in human maternal and fetal biological specimens from women undergoing voluntary, second trimester pregnancy terminations and generating original human data on whether fetal exposures to PBDEs alter gene expression of cytochrome P450 (CYP) enzymes in the second-trimester human fetal liver and placenta. Dr. James Olson of SUNY Buffalo is conducting a qualitative and quantitative characterization of the human CYP-specific *in vitro* metabolism of

Table 3-7. Ongoing Research for PBDEs in Humans

Investigator	Affiliation	Research description	Sponsor
Baccarelli, A	Harvard School of Public Health	Prenatal exposure to PBDEs and visual skills, attention, and fine motor skills in children	NIEHS
Barr, DB	Emory University	Prenatal exposure to PBDEs and birth outcomes	NIHES
Blumberg, B	University of California-Irvine	Endocrine disrupter modulation of the steroid and xenobiotic receptor, SXR, in development and lymphomagenesis	NIEHS
Caudle, WM	Emory University	Vesicular monoamine transporter 2 as a mediator of PBDE neurotoxicity	NIEHS
Chen, A	University of Cincinnati	Longitudinal study of exposure to PBDEs and child neurobehavior	NIEHS
Chen, S	Beckman Research Institute/City of Hope	Determine role and mechanism of PBDEs on development of breast cancer during the menopausal transition	NIEHS
Costa, LG	University of Washington	Low-level exposure to PBDEs: Testing the hermetic and epigenetic hypotheses	NIEHS
Croen, LA	Kaiser Foundation Research Institute	Prenatal and neonatal biologic markers for autism	NIEHS
Darrow, L	Emory University	PBDE body burdens, house dust concentrations, and associations with thyroid hormone	NIEHS
Di Giulio, R	Duke University	Thyroid metabolism disruption key in toxicant-induced development	NIEHS
Eskenazi, B	University of California Berkeley	Pesticides and PBDEs on neurobehavior	NIEHS
Eskenazi, B	University of California Berkeley	PBDEs, DDT, and neurodevelopment in school-aged Mexican-American children	NIEHS
Eskenazi, B	University of California Berkeley	DDT and PBDE exposure, puberty onset, and neurodevelopment in Mexican-American girls	NIEHS
Eubig, P	University of Illinois Urbana-Champaign	Effects of PCBs and PBDEs on three distinct components of response inhibition	NIEHS
Ferguson, PL	University of South Carolina at Columbia	Mechanisms of xenoestrogen stress: a proteomic and functional genomic approach	NIEHS
Fitzgerald, EF	State University of New York at Albany	Persistent organic pollutants and cognitive decline in the elderly	NIEHS
Getahun, D	Kaiser Foundation Research Institute	Flame retardant and adverse perinatal outcome	NIEHS
Giese, RW	Northeastern University	Discovery of xenobiotics associated with preterm birth	NIEHS
Hauser, RB	Harvard School of Public Health	Maternal and paternal flame retardant exposure, impact on fertility and pregnancy	NIEHS
Herbert, C	Southern Research Institute	Studies to evaluate the toxicologic and carcinogenic potential	NIEHS
Herbstman, JB	Columbia University	Pre- and postnatal PBDE exposure, thyroid hormones, and neurodevelopment	NIEHS

Table 3-7. Ongoing Research for PBDEs in Humans

Investigator	Affiliation	Research description	Sponsor
Hertz-Picciotto, I	University of California at Davis	Epidemiology and the environment in autism	NIEHS
Hertz-Picciotto, I	University of California at Davis	The CHARGE study: Childhood Autism Risk from Genetics and Environment	NIEHS
Hertz-Picciotto, I	University of California at Davis	Autism risk, prenatal environmental exposures, and pathophysiologic markers	NIEHS
Holland, NT	University of California Berkeley	Epigenetic effects of DDT/E and PBDEs on puberty	NIEHS
Kamen, DL	Medical University of South Carolina	Environmental determinants of autoimmunity among African Americans in coastal South Carolina	NIEHS
Klaassen, CD	University of Washington	Developmental exposure to PBDEs and long-lasting modifications of drug metabolism in children	NIEHS
Lasalle, JM	University of California at Davis	Epigenetic interaction of MECP2 and organic pollutants in neurodevelopment	NIEHS
Lasalle, JM	University of California at Davis	Methylomic and genomic impacts of organic pollutants in Dup15q syndrome	NIEHS
Loch-Caruso, RK	University of Michigan	Mechanisms of inflammation in gestational membranes	NIEHS
Louis, G	Eunice Kennedy Shriver National Institute of Child Health & Human Development	Longitudinal investigation of fertility and the environment	NICHD
Miller, PK	Alaska Community Action on Toxics (ACAT)	Protecting the health of future generations: assessing and preventing exposures	NIEHS
Morello-Frosch, RA	University of California, San Francisco	Effects of endocrine disrupting chemicals and chronic psychosocial stress on fetal growth	NIEHS
Olson, JR	State University of New York at Buffalo	Bioactivation of PBDEs by human cytochrome P-450	NIEHS
Pessah, IN	University of California at Davis	Calcium signaling defects in autism	NIEHS
Pessah, IN	University of California at Davis	Neurodevelopmental toxicology of autism	NIEHS
Peterson, M	University of Utah	Environment chemicals and gynecologic health	NICHD
Rappaport, SM	University of California Berkeley	Exposure Assessment for childhood leukemia	NIEHS
Robinson, JF	University of California, San Francisco	Polybrominated diphenyl ether effects on human neuronal development	NIEHS
Sable, HJ	University of Memphis	Assessment of psychostimulant addiction risk following developmental PCB exposure	NIEHS
Sabo-Attwood, TL	University of South Carolina at Columbia	Mechanisms of xenoestrogen stress: a proteomic and functional genomic approach	NIEHS

Table 3-7. Ongoing Research for PBDEs in Humans

Investigator	Affiliation	Research description	Sponsor
Schantz, SL	University of Illinois Urbana-Champaign	PCBs, PBDEs, hearing loss, and attention/impulsivity: mechanistic studies in animals	NIEHS
Schantz, SL	University of Illinois Urbana-Champaign	Health effects of PCB exposure from contaminated fish	ATSDR
Seegal, RF	Wadsworth Center	Developmental neuroendocrine effects of PCBs and PBDEs	NIEHS
Small, DJ	University of New England	Effect of polybrominated diphenyl ether flame retardant exposure on osteogenesis	NIEHS
Stapleton, HM	Duke University	Children's exposure to flame retardants: effects on thyroid hormone regulation	NIEHS
Stapleton, HM	Duke University	Deiodinase activity as a biomarker of response to brominated flame retardants	NIEHS
Taylor, LU	Battelle Memorial Institute	Studies to evaluate the toxicological potential of test articles	NIEHS
Turyk, ME	University of Illinois at Chicago	Diabetes and persistent organic pollutants	NIEHS
Van de Water, JA	University of California at Davis	Immune environment interaction and neurodevelopment	NIEHS
Wapner, R	Columbia University Health Sciences	Endocrine disruption in pregnant women: thyroid disruption and infant development	NIEHS
Webster, TF	Boston University Medical Campus	Measuring human exposure to PBDEs	NIEHS
Woodruff, TJ	University of California, San Francisco	Human maternal/fetal exposures to PBDEs and their metabolites during development	NIEHS
Woodruff, TJ	University of California, San Francisco	Mid-gestational exposure to endocrine disrupting chemicals and effects on placental development	NIEHS
Zhang, Y	Yale University	Polyhalogenated aromatic hydrocarbons and thyroid cancer risk in the Department of Defense Serum Repository (DoDSR) cohort	NIEHS
Zota, AR	George Washington University	Role of endocrine-disrupting chemicals and social stress on perinatal outcomes	NIEHS

Source: RePORTER 2014, 2016

NIEHS = National Institute of Environmental Health Sciences; PBDE = polybrominated diphenyl ether; PCB = polychlorinated biphenyl

2,2',4,4'-tetraBDE (BDE 47), 2,2',4,5'-tetraBDE (BDE 49), 2,2',4,4',5-pentaBDE (BDE 99), and 2,2',4,4',6- pentaBDE (BDE 100) as well as quantify OH-PBDEs in human milk and serum and assess the potential impact of CYP2B6 genotype on the body burden of PBDEs. Dr. Heather Stapleton of Duke University is identifying the products of hepatic metabolism of PBDEs (no further details available).

Mechanistic Studies. Several studies are investigating mechanisms of neurotoxicity. At the University of California at Davis, Dr. Janine Lasalle is investigating epigenetic changes in the genome of Mecp2 mutant mouse models of Rett syndrome and autism following *in vivo* exposure to BDE 47, Drs. Janine Lasalle and Isaac Pessah are investigating potential mechanisms of PBDE toxicity in susceptible neuronal cell models (chromosome 15q11-13 duplication syndrome [Dup15q] or fragile X syndrome [FMRI]), and Dr. Judy Van de Water is investigating mechanisms of PBDE toxicity in peripheral blood mononuclear cells from children with autism. Dr. Joshua Robinson of the University of California San Francisco is investigating the effects *in vitro* exposure to BDE 47 and BDE 99 on neuronal differentiation and gene expression in human embryonic stem cells. Dr. Lucio Costa of University of Washington is investigating genetic and epigenetic changes in cultured mouse neurons exposed to low, environmentally-relevant concentrations of BDE 47. Dr. William Caudle of Emory University is investigating the potential mechanisms by which the commercial pentaBDE mixture DE-71 disrupts VMAT2, which is a key mediator of cytosolic dopamine.

Additional studies are investigating mechanisms of thyroid hormone disruption. Dr. Heather Stapleton of Duke University is investigating the effects of PBDEs and their metabolites on intra- and extra-cellular thyroid hormone regulation *in vitro* to elucidate the mechanisms of action for thyroid toxicity. Dr. Deena Small of the University of New England is measuring thyroid hormone receptor- mediated transcription and thyroid hormone receptor binding of pentaBDEs *in vitro.* Dr. Richard Di Guilio of Duke University is investigating the potential role of inhibition of diodinases by PBDEs in altered thyroid hormone homeostasis, using zebrafish as a model.

Other mechanistic studies are investigating mechanisms of xenoestrogen stress (Drs. P. Lee Ferguson and Tara Sabo-Attwood of the University of South Carolina at Columbia), potential mechanisms behind the etiology of inflammation of extra-placental gestational membranes and associated release of cytokines and prostaglandins (which are associated with preterm birth) (Dr. Rita Loch-Caruso of the University of Michigan), the potential role of the steroid and xenobiotic receptor SXR in the development of lymphoma and leukemia in individuals exposed to PBDEs (Dr. Bruce Blumberg of the University of California-Irvine), and development of biomarkers of mitochondrial function in primary mouse hepatocytes exposed

to BDE 47 using metabolomic analysis (Dr. Hockenberry from the Fred Hutchison Cancer Research Center.