5. HEALTH EFFECTS

5.1 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 polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (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.

PBBs and PBDEs are classes of brominated hydrocarbons that are used as flame retardant additives in plastics, textiles, and other materials. Both classes of chemicals are comprised of 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 PBBs and PBDEs (mono-brominated through decabrominated), each containing one or more isomers. PBBs and 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 PBBs and PBDEs are necessarily similar.

Commercial production of PBBs began in approximately 1970, and manufacture was discontinued in the United States in 1976 following a contamination episode that occurred in Michigan in 1973–1974. Three main commercial mixtures of PBBs were produced: hexabromobiphenyl, octabrombiphenyl, and deca-bromobiphenyl. The most prevalent hexabromobiphenyl PBB mixtures had the trade names FireMaster BP-6 and FireMaster FF-1. FireMaster FF-1 was produced by grinding FireMaster BP-6 and adding 2% calcium polysilicate as an anticaking agent. The hexabromobiphenyl mixtures contained varying proportions (depending on lot number) of di- through octabrominated homologues, and 2,2',4,4',5,5'-hexa-bromobiphenyl (BB 153) was the most abundant congener (53.9–68.0%) followed by 2,2',3,4,4',5,5'-heptabromobiphenyl (7.0–27.3%). Commercial octabromobiphenyl PBB mixtures contained a large proportion (47.4–60.0%) of nonabromobiphenyl congeners, whereas commercial decabromobiphenyls contained predominately (96.8%) decabromobiphenyl congener. The general names hexabromobiphenyl,

octabromobiphenyl, and decabromobiphenyl are used in this profile to refer to unspecified commercial mixtures of these PBBs.

Concern regarding the health effects of PBBs is largely related to exposures that resulted from the Michigan contamination episode. Livestock on farms in Michigan were exposed to FireMaster FF-1 over a period of approximately 10 months after it was accidentally mistaken for the feed supplement magnesium oxide and mixed with animal feed that was distributed within the state. Health problems in dairy cattle (decreased feed consumption and decreased milk production), reported in the fall of 1973, were the first signs that the contamination episode occurred, but accidental addition of PBBs to animal feed was not identified as the cause of the problem until late spring of 1974 (Fries 1985a; Jackson and Halbert 1974). The U.S. Food and Drug Administration (FDA) established tolerances of 1 ppm in milk and meat fat and 0.1 ppm in eggs in May 1974, which were revised downward to 0.3 and 0.05 ppm, respectively, in November 1974 due to improved analytical sensitivity (Dunckel 1975; Fries 1985a). The Michigan Department of Agriculture (MDA) subsequently lowered the FDA tolerance in meat fat from 0.3 to 0.02 ppm, but there currently are no FDA or MDA tolerances for PBBs (FDA 1989; Fries 1985a). As a result of a farm animal testing and quarantining program established by the MDA in May 1974, about 30,000 dairy cattle, 2,000 swine, 400 sheep, and over 2,000,000 chickens were found to contain PBBs at concentrations requiring their destruction (Dunckel 1975; Fries 1985a; Mercer et al. 1976).

Most of the information that is available on health effects of PBBs in humans comes from studies of Michigan residents who ingested milk, meat, and eggs that were produced on farms that used the FireMaster-contaminated animal feed. In the interval of more than 9 months between the accident, the detection and identification of its cause, and the beginning of testing and the establishment of quarantines, PBB-contaminated food products were consumed, not only by farm families and people that acquired produce directly from PBB-contaminated farms, but also by people who purchased food from markets (Anderson et al. 1979). The Michigan PBB contamination episode led to the establishment of epidemiological studies (that are still ongoing) of Michigan residents who were expected to have consumed PBB-contaminated food, as well as to a substantial increase in research activity regarding the health effects of PBBs in cattle, poultry, and laboratory animals. Compared to the FireMaster (commercial hexabromobiphenyl) PBBs, relatively limited data are available on health effects of commercial mixtures of octabromobiphenyl and decabromobiphenyl. Reviews of the research results on the toxicity of PBBs include those by Damstra et al. (1982), DiCarlo et al. (1978), Fries (1985a), Kay (1977), Kimbrough (1987), Kimbrough et al. (1978a), and WHO (1994a).

5. HEALTH EFFECTS

This profile discusses information regarding health effects of PBBs in humans and laboratory animals; some research on cattle and poultry is also discussed, but its relevance to human health effects is uncertain due to interspecies physiological differences. Although the toxicity in livestock from Michigan farms that used large amounts of contaminated feed is generally attributed to PBBs, data on effects in animals from farms with low PBB contamination have generated some controversy, because the signs of toxicosis in these animals have not been reproduced in cattle experimentally exposed to PBBs at levels that caused tissue residue concentrations ≈100 times greater than those in the farm animals (Jackson and Halbert 1974; Moorhead et al. 1977). This led some investigators to suggest that some signs of toxicosis reported in Michigan cattle reflected farm management procedures, nutritional deficiencies, microbial and parasitic infections, or exposure to unknown contaminants in the feed (Durst et al. 1977; Fries 1985a; Moorhead et al. 1977). Although exposure by ingestion occurred during the Michigan contamination episode, existing information on the metabolism of PBBs in livestock is insufficient to ascertain whether the people ingested PBBs or metabolic products of PBBs. However, based on available data discussed in Section 5.4.2.2, it is reasonable to assume that mainly unchanged penta-, hexa-, and heptabromobiphenyl congeners in animal products were consumed.

Unlike PBBs, PBDEs have been continuously produced and used as flame-retardant additives since the 1970s. Concern for health effects of PBDEs has heightened due to relatively recent evidence that some PBDE congeners have become ubiquitously distributed in the environment and are present in tissues and breast milk of the general population at levels that continue to increase. Three commercial PBDE mixtures have been produced: decabromodiphenyl ether (decaBDE), octabromodiphenyl ether (octaBDE), and pentabromodiphenyl ether (pentaBDE). DecaBDE has accounted for more than 80% of PBDE usage. The composition of commercial decaBDE is ≥97% of the pure congener with the remainder mainly nonaBDE. Commercial octaBDE is a mixture of congeners ranging from nona- to hexaBDE, and mixtures of pentaBDE are comprised of tetra-, penta-, and hexaBDE congeners. Congeners with less than four bromine atoms are generally not found in commercial PBDEs. Reviews on the health effects and other aspects of PBDEs include those by Darnerud et al. (2001), de Boer et al. (2000), de Wit (2002), Hardy (1999, 2002a, 2002b), McDonald (2002), NAS (2002), Rahman et al. (2001), Silberhorn et al. (1990), and WHO (1994b). Penta- and octa-BDEs are apparently being phased out of production and use. Great Lakes Chemical Corporation will cease production of penta- and octaBDE by the end of 2004 (Tullo 2003) and the European Union has banned the sale of all products containing more than 0.1% (by mass) penta- or octaBDE (EU 2003b).

Toxicity data for individual PBB and PBDE congeners are included in some discussions in this profile when these data corroborate or provide information on effects not documented for the PBB and PBDE mixtures. Congener-specific toxicity data are currently not practical for determining exposure levels of PBB and PBDE mixtures associated with adverse health effects at hazardous waste sites. This is due in part to the fact that standardized analytical procedures for congener mixtures and commercially available standards for all congeners are lacking, and congener-specific analyses are not routinely performed. Additionally, using current health effects evaluation procedures, toxicity data for individual congeners may overestimate or underestimate the actual health risk of PBB and PBDE mixtures because congeners vary in toxic potency and may be influenced by other congeners in an additive or less-than-additive way. It is also important to recognize that the PBBs and PBDEs to which people may be exposed may be different from the original PBB and 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.

5.2 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.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for PBBs and PBDEs. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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.

5.2.1 Inhalation Exposure

A few studies have examined groups of chemical workers involved in the manufacture and distribution of PBBs and/or 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 predominately 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 5.2.3. 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 5-1 and plotted in Figure 5-1; due to the general lack of data regarding inhalation exposure to PBBs, no equivalent table is presented for PBBs.

5.2.1.1 Death

Polybrominated Biphenyls. No studies were located regarding death in humans after inhalation exposure to PBBs.

Nose-only exposure to the highest attainable dust concentration of octabromobiphenyl mixture for 4 hours (960 mg/m^3 as a time-weighted average) was not lethal to six male rats observed for 7 days (Waritz et al. 1977). No deaths occurred in groups of five male and five female rats that were exposed to a decabromobiphenyl dust mixture at concentrations ranging from 5 to 5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). Information on lethality of inhaled hexabromobiphenyl PBB mixtures was not located.

Polybrominated Diphenyl Ethers. 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 levels of 174 mg/m^3 for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978), ≤250 mg/m^3 for 6 hours/day, 5 days/week for 14 days (Great Lakes Chemical Corporation 2000), or ≤202 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2001a, 2001b).

Table 5-1 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Inhalation

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form
	ACUTE EXPOSURE						
	Systemic						
1	Rat (CD)	14 d 8 h/d	Resp	3.7	24 (reversible rapid breathing)		Great Lakes Chemical Corporation 1978 OctaBDE
			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 5-1 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form
2	Rat (CD)	14d 5 d/wk 6 h/d	Resp	1			Great Lakes Chemical Corporation 2000 OctaBDE
			Hepatic	1	10		
			Renal	250			
			Bd Wt	250			

Table 5-1 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form
	INTERMEDIATE EXPOSURE						
	Systemic						
3	Rat (CD)	13 wk 5 d/wk 6 h/d	Resp	16	202 (alveolar histiocytosis, chronic active lung inflammation)		Great Lakes Chemical Corporation 2001 OctaBDE
			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						
4	Rat (CD)	13 wk 5 d/wk 6 h/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 2001 OctaBDE

Table 5-1 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form
	Reproductive						
5	Rat (CD)	13 wk 5 d/wk 6 h/d		16 F	202 F (absence of corpora lutea in ovaries)		Great Lakes Chemical Corporation 2001 OctaBDE

a The number corresponds to entries in Figure 5 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).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; gastro = gastrointestinal; hemato = hematological; hr = hour(s); LOAEL = lowest observed adverse effect level; M = male; metab = metabolic; min = minute; mo = month(s); Musc/skel = muscular/skeletal; NOAEL = no observed adverse effect level; Resp = respiratory; T4 = thyroxine ; TSH = thyroid stimulating hormone; wk = week(s)

Figure 5-1. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers- Inhalation

Acute (≤14 days)

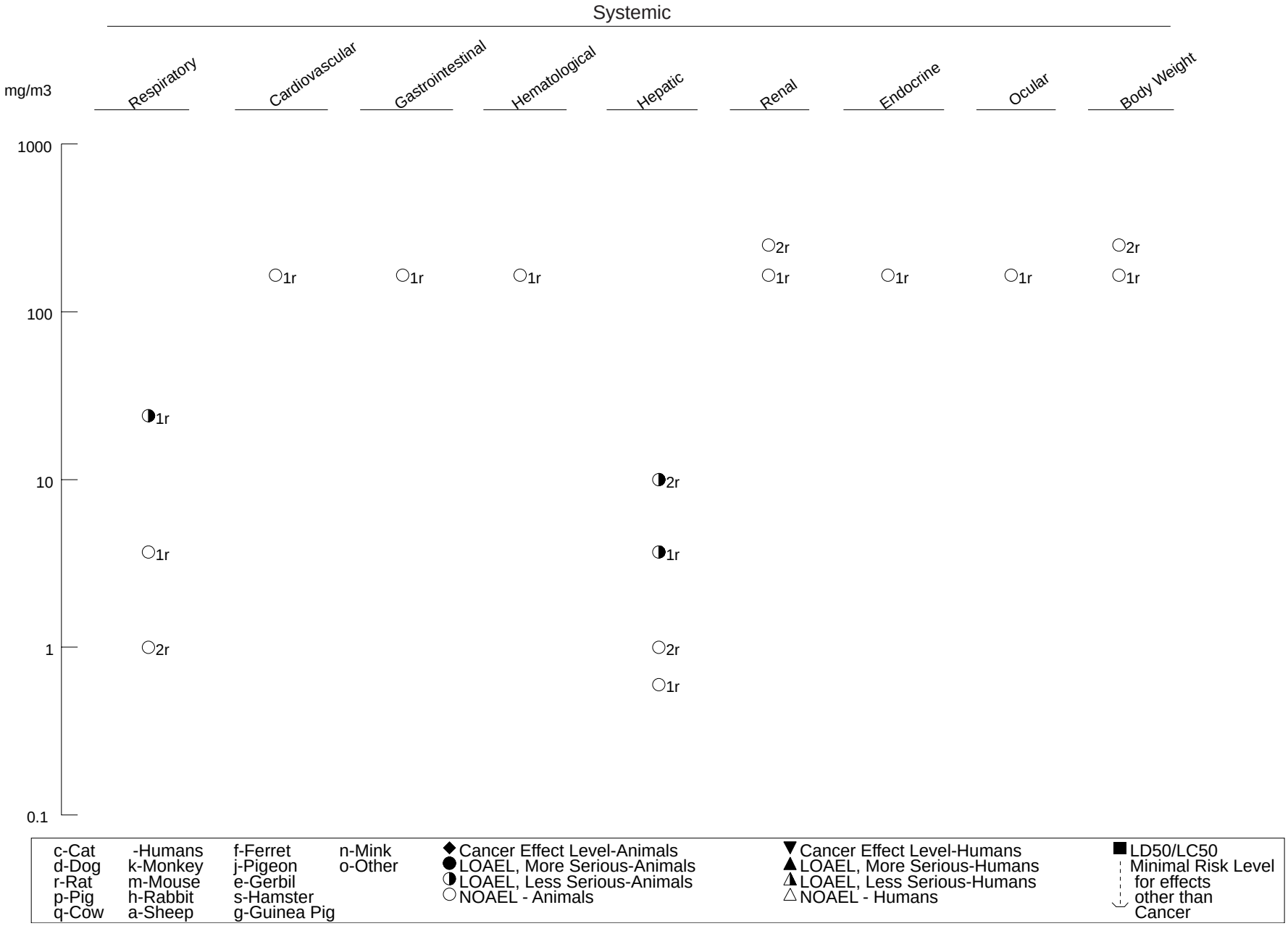
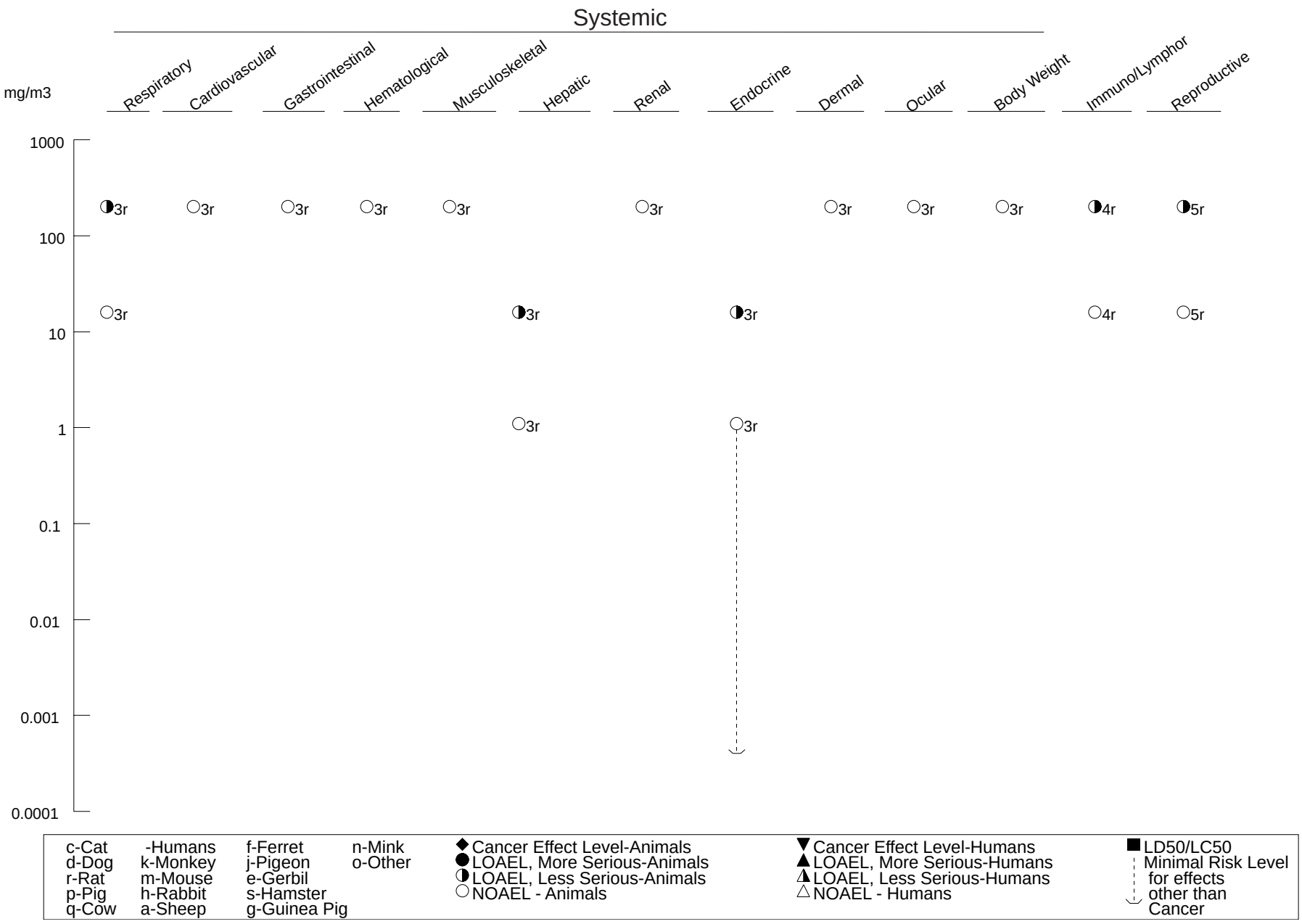


Figure 5-1. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers- Inhalation (*Continued*)

Intermediate (15-364 days)



5.2.1.2 Systemic Effects

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

Respiratory Effects.

Polybrominated Biphenyls. No studies were located regarding respiratory effects in humans after inhalation exposure to PBBs.

Slight dyspnea was observed in five male and five female rats that were exposed to a decabromobiphenyl dust mixture at 5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). This effect was not observed at 500 mg/m^3 and lower concentrations or in air only-exposed controls, and there were no changes in pulmonary resistance and compliance in urethane-anesthetized rats, blood gases, and lung histology at any of the exposure levels. Lung function and blood gases were not evaluated in starch-exposed controls, but this is unlikely to be a serious study deficiency as the ratio of PBB to starch was ≈1,000 in the high exposure group.

Polybrominated Diphenyl Ethers. No studies were located regarding respiratory effects in humans after inhalation exposure to PBDEs.

Transient signs of respiratory distress that included tachynpea 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.

Two 14-day inhalation studies of commercial octaBDE have been conducted. In one 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 levels. 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). In the other study, rats were nose-only exposed to 0, 1.0, 10, 110, or 250 mg/m^3 as dust aerosol for 6 hours/day, 5 days/week for 2 weeks (Great Lakes Chemical Corporation 2001a, 2001b). Histological examinations showed nasal alterations, consisting of minimal to mild goblet cell hyperplasia and/or hypertrophy at ≥1 mg/m^3 in males and ≥10 mg/m^3 in females. The nasal goblet cell changes occurred in nasal levels II and III at 1 mg/m^3 and generally in levels II-VI at ≥10 mg/m^3, and were considered indicative of very slight nasal irritation. No histopathology in the lungs or toxicolgically significant clinical signs were observed.

Histological changes in the lungs, but not clearly 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 2001a, 2001b). The pulmonary effects included alveolar histiocytosis and chronic active inflammation, 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 incidences were not clearly dose-related and there was essentially no increase in severity from minimal 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.

Polybrominated Biphenyls. No studies were located regarding cardiovascular effects in humans or animals after inhalation exposure to PBBs.

Polybrominated Diphenyl Ethers. 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 levels 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 2001a, 2001b).

Gastrointestinal Effects.

Polybrominated Biphenyls. No studies were located regarding gastrointestinal effects in humans or animals after inhalation exposure to PBBs.

Polybrominated Diphenyl Ethers. 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 tissues of rats that were exposed to dusts of commercial octaBDE products at levels 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 2001a, 2001b).

Hematological Effects.

Polybrominated Biphenyls. No studies were located regarding hematological effects in humans after inhalation exposure to PBBs.

Hematology was normal in groups of five male and five female rats that were exposed to a decabromobiphenyl dust mixture at concentrations of 5 or 5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). The evaluation included erythrocyte and leucocyte counts, differential leukocyte count, hematocrit, and hemoglobin level.

Polybrominated Diphenyl Ethers. 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 remarkable 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 2001a, 2001b).

Hepatic Effects.

Polybrominated Biphenyls. No studies were located regarding hepatic effects in humans after inhalation exposure to PBBs.

No significant ($p<0.05$) increase in relative liver weight or hepatic histological changes were found in six male rats nose-only exposed to a octabromobiphenyl dust mixture at 960 mg/m^3 for 4 hours (time-weighted average, highest attainable concentration), and observed for 7 days (Waritz et al. 1977). Toxicity of octabromobiphenyl mixture vapor was investigated in groups of six rats almost continuously exposed (23 hours/day, 7 days/week) for 2, 4, 7, 9, 11, 13, or 15 weeks (Waritz et al. 1977). The exposure level was 0.00035 µg/m^3, which is the reported equilibrium concentration at 28 °C. Gross pathologic examination and measurement of relative liver weight showed no exposure-related changes at any of the sacrifices, but it is unclear if liver histology was evaluated.

Relative liver weight was increased ≈25% in groups of 5 or 10 rats that were exposed to a decabromo-biphenyl dust mixture at concentrations of 50–5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). The increased liver weight was not accompanied by hepatic histologic changes, and therefore may be an adaptive response because PBBs are hepatic inducers and cause cellular proliferation (see Section 5.2.2.2 Hepatic Effects). No effects on liver weight or histology were observed at 5 mg/m^3.

Polybrominated Diphenyl Ethers. No studies were located regarding hepatic effects in humans after inhalation exposure to PBDEs.

Hepatic effects were observed in 14-day inhalation studies of dusts of commercial octaBDE mixtures. In one 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 at 3.7 mg/m^3 and higher levels of exposure. 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 at ≥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. In another study, rats were nose-only exposed to 0, 1.0, 10, 110, or 250 mg/m^3 as dust aerosol for 6 hours/day, 5 days/week for 2 weeks (Great Lakes Chemical Corporation 2001a, 2001b). Hepatic effects included increased mean absolute and/or relative liver weights at ≥10 mg/m^3 in males and ≥110 mg/m^3 in females, with the greatest increases at 110 and 250 mg/m^3 (21–44%). Centribobular hypertrophy similarly occurred in the liver at ≥10 mg/m^3 in both sexes (100% incidences except for 4/5 females at 10 mg/m^3).

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 2001a, 2001b). The liver was affected in both sexes as shown by dose-related increased 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 (39.8% less 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 the 202 mg/m^3 females, but were not considered exposure-related due to small magnitudes of changes and lack of similar changes in the males.

Renal Effects.

Polybrominated Biphenyls. No studies were located regarding renal effects in humans after inhalation exposure to PBBs.

Groups of six rats were exposed to 0.00035 µg/m^3 of octabromobiphenyl mixture vapor (equilibrium concentration) 23 hours/day, 7 days/week for 2, 4, 7, 9, 11, 13, or 15 weeks (Waritz et al. 1977). Gross pathologic examination at each sacrifice and measurement of relative kidney weight at the last sacrifice showed no exposure-related changes, but it is unclear if kidney histology was evaluated.

Urinalysis was normal in groups of five male and five female rats that were exposed to a decabromobiphenyl dust mixture at concentrations ranging from 5 to 5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). The analysis included pH, specific gravity, proteins, glucose, ketone bodies, biliary pigments, urobilinogen, blood, and microscopic examination of sediment. A comprehensive histology evaluation was performed in this study, but the only tissues specifically mentioned as having been examined are the liver and lungs. However, a total of 21 tissues were examined; therefore, it is probable that the kidney was examined, but was not discussed because no histological alterations were found.

Polybrominated Diphenyl Ethers. No studies were located renal respiratory 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 levels of 174 mg/m^3 for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978), ≤250 mg/m^3 for 6 hours/day, 5 days/week for 14 days (Great Lakes Chemical Corporation 2000), or ≤202 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2001a, 2001b). Urinalyses were not performed in any of these studies.

Endocrine Effects.

Polybrominated Biphenyls. Hypothyroidism was diagnosed in 4 of 35 men who were occupationally exposed to unspecified PBBs and/or decaBDE (Bahn et al. 1980). The cohort consisted of workers (mean age 35.9 years) who had been employed at a production plant for at least 6 weeks during a 52-month period during which PBBs and decaBDE were the only chemicals manufactured and who had volunteered for a comprehensive medical evaluation performed 3 months after the end of the 52-month period. There was no further description of exposure, and it was assumed to have occurred by both inhalation and dermal routes. The cohort was matched by sex, race, and age to 89 unexposed control subjects. Four subjects (22–50 years old, employed for 9–46 months not entirely during the 52-month production period) had elevated serum thyrotropin levels (mean 37.5 versus ≤1.5–8 µU/ml normal range), low or borderline low serum T_4 levels (4.4 versus 4.5–11.5 µg/dL) and free-thyroxine indices (3.7 versus 3.8–10.8), and markedly elevated thyroid antimicrosomal antibody titers (1:6,400 or above). Serum T_4 levels measured 7 months earlier in two of the four men were normal. Antithyroglobulin antibodies were elevated in one of the four subjects (not evaluated in other workers). The exposed cohort had significantly more subjects with elevated serum thyrotropin ($p=0.006$), but free thyroxine index ($p=0.06$), serum T_4 level ($p=0.11$) and antimicrosomal antibody titer ($p=0.06$) did not differ significantly from the controls. Questioning

about exposure to 74 occupational toxicants showed that three of the four hypothyroid subjects had only three common chemical exposures (PBBs, decaBDE, and bromine); the fourth worker was hired after PBB production ceased and was exposed only to decaBDE and bromine, but it is not clear if PBBs were still present in the work environment. Except for one control subject who had an enlarged thyroid, none of the exposed or control subjects had signs of thyroid enlargement, thyroid nodularity or hypothyroidism on physical examination, or had reported taking thyroid medication or having thyroid problems within the previous 5 years. Reevaluation of three of the four subjects 1 year later (none had been treated with thyroid hormone) showed that two still had low free-thyroxine indices and high serum thyrotropin, one had a normal free-thyroxine index and a high-normal serum thyrotropin, and all three still had markedly elevated thyroid antimicrosomal antibody titers. The findings of this study suggest that occupational exposure to PBBs, decaBDE, and/or bromine affected the thyroid, but the mixed chemical exposure and a lack of data on serum or tissue levels of the chemicals preclude attributing effects solely to any particular congener or mixture of congeners.

Polybrominated Diphenyl Ethers. There is suggestive evidence of hypothyroidism in a small group of workers who were occupationally exposed to decaBDE as well as PBBs (Bahn et al. 1980), as summarized in the preceding subsection on endocrine effects of PBBs. 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 congeners.

Inhalation studies 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), or in the adrenals (only endocrine tissue examined) following nose-only exposure to ≤250 mg/m^3 as dust aerosol for 6 hours/day, 5 days/week for 14 days (Great Lakes Chemical Corporation 2000). Rats that were nose-only exposed to commercial octaBDE at levels 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 2001a, 2001b). Measurements of serum levels of thyroid hormones in the 13-week rat study, however, showed exposure-related decreases in mean thyroxine (total T_4) at ≥16 mg/m^3 in both sexes, and increases in thyroid stimulating hormone (TSH) at ≥16 mg/m^3 in males and 202 mg/m^3 in females. 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 5-1 and discussed in Chapter 4 and Appendix A.

Dermal Effects.

Polybrominated Biphenyls. In a medical history survey study, 7 of 10 (70%) workers in the production department of a PBB manufacturing plant reported that they experienced symptoms of skin disorders, compared with 31% of 45 workers in other departments in the same plant and 18% in a control group of 153 Wisconsin farm residents (Chanda et al. 1982). The survey covered a period of 3 years of potential exposure, but exposure levels were not reported. The dermatological symptoms were described as "almost uniformly" halogen acne (bromacne). Mean serum PBB levels for the respective PBB groups (with ranges listed in parentheses) were 603.9 ppb (11.4–1,729 ppb) and 16.5 ppb (4–234 ppb); PBBs were not detected in serum of the control subjects (Chanda et al. 1982). Physical examination confirmed the occurrence of bromacne in 13% of PBB workers compared with no acne in the control group. No other studies were located regarding dermal effects in humans after occupational exposure to PBBs.

No studies were located regarding dermal effects in animals after inhalation exposure to PBBs.

Polybrominated Diphenyl Ethers. 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 levels of ≤202 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks (Great Lakes Chemical Corporation 2001a, 2001b).

Ocular Effects.

Polybrominated Biphenyls. No studies were located regarding ocular effects in humans after inhalation exposure to PBBs.

Signs of ocular irritation (no further description) were observed in five male and five female rats that were exposed to a decabromobiphenyl dust mixture at 5,000 mg/m^3 concurrently with starch dust (6 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks (Millischer et al. 1980). The seriousness of this

effect is unclear as severity was not reported and recovery was not assessed. Ocular irritation was not observed at 500 mg/m^3 and lower concentrations.

Polybrominated Diphenyl Ethers. 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 2001a, 2001b).

5.2.1.3 Immunological and Lymphoreticular Effects

Polybrominated Biphenyls. Several immunological parameters were evaluated in a group of 28 workers from the Michigan Chemical Company who were involved in manufacturing and distributing PBBs (Stross et al. 1981). This company manufactured the FireMaster FF-1 that was involved in the agricultural contamination episode in Michigan in 1973–1974. The subjects had worked directly with PBBs during the previous 5 years, but exposure levels were not reported. Immunological analyses included determination of immunoglobulin levels, skin testing, and lymphocyte transformation studies. No abnormalities in lymphocyte number or function could be determined when compared to an unexposed group. One of three blastogenic responses (pokeweed mitogen [PWM]) was significantly reduced ($p<0.01$) relative to concurrent controls, but was within the normal control range for the laboratory. PWM is a mitogenic lectin that stimulates both human T and B cells. No specific information was provided regarding the skin testing and immunoglobulin levels.

No studies were located regarding immunological or lymphoreticular effects in animals after inhalation exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding immunological or lymphoreticular effects in humans after inhalation exposure to PBDEs.

No histopathological changes were observed in 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).

Inhalation studies of commercial octaBDE dusts in rats showed no histopathological changes in the spleen, mesenteric or mediastinal lymph nodes, bone marrow, or spleen following chamber exposure to 174 mg/m^3 as powdered dust for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978), or in the spleens (only lymphoreticular tissue reported) following nose-only exposure to ≤250 mg/m^3 as dust aerosol for 6 hours/day, 5 days/week for 14 days (Great Lakes Chemical Corporation 2000). Rats that were nose-only exposed to commercial octaBDE at levels 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. The effects included discolored and/or enlarged bronchial and mediastinal lymph nodes, and appeared to be associated with concurrent granulomatous inflammation of the lungs.

5.2.1.4 Neurological Effects

Polybrominated Biphenyls. Twenty-five workers at a PBB-manufacturing plant (exposure duration and levels not reported) displayed mean scores on tests of memory and learning that were typical for people of their age, and educational, occupational, and cultural backgrounds, even though they had an elevated mean PBB concentration in adipose tissue (9.33 ppm) (Brown et al. 1981). Workers with the highest concentrations of PBBs in adipose tissue showed no evidence of memory dysfunction in these tests.

No studies were located regarding neurological effects in animals after inhalation exposure to PBBs.

Polybrominated Diphenyl Ethers. 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 levels 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 2001a, 2001b). 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).

5.2.1.5 Reproductive Effects

Polybrominated Biphenyls. Eleven workers in a PBB manufacturing company (exposure duration and levels not reported) displayed no differences in the distribution of sperm counts, motility, or sperm morphology compared with a control group of 52 nonexposed men (Rosenman et al. 1979). PBBs were detected in the serum of all exposed subjects and in only one nonexposed subject, but no mean or individual serum PBB values were reported.

No studies were located regarding reproductive effects in animals after inhalation exposure to PBBs.

Polybrominated Diphenyl Ethers. 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 of ≤174 mg/m^3 as powdered dust for 8 hours/day for 14 consecutive days (Great Lakes Chemical Corporation 1978), or ≤250 mg/m^3 as dust aerosol for 6 hours/day, 5 days/week for 2 weeks (Great Lakes Chemical Corporation 2001a, 2001b). 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 2001a, 2001b). 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).

5.2.1.6 Developmental Effects

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

5.2.1.7 Cancer

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

5.2.2 Oral Exposure

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 5-2 (PBBs), 5-3 (lower PDBEs), or 5-4 (decaBDE) and plotted in Figures 5-2 (PBBs), 5-3 (lower PDBEs), or 5-4 (decaBDE).

5.2.2.1 Death

Polybrominated Biphenyls. No studies were located regarding death in humans after oral exposure to PBBs.

Limited information is available on lethal amounts of PBBs in animals. In general, dosing regimen and magnitude affect response. The lack of decreased survival in some studies does not necessarily indicate low toxicity because observation periods may not be sufficient to observe effects that develop slowly.

Except as noted below, acute-duration studies administered PBBs by gavage in oil vehicle. A single 1,000 mg/kg dose of FireMaster FF-1 did not significantly increase mortality in rats observed for ≤2 years posttreatment (Kimbrough et al. 1978b, 1981). Exposing pregnant rats to ≤800 mg/kg FireMaster BP-6 on one of gestation days 6–14 did not significantly increase mortality, but the animals were not observed beyond pregnancy (Beaudoin 1977). Administration of 1,000 mg/kg/day FireMaster FF-1 for 6–10 doses (5 days/week), however, caused 100% mortality in rats; the mean time to death was 12.3 days in females and 11.0 days in males (Gupta and Moore 1979). The cause of death was not specifically reported, but a

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE						
	Death						
1	Rat (Fischer 344/N)	2 wk 5d/wk 1x/d (GO)				1000 (18/18 died)	Gupta and Moore 1979 (FF-1)
2	Mouse (Balb/c)	14 d ad lib (F)				130 F (63% lethality)	Fraker 1980;Fraker and Aust 1978 (BP-6)
	Systemic						
3	Rat (Sprague-Dawley)	once (GO)	Endocr	286 M			Allen-Rowlands et al. 1981 (NS)
4	Rat (Sprague-Dawley)	10 d 1x/d (GO)	Endocr	1[b]	3 M (decreased thyroid plasma T4 hormone)		Allen-Rowlands et al. 1981 (NS)
5	Rat (Wistar)	once Gd 6-14 (GO)	Bd Wt	400 F	800 F (unknown percent maternal weight loss)		Beaudoin 1977 (BP-6)
6	Rat (Sherman)	once 18 mo observ (GO)	Hepatic		500 M (increased hepatic phospholipids and serum cholesterol)		Bernert et al. 1983 (FF-1)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
7	Rat (Fischer 344/N)	2 wk 5 d/wk 1x/d (GO)	Hepatic		1000	(hepatocytic swelling, fatty infiltration, multinucleation, necrosis, and cytolysis)			Gupta and Moore 1979 (FF-1)
			Renal		1000	(darkened kidneys)			
			Endocr		1000	(darkened adrenal glands)			
			Bd Wt				1000	(unknown percent weight loss, emaciation)	

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
8	Rat (Fischer 344/N)	2 wk 5 d/wk 1x/d (GO)	Resp	30			Gupta et al. 1981 (FF-1)
			Cardio	30			
			Gastro	30			
			Hemato	30			
			Musc/skel	30			
			Hepatic	0.3	3 (dose-related hepatocyte enlargement and single-cell necrosis)		
			Renal	30			
			Endocr	30			
			Dermal	30			
			Ocular	30			
			Bd Wt	30			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
9	Rat (Sherman)	once 2-14 mo observ (GO)	Resp	1000			Kimbrough et al. 1978 (FF-1)
			Cardio	1000			
			Gastro	1000			
			Hepatic			1000 (vacuolation, necrosis, and fibrosis, porphyria, multinucleation)	
			Renal	1000			
			Endocr	1000			
			Bd Wt	1000			
10	Rat (Sherman)	once 15wk observ (GO)	Hepatic		500 M (vacuolation of hepatocytes)		Kimbrough et al. 1980 (FF-1)
			Bd Wt	500 M			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
11	Rat (Sherman)	once 18-22mo observ (GO)	Resp	200 F			Kimbrough et al. 1981 (FF-1)
			Cardio	200 F			
			Hepatic		200 F (porphyrin accumulation)		
			Renal	200 F			
			Endocr	200 F			
			Dermal	200 F			
			Bd Wt	200 F			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
12	Rat (Sherman)	once 23mo observ (GO)	Resp	1000 F			Kimbrough et al. 1981 (FF-1)
			Cardio	1000 F			
			Gastro	1000 F			
			Musc/skel	1000 F			
			Hepatic		1000 F (hepatomegaly, hepatocyte enlargement and vacuolation, porphyrin accumulation)		
			Renal	1000 F			
			Endocr	1000 F			
			Ocular	1000 F			
			Bd Wt		1000 F (12% decreased body weight gain)		

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
13	Rat (Sprague-Dawley)	once 28d observ (GO)	Hepatic		1000 M (fatty changes in centrilobular hepatocytes)		Lee et al. 1975a (OBB)
			Bd Wt	1000 M			
14	Rat (Sprague-Dawley)	2 d 1x/d (GO)	Hepatic		3000 M (fatty changes in centrilobular hepatocytes)		Lee et al. 1975a (OBB)
			Bd Wt	1000 M			
15	Rat (Sprague-Dawley, Spartan)	once (GO)	Bd Wt	2000 F			Norris et al. 1975b (OBB)
16	Rat (Fischer 344)	10 d ad lib (F)	Hepatic		5 M (hepatomegaly and fatty changes in weanlings)		Raber and Carter 1986 (BP-6)
			Bd Wt	5 M			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
17	Rat (Sprague-Dawley)	2 wk ad lib (F)	Hepatic	0.66 M	6.53 M (hyperplasia and fatty changes)		Waritz et al. 1977; Lee et al. 1975b (OBB)
			Renal	71 M			
			Endocr	71 M			
			Bd Wt	71 M			
18	Mouse (Swiss-Webster)	2 wk ad lib (F)	Hepatic	36 F			Cagen et al. 1977 (BP-6)
19	Mouse (Swiss/ IRC)	11 d ad lib (F)	Hepatic		130 F (focal areas of coagulative necrosis)		Corbett et al. 1975 (BP-6)
20	Mouse (Swiss/ IRC)	4-14 d ad lib (F)	Bd Wt			130 M (30% decreased body weight)	Corbett et al. 1978 (BP-6)
21	Mouse (Balb/c)	14 d ad lib (F)	Bd Wt			130 F (23% weight loss)	Fraker 1980;Fraker and Aust 1978 (BP-6)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
22	Mouse (B6C3F1)	2 wk 5 d/wk 1x/d (GO)	Resp	30			Gupta et al. 1981 (FF-1)
			Cardio	30			
			Gastro	30			
			Hemato	30			
			Musc/skel	30			
			Hepatic	0.3	3 (dose-related increase in incidence of hepatocyte enlargement and single-cell necrosis)		
			Renal	30			
			Endocr	30			
			Ocular	30			
			Bd Wt	30			
			Other	30			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Immuno/ Lymphoret						
23	Rat (Fisher 344/N)	10 d 5 d/wk 1x/d (GO)				1000 (atrophy of thymus; necrosis of splenic lymphoblasts)	Gupta and Moore 1979 (FF-1)
24	Mouse (Balb/c)	14 d ad lib (F)				130 F (suppressed antibody-mediated response to SRBC, thymic atrophy)	Fraker 1980;Fraker and Aust 1978 (BP-6)
	Reproductive						
25	Rat (Sherman)	once 23mo observ (GO)			1000 F (9% increased incidence of uterine polyps)		Kimbrough et al. 1981 (FF-1)
26	Mouse (C57BL)	9 d Gd 6-15 1x/d (F)		21 F		63 F (29% reduction in success of pregnancy)	Welsch and Morgan 1985 (HBB)
	Developmental						
27	Rat (Wistar)	once Gd 6-14 (GO)		40		200 (9.1-31.4% resorptions)	Beaudoin 1977 (BP-6)
28	Rat (Sprague-Dawley)	14 d Gd 7-20 (F)		5	50 (12% decrease in fetal body weight)		Corbett et al. 1975 (BP-6)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
29	Rat (Sherman)	Gd 7-14 1x/d (GO)					200	(increased mortality and liver neoplasms in offspring)	Groce and Kimbrough 1984 (FF-1)
30	Rat (NS)	9 d Gd 7-15 1x/d (GO)			42.9	(12-20% decreased mean body weight in treated pups at post-parturition day 60)			Harris et al. 1978 (BP-6)
31	Rat (Sprague-Dawley, Iffa credo)	10 d Gd 6-15 1x/d (GO)		1000					Millischer et al. 1980 (DBB)
32	Rat (ChR-CD)	10 d Gd 6-15 ad lib (F)		9.1	86	(increased incidence of extra ribs)			Waritz et al. 1977 (OBB)
33	Mouse (Swiss/ IRC)	12 d Gd 7-18 (F)		5			50	(cleft palate)	Corbett et al. 1975 (BP-6)
	Cancer								
34	Rat (Sherman)	Gd 7-14 1x/d (GO)					200	(CEL: hepatocellular carcinoma in offspring)	Groce and Kimbrough 1984 (FF-1)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
35	Rat (Sherman)	once (GO)				1000 F (CEL: hepatocellular carcinoma)	Kimbrough et al. 1981 (FF-1)
	INTERMEDIATE EXPOSURE						
	Death						
36	Rat (Fischer 344/N)	4.5 wk 5 d/wk 22 d (GO)				149 M (90-day LD50) 65[c] F (90-day LD50)	Gupta and Moore 1979 (FF-1)
37	Rat (Fischer 344/N)	25 wk 5 d/wk 1x/d (GO)				0.3 M (decreased mean survival time)	NTP 1983 (FF-1)
38	Mouse (B6C3F1)	25 wk 5 d/wk 1x/d (GO)				10 F (decreased mean survival time)	NTP 1983 (FF-1)
39	Gn Pig (NS)	30 d ad lib (F)				4 M (4/6 died)	Sleight and Sanger 1976 (NS)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
40	Gn Pig (NS)	45 d ad lib (F)				2 F (7/8 died)	Vos and van Genderen 1973 (BP-6)
41	Mink (NS)	313 d ad lib (F)				0.47[c] M (LD50) 0.61 F (LD50)	Aulerich and Ringer 1979 (FF-1)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Systemic						
42	Monkey (Rhesus)	137 d ad lib (F)	Gastro		18 F (hyperplastic gastroenteritis)		Allen et al. 1978 (FF-1)
			Hemato		18 F (decreased RBC, PCV, and WBC)		
			Hepatic		18 F (enlarged hepatocytes, hyperplasia of bile duct epithelium, increased SGPT, decreased serum cholesterol)		
			Renal		18 F (hyperplasia of bladder epithelium)		
			Endocr			18 F (adrenal hemorrhage)	
			Dermal		18 F (edema, atrophy, and squamous metaplasia of sebaceous glands)		
			Bd Wt			18 F (27% body weight loss)	

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
43	Monkey (Rhesus)	25-50 wk ad lib (F)	Cardio		0.73 M (enlarged heart at necropsy)		Allen et al. 1978; Lambrecht et al. 1978 (FF-1)
			Gastro			0.73 M (proliferation of mucosal cells, chronic inflammatory cells, severe ulcerative colitis)	
			Hemato		0.73 M (decreased PCV and total serum protein)		
			Hepatic		0.73 (enlarged hepatocytes with increased lipid droplets, increased SGPT, decreased serum cholesterol, hyperplasia of bile duct epithelium)		
			Dermal			0.73 (edema and alopecia, keratinization of hair follicles and sebaceous glands)	
			Bd Wt			0.73 (34% weight loss in adult male, 0% weight gain in juvenile)	

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
44	Rat (Sprague-Dawley)	30 d ad lib (F)	Resp	10 M			Akoso et al. 1982a (BP-6)
			Cardio	10 M			
			Gastro	10 M			
			Hemato	10 M			
			Musc/skel	10 M			
			Hepatic		0.1 M (hepatocyte swelling, vacuolation)		
			Renal	10 M			
			Endocr	10 M			
			Dermal	10 M			
			Ocular	10 M			
			Bd Wt	10 M			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
45	Rat (Sprague-Dawley)	30 d ad lib (F)	Endocr		0.05 M (altered thyroid follicular ultrastructure)		Akoso et al. 1982b (BP-6)
46	Rat (Sprague-Dawley)	20 d 1x/d (GO)	Endocr		1 M (decreased serum thyroid hormone T4)		Allen-Rowlands et al. 1981 (NS)
47	Rat (Sprague-Dawley)	7 mo ad lib (F)	Endocr		0.45 F (decreased thyroid serum T3 and T4 hormones)		Byrne et al. 1987 (BP-6)
48	Rat (Sprague-Dawley)	5-7 mo ad lib (F)	Endocr	0.05 F	0.25 F (decreased adrenal serum corticosterone B, DHEA and DHS hormones)		Byrne et al. 1988 (BP-6)
49	Rat (Sprague-Dawley)	20 d 1x/d (GO)	Endocr	6 M			Castracane et al. 1982 (NS)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
50	Rat (Sprague-Dawley)	28 d ad lib (F)	Resp	2 M			Chu et al. 1980 (BP-6)
			Cardio	2 M			
			Gastro	2 M			
			Hemato	2 M			
			Hepatic		2 M (increased liver weight, increased liver microsomal enzymes, fatty degeneration of liver)		
			Renal	2 M			
			Endocr		2 M (reduction of follicular size and colloid density and exfoliation of epithelium in thyroid)		
			Dermal	2 M			
			Bd Wt	2 M			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
51	Rat (Sprague-Dawley)	82 d ad lib (F)	Hepatic		0.5 M (bile duct hyperplasia)		Darjono et al. 1983 (BP-6)
			Ocular		5 M (xerophthalmia)		
			Bd Wt	5 M			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
52	Rat (Fischer 344/N)	4.5 wk 5 d/wk 1x/d (GO)	Cardio	1000			Gupta and Moore 1979 (FF-1)
			Gastro	1000			
			Hemato		30 (decreased hemoglobin, PCV, and platelet count)		
			Hepatic		30 (hepatocyte enlargement, fatty infiltration and multinucleation, porphyrin accumulation)		
			Renal		30 (dilation of Bowman's capsule with serous fluid)		
			Endocr		30 (unspecified altered thyroid histology)		
			Bd Wt		30 (19% decreased body weight gain)	100 (emaciation)	

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
53	Rat (Fischer 344/N)	30 d 5 d/wk 1x/d (GO)	Resp	30			Gupta et al. 1981 (FF-1)
			Cardio	30			
			Gastro	30			
			Hemato	30			
			Musc/skel	30			
			Hepatic	0.3	3 (increased liver weight, hepatocyte swelling, and necrosis)		
			Renal	30			
			Bd Wt	3	30 (significant decrease in body weight)		
54	Rat (Holtz- man)	5 wk ad lib (F)	Endocr		0.25 M (colloid droplets, abnormal microvilli and other changes in thyroid follicle ultrastructure)		Kasza et al. 1978a (BP-6)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
55	Rat (Holtz- man)	5 wk ad lib (F)	Hepatic	0.25 M	2.5 M (hepatocyte hypertrophy and degeneration)		Kasza et al. 1978a (BP-6)
56	Rat (Fischer 344)	6 mo 5 d/wk (GO)	Hemato		10 F (increased white blood cell count)		Luster et al. 1980 (FF-1)
			Bd Wt	1 F	3 F (15% decreased weight gain)		
57	Rat (Sprague-Dawley)	3 mo ad lib (F)	Hepatic		5 F (enlarged and vacuolated hepatocytes, focal necrosis)		McCormack et al. 1978 (BP-6)
			Renal		5 F (degenerative changes in glomeruli)		
			Bd Wt		5 F (10% decreased body weight gain)		

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)	Reference Chemical Form
58	Rat (Sprague-Dawley CFY)	13 wk (F)	Gastro	100				Millischer et al. 1980 (DBB)
			Hemato	100				
			Hepatic	25	100	(11% increased liver weight, hepatocyte hypertrophy and vacuolization, slightly increased liver lipids)		
			Renal	100				
			Bd Wt	100				

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
59	Rat (Sprague-Dawley)	30 d ad lib (F)	Cardio	800 M			Norris et al. 1975b (OBB)
			Hemato	80 M	800 M (decreased PCV and RBC counts)		
			Hepatic		8 M (enlargement and vacuolation)		
			Renal		8 M (hyaline degenerative changes)		
			Endocr		8 M (thyroid hyperplasia)		
			Bd Wt	800 M			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
60	Rat (Sprague-Dawley)	8 mo ad lib (F)	Cardio	1			Norris et al. 1975b (OBB)
			Hemato	1			
			Hepatic	1			
			Renal	1			
			Endocr	1			
			Bd Wt	1			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
61	Rat (Fischer 344/N)	25 wk 5 d/wk 1x/d (GO)	Resp	10			NTP 1983 (FF-1)
			Cardio	10			
			Gastro	0.3	1 (gastric ulcers)		
			Hemato	0.1	0.3 (decreased hemoglobin, MCH, PCV, and MCV)		
			Musc/skel	10			
			Hepatic	0.1	0.3 (lipid accumulation; increased atypical foci; porphyrin accumulation)		
			Renal	0.3		1 (chronic progressive nephropathy)	
			Endocr	0.1	0.3 (decreased serum thyroid T4 hormone)		
			Ocular	10			
			Bd Wt	10			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
62	Rat (Sprague-Dawley)	7 mo ad lib (F)	Hepatic	2.5 F			Sepkovic and Byrne 1984 (OBB)
			Endocr	2.5 F			
63	Rat (Sprague-Dawley)	7 mo ad lib (F)	Endocr	2.5 F			Sepkovic and Byrne 1984 (HBB)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
64	Rat (Sprague-Dawley)	30 d ad lib (F)	Resp	50 M			Sleight and Sanger 1976 (NS)
			Cardio	50 M			
			Gastro	50 M			
			Hemato	50 M			
			Musc/skel	50 M			
			Hepatic		1 M (hepatocyte vacuolation)		
			Renal	10 M	50 M (unquantified, but significantly increased BUN)		
			Endocr	50 M			
			Bd Wt	10 M	50 M (16% decreased weight gain)		

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
65	Rat (Sprague-Dawley)	30 d ad lib (F)	Resp	5 M			Sleight et al. 1978 (BP-6)
			Cardio	5 M			
			Gastro	5 M			
			Hemato	5 M			
			Hepatic	0.5 M	5 M (hepatocyte swelling and vacuolation)		
			Renal	5 M			
			Endocr	0.5 M	5 M (hyperplasia of thyroid follicular epithelium)		
			Bd Wt	0.5 M	5 M (27-36% reduced body weight gain)		

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
66	Rat (Sprague-Dawley)	4 wk ad lib (F)	Hepatic	0.66 M	6.53 M (hyperplasia and progressive lipid changes)		Waritz et al. 1977; Lee et al. 1975b (OBB)
			Renal	71 M			
			Endocr	71 M			
			Bd Wt	71 M			
67	Mouse (Balb/c)	30 d ad lib (F)	Bd Wt	13 F			Fraker 1980;Fraker and Aust 1978 (BP-6)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
68	Mouse (B6C3F1)	30 d 5 d/wk 1x/d (GO)	Resp	30			Gupta et al. 1981 (FF-1)
			Cardio	30			
			Gastro	30			
			Hemato		30 F (decreased PCV)		
			Musc/skel	30			
			Hepatic	0.3	3 (hepatocyte enlargement and necrosis)		
			Renal	30			
			Ocular	30			
			Bd Wt	3	30 M (significant decrease in weight gain)		
			Other	30			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
69	Mouse (Balb/ cBYJ)	6 wk ad lib (F)	Resp	21.7 M			Loose et al. 1981 (FF-1)
			Cardio	21.7 M			
			Hepatic	0.65 M		21.7 M (hepatocellular necrosis and vacuolation)	
			Renal	21.7 M			
			Endocr	0.65 M			
			Bd Wt	0.65 M		21.7 M (33% reduction in body weight)	
70	Mouse (B6C3F1)	6 mo 5 d/wk (GO)	Hemato	10			Luster et al. 1980 (FF-1)
			Endocr		10 (increased adrenal weight gain)		
			Bd Wt	10			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
71	Mouse (B6C3F1)	25 wk 5 d/wk 1x/d (GO)	Resp	10			NTP 1983 (FF-1)
			Cardio	10			
			Gastro	10			
			Hemato	0.1	0.3 (decreased erythrocyte count and MCV)		
			Musc/skel	10			
			Hepatic	0.1	0.3 (increased liver weight, SGOT and porphyrin accumulation)		
			Renal	10			
			Endocr	10			
			Ocular	10			
			Bd Wt	3 M		10 M (25% decreased body weight gain)	

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
72	Gn Pig (NS)	30 d ad lib (F)	Resp	20 M			Sleight and Sanger 1976 (NS)
			Hepatic		0.04 M (vacuolation and fatty changes)		
			Bd Wt	0.4 M		4 M (severe weight loss prior to death)	

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
73	Pig (NS)	16 wk ad lib (F)	Cardio	8			Ku et al. 1978 (NS)
			Gastro	1	8 (gross hyperplasia glandular stomach)		
			Hemato	8			
			Hepatic		1 (LDH increased)		
			Renal	8			
			Endocr		8 (increased adrenal weight)		
			Dermal	1	8 (dermatosis)		
			Bd Wt		1 (12.9% reduced weight gain and food intake)		
74	Pig (NS)	12 wk Gwk 8-ppwk 4 ad lib (F)	Hepatic	0.125 F	1.25 F (fatty changes and necrosis)		Werner and Sleight 1981 (BP-6)
			Endocr	1.25 F	2.5 F (significant decrease in thyroid serum T3 and T4 hormones)		

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
75	Mink (NS)	313 d ad lib (F)	Cardio	2.4			Aulerich and Ringer 1979;Ringer and Aulerich 1981 (FF-1)
			Hepatic		0.24 F (48% increased relative liver weight, fatty infiltration)		
			Renal	2.4			
			Bd Wt		0.39 F (14% decreased prebreeding body weight gain)	1.86 F (up to 19% mean body weight loss prior to death)	
	Immuno/ Lymphoret						
76	Rat (Fischer 344)	5 wk 5 d/wk (GO)		0.03 M	3 M (decreased lymphocytic response to mitogen stimulation; decrease in absolute and relative thymus weight)		Luster et al. 1978 (FF-1)
77	Rat (Fischer 344)	6 mo 5 d/wk 1x/d (GO)		1 F	3 F (decreased lymphoproliferative responses and decreased delayed hypersensitivity responses)		Luster et al. 1980 (FF-1)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
78	Mouse (Balb/c)	30 d ad lib (F)		0.13 F	1.3 F (reduced antibody mediated response to SRBC and 21% reduction in thymus weight)		Fraker 1980;Fraker and Aust 1978 (BP-6)
79	Mouse (Balb/ cBYJ)	6 wk ad lib (F)		0.65 M		21.7 M (increased lethality due to endotoxin challenge)	Loose et al. 1981 (FF-1)
80	Mouse (B6C3F1)	6 mo 5 d/wk 1x/d (GO)		3		10 (increased lethality due to infection with L monocytogenes; decreased response to mitogen stimulation)	Luster et al. 1980 (FF-1)
81	Gn Pig (NS)	45 d ad lib (F)			0.4 F (reduced antitoxin titers following toxoid challenge)	4 F (thymic atrophy and follicular depletion in spleen)	Vos and van Genderen 1973 (BP-6)
82	Pig (NS)	12 wk Gwk 8- ppwk 4 ad lib (F)		1.25 F	2.5 F (reduced lymphocyte response to mitogen stimulation)		Howard et al. 1980 (BP-6)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Neurological						
83	Rat (NS)	6 mo 5 d/wk 1x/d (GO)			10 (decreased limb strength)		Cabe and Tilson 1978 (FF-1)
84	Rat (Sprague-Dawley Holtz- man)	4 wk 5 d/wk 1x/d (G)		3 M		6 M (decreased motor activity)	Geller et al. 1979 (FF-1)
85	Rat (Sprague-Dawley)	40d Gd 6-Ppd 24 (F)		0.2	2 (delayed acquisition of locomotion and reduced open field activity in offspring)		Henck et al. 1994 (BP-6)
86	Rat (Fischer 344/N)	6 mo 5 d/wk (GO)				3 (decreased motor activity, grip strength, and startle responsiveness)	Tilson and Cabe 1979 (FF-1)
87	Rat (Fischer 344/N)	4 wk 5 d/wk 1x/d (GO)				30 (decreased open field motor activity and grip strength)	Tilson and Cabe 1979 (FF-1)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Reproductive						
88	Monkey (Rhesus)	25-50 wk ad lib (F)			0.73 M (hypoactive seminiferous tubules)		Allen et al. 1978; Lambrecht et al. 1978 (FF-1)
89	Monkey (Rhesus)	6 mo ad lib (F)				0.012 F (increased menstrual cycle duration in 4/7; implantation bleeding in 2/7)	Lambrecht et al. 1978; Allen et al. 1978; 1979 (FF-1)
90	Rat (Wistar)	15 d Gd 0-14 8x (GO)		14.3 F		28.6 F (no implantations in 2/5 rats)	Beaudoin 1979 (BP-6)
91	Rat (Fischer 344/N)	4-5 wk 5 d/wk 22 d (GO)		30[c] M 1000 F	100 M (squamous metaplasia, hyperplasia, and necrosis in epithelium of ductus deferens)		Gupta and Moore 1979 (FF-1)
92	Rat (Sprague-Dawley)	42 d Gd 8-ppd 28 ad lib (F)		5 F			McCormack et al. 1981 (BP-6)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
93	Mouse (B6C3F1)	4-5 wk 5 d/wk 1x/d (GO)		30				Gupta et al. 1981 (FF-1)
94	Mink (NS)	313 d ad lib (F)		0.24	0.39 F (10% reduction in body weight)			Aulerich and Ringer 1979 (FF-1)
	Developmental							
95	Rat (Wistar)	15 d Gd 0-14 8x (GO)		2.9		14.3	(increased resorptions)	Beaudoin 1979 (BP-6)
96	Rat (Holtz- man Sprague-Dawley)	4 wk 5 d/wk (G)		5				Geller et al. 1985 (FF-1)
97	Rat (Sprague-Dawley)	40 d Gd 6- ppd24 ad lib (F)				0.2	(deficits in learning behavior in offspring, 6 months after prenatal and lactational exposure)	Henck and Rech 1986 (BP-6)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
98	Rat (Sprague-Dawley)	40d Gd 6-Ppd 24 (F)			0.2 M (reduced crown-rump length)		Henck et al. 1994 (BP-6)
99	Rat (Sprague-Dawley)	42 d Gd 8-ppd 28 ad lib (F)			0.5 (increased liver weight, hepatocyte vacuolation, decreased hepatic vitamin A content in F1 but not F2)	5 (decreased pup survival during lactation in F1)	McCormack et al. 1981 (BP-6)
100	Rat (Sprague-Dawley)	42-126 d Gd 8-ppd 28-112 ad lib (F)			5 (20% decrease in pup body weight gain, 50% decreased hepatic vitamin A, 256-285% decreased urinary uro- and coproporphyrins in pups)		McCormack et al. 1982a (BP-6)
101	Rat (Sprague-Dawley)	37 d Gd 0-ppd 15 ad lib (F)			2.5 (decreased body weight, increased relative liver weight, and decreased serum T4 in offspring)		Meserve et al. 1992 (BP-6)
102	Rat (Fischer 344/N)	77 d Gd 0-ppd 56 ad lib (F)			0.5 (hepatic vacuolization and altered foci in pups)		NTP 1992, Chhabra et al. 1993 (FF-1)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
103	Mouse (B6C3F1)	42 d Gd 0- weaning 1x/2d (GO)		2	3	(decreased hematocrit in offspring)	10	(early postnatal death; no details provided)	Luster et al. 1980 (FF-1)
104	Mouse (B6C3F1)	77 d Gd 0- ppd 56 ad lib (F)			1.5	(hepatic cytomegaly and altered foci in pups)			NTP 1992, Chhabra et al. 1993 (FF-1)
105	Mouse (C57B1/6)	Gd 0-ppd 21 1x/2d (GO)			3	(performance deficits in offspring in a learned task)			Tilson 1992 (FF-1)
106	Pig (NS)	12 wk Gwk 8- ppwk 4 ad lib (F)		0.125			1.25	(increased relative liver weight, decreased serum thyroid hormone levels, and slight thyroid hyperplasia in offspring)	Werner and Sleight 1981 (BP-6)
107	Mink (NS)	313 d (F)			0.155	(decreased birth and 4-week weights in kits)			Aulerich and Ringer 1979;Ringer and Aulerich 1981 (FF-1)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
	Cancer							
108	Rat (Sherman)	4 mo 12 x (GO)				100	(CEL: hepatocellular carcinoma)	Kimbrough et al. 1981 (FF-1)
109	Rat (Fischer 344/N)	25 wk 5 d/wk 1x/d (GO)				3	(CEL: hepatocellular carcinoma)	NTP 1983 (FF-1)
110	Mouse (B6C3F1)	25 wk 5 d/wk 1x/d (GO)				10	(CEL: hepatocellular carcinomas)	NTP 1983 (FF-1)
111	Mouse (B6C3F1)	77 d Gd 0-ppd 56 ad lib (F)				1.5	(CEL: hepatocullular adenoma and carcinoma in offspring)	NTP 1992, Chhabra et al. 1993 (FF-1)
	CHRONIC EXPOSURE							
	Death							
112	Rat (Fischer 344/N)	115 wks Gd 0-ppd 56 (weaning) 104 wks post-weaning ad lib (F)				0.5 M	(18% decreased survival)	NTP 1992, Chhabra et al. 1993 (FF-1)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
113	Mouse (B6C3F1)	116 wks Gd 0-ppd 56 (weaning) 105wks post-weaning ad lib (F)				1.3 F (44% decreased survival)	NTP 1992, Chhabra et al. 1993 (FF-1)
	Systemic						
114	Monkey (Rhesus)	66 wk ad lib (F)	Hemato	0.012			Lambrecht et al. 1978 (FF-1)
			Bd Wt		0.012 (7.4% weight loss)		

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
115	Rat (Fischer 344/N)	115 wks Gd 0-ppd 56; 104 wks post-weaning (F)	Resp	1.5			NTP 1992, Chhabra et al. 1993 (FF-1)
			Cardio	1.5			
			Gastro	0.5	1.5 M (forestomach hyperplasia, inflammation, ulceration)		
			Hemato	0.5	1.5 F (mild anemia)		
			Musc/skel	1.5			
			Hepatic		0.5 (hypertrophy, vacuolation, altered foci, increased serum cholesterol, decreased serum triglycerides)		
			Renal	1.5			
			Endocr	1.5			
			Dermal	1.5			
			Bd Wt		0.5 (11-18% decreased final body weight)		

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
116	Mouse (B6C3F1)	116 wks Gd 0-ppd 56 (weaning) 105 wks post-weaning ad lib (F)	Resp	3.9			NTP 1992, Chhabra et al. 1993 (FF-1)
			Cardio	3.9			
			Gastro	3.9			
			Musc/skel	3.9			
			Hepatic		1.3 (hypertrophy, vacuolization, single-cell necrosis, altered foci, bile duct hyperplasia)		
			Renal	1.3		3.9 (increased chronic nephropathy)	
			Endocr		1.3 (thyroid follicular cell hyperplasia)		
			Dermal	3.9			
			Bd Wt	3.9			

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Immuno/ Lymphoret						
117	Rat (Fischer 344/N)	115 wks Gd 0-ppd 56 (weaning) 104 wks post-weaning ad lib (F)		0.5 M	1.5 M (splenic fibrosis)		NTP 1992, Chhabra et al. 1993 (FF-1)
118	Mouse (B6C3F1)	116 wks Gd 0-ppd 56 (weaning) 105 wks post-weaning ad lib (F)		1.3	3.9 (increased splenic hematopoiesis)		NTP 1992, Chhabra et al. 1993 (FF-1)
	Reproductive						
119	Rat (Fischer 344/N)	115 wks Gd 0-ppd-56 (weaning) 104 wks post-weaning ad lib (F)		1.5 M 0.5[c] F	1.5 F (cystic endometrial hyperplasia)		NTP 1992, Chhabra et al. 1993 (FF-1)
120	Mouse (B6C3F1)	116 wks Gd 0-ppd 56 (weaning) 105 wks post-weaning ad lib (F)		3.9			NTP 1992, Chhabra et al. 1993 (FF-1)

Table 5-2 Levels of Significant Exposure to Polybrominated Biphenyls - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
	Developmental							
121	Monkey (Rhesus)	359-469 d ad lib (F)				0.012	(1/7 fetuses were aborted,1/7 fetuses stillborn, 12% decreased birth weight and 22% decreased postnatal weight gain in 4/7 survivors)	Lambrecht et al. 1978; Allen et al. 1978; Allen et al. 1979 (FF-1)
	Cancer							
122	Rat (Fischer 344/N)	115 wks Gd 0- ppd 56 104 wks post-weaning (F)				1.5	(CEL: leukemia)	NTP 1992, Chhabra et al. 1993 (FF-1)
						0.5	(CEL: hepatocellular adenoma and carcinoma)	
123	Mouse (B6C3F1)	116 wks Gd 0- ppd 56 105wks post-weaning (F)				3.9	(thyroid follicular cell adenoma)	NTP 1992, Chhabra et al. 1993 (FF-1)
						1.3	(CEL: hepatocellular adenoma and carcinoma)	

a The number corresponds to entries in Figure 5-2.

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.01 mg/kg/day. The MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 for extrapolation from animals to humans, 10 for human variability.

c Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 5-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

Ad lib - ad libitum; Bd Wt = body weight; BP-6 = FireMaster BP-6; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); DBB = deca=brominated biphenyl; DW = drinking water; endocr = endocrine; (F)= feed; F = female; FF-1 = FireMaster FF-1; (G) = gavage; gastro = gastrointestinal; Gd = gestation day; Gn Pig - Guinea Pig; (GO) = gavage in oil; Gwk = gestation week; HBB = hexa-brominated biphenyl; hemato = hematological; hr = hour(s); LD50 = lethal dose; 50% kill; LOAEL = lowest observed adverse effect level; M = male; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no observed adverse effect level; NS = not specified; OBB octa-brominated biphenyl; observ = observation; ppd = post partum day; ppwk = post partum week; Resp = respiratory; T4 = thyroxine; wk = week(s); x = time(s)

Figure 5-2. Levels of Significant Exposure to Polybrominated Biphenyls- Oral

Acute (≤14 days)

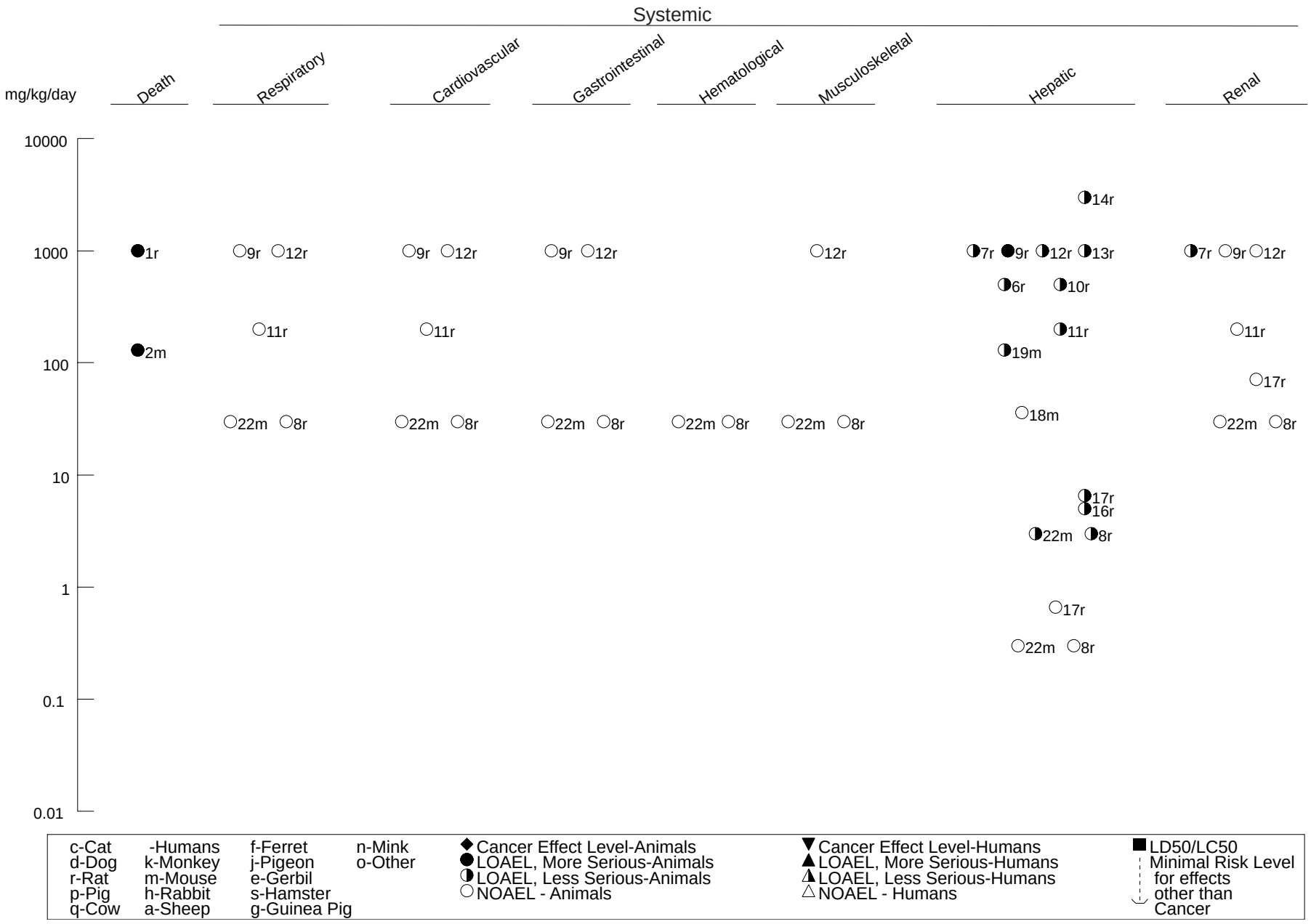


Figure 5-2. Levels of Significant Exposure to Polybrominated Biphenyls- Oral (*Continued*)

Acute (≤14 days)

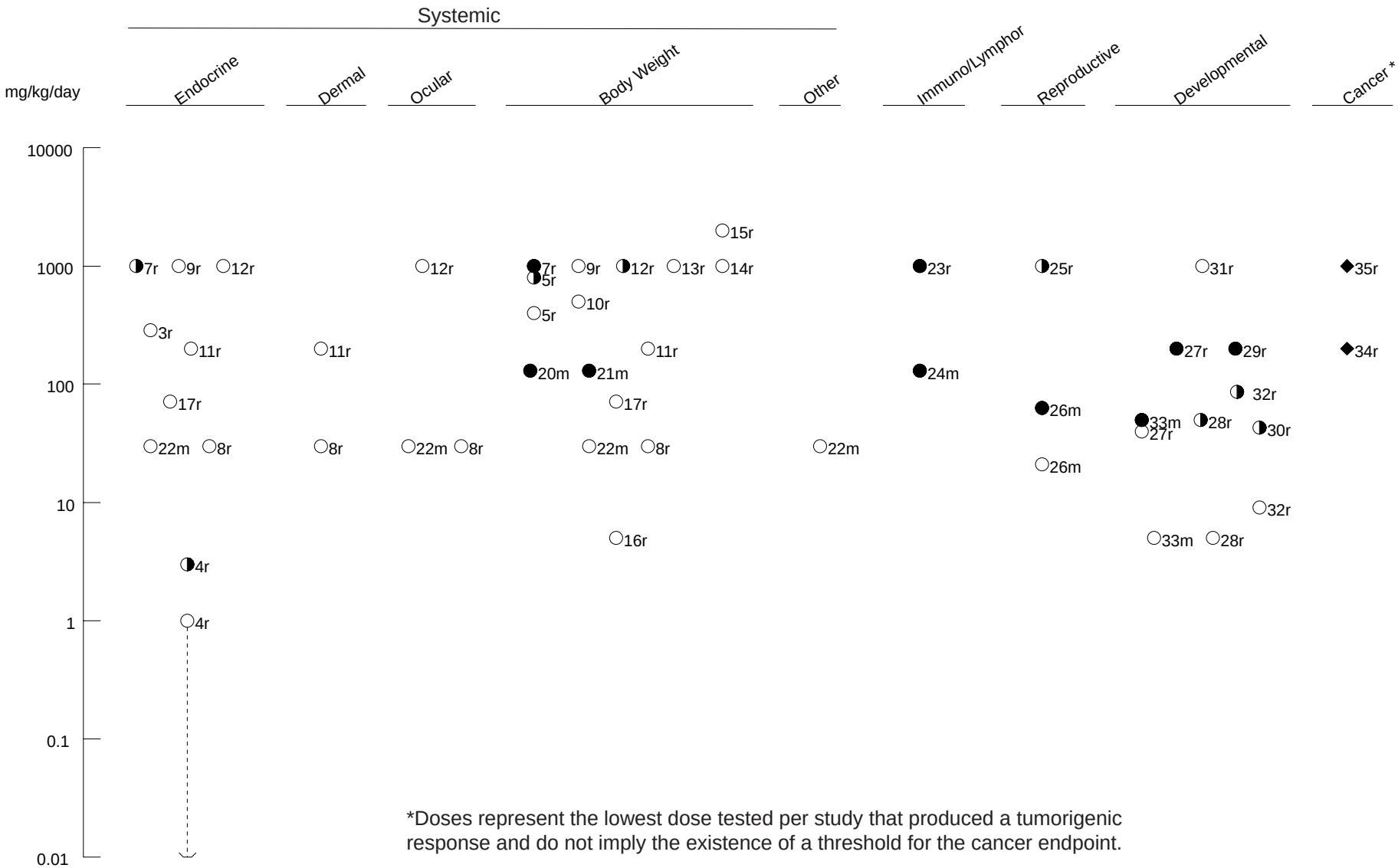


Figure 5-2. Levels of Significant Exposure to Polybrominated Biphenyls- Oral (*Continued*)

Intermediate (15-364 days)

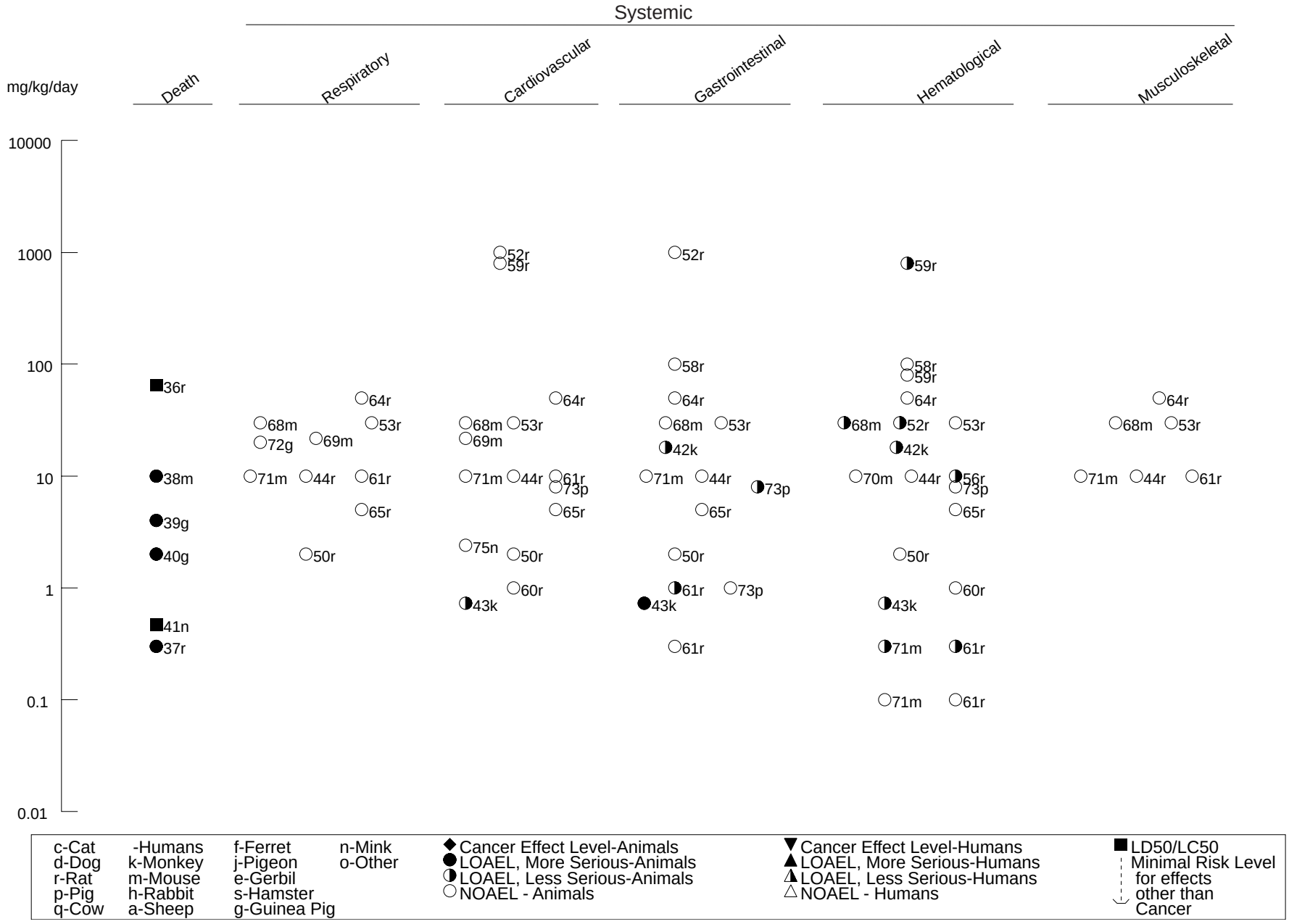


Figure 5-2. Levels of Significant Exposure to Polybrominated Biphenyls- Oral (*Continued*)

Intermediate (15-364 days)

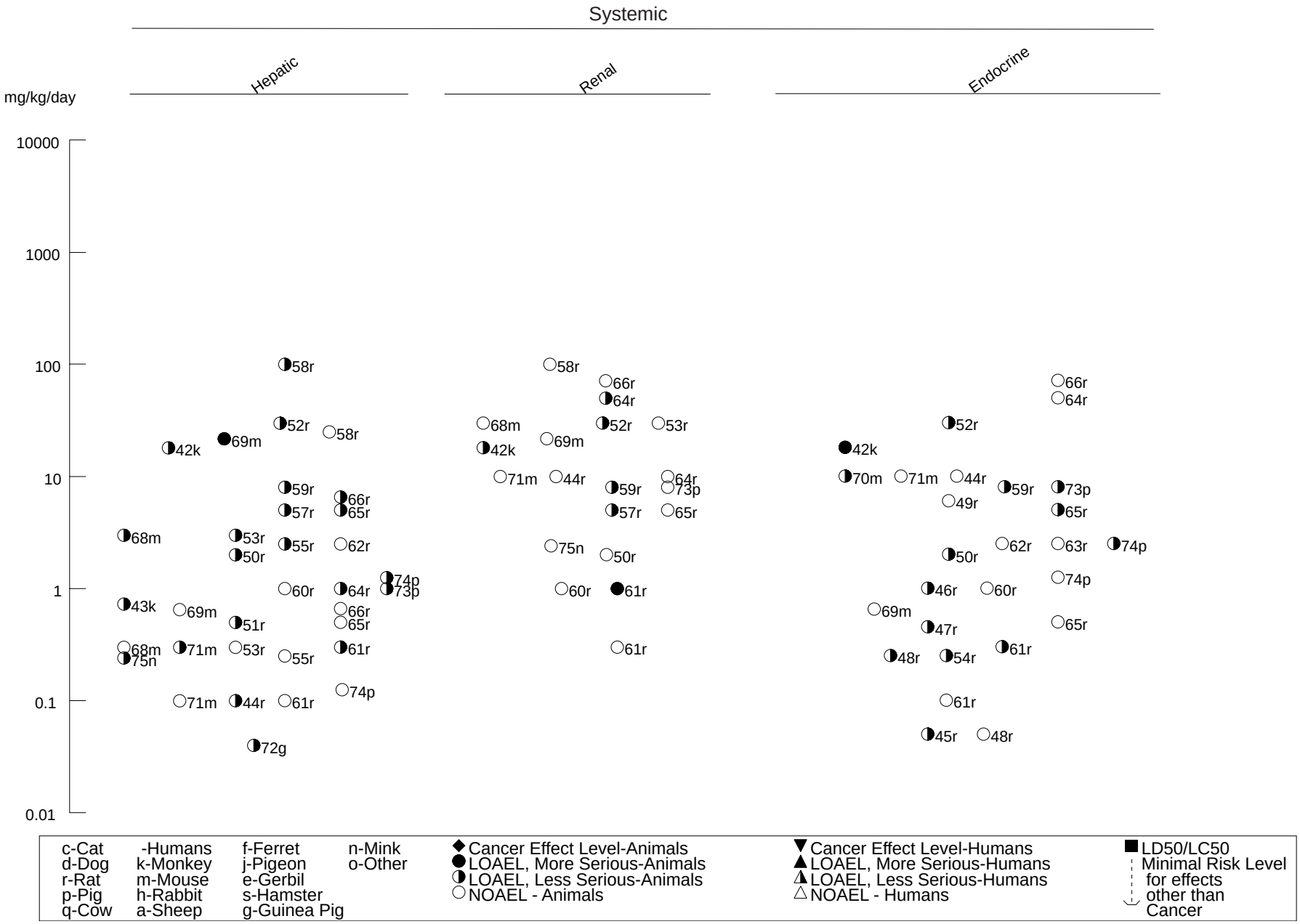


Figure 5-2. Levels of Significant Exposure to Polybrominated Biphenyls- Oral (*Continued*)

Intermediate (15-364 days)

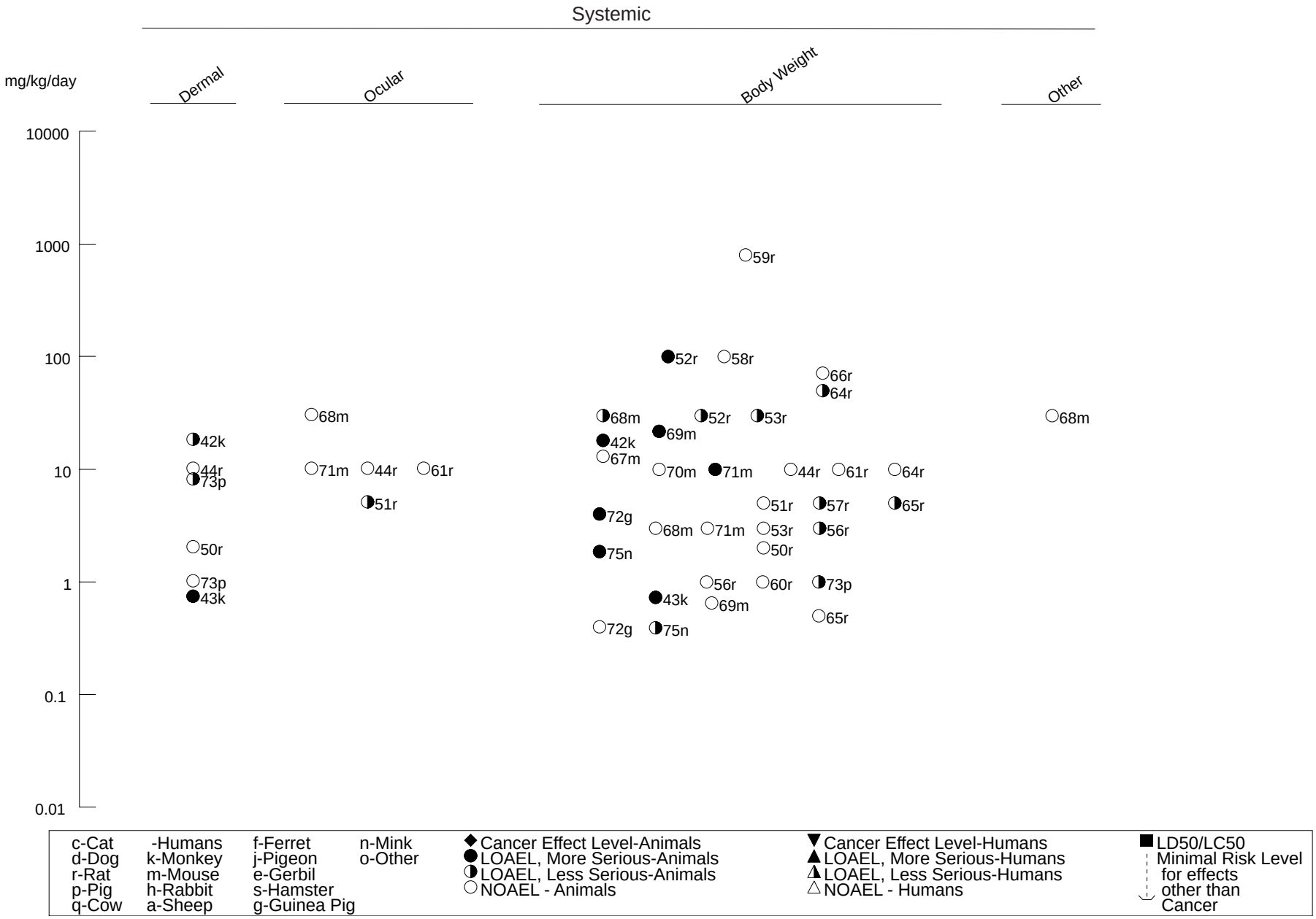


Figure 5-2. Levels of Significant Exposure to Polybrominated Biphenyls- Oral (*Continued*)

Intermediate (15-364 days)

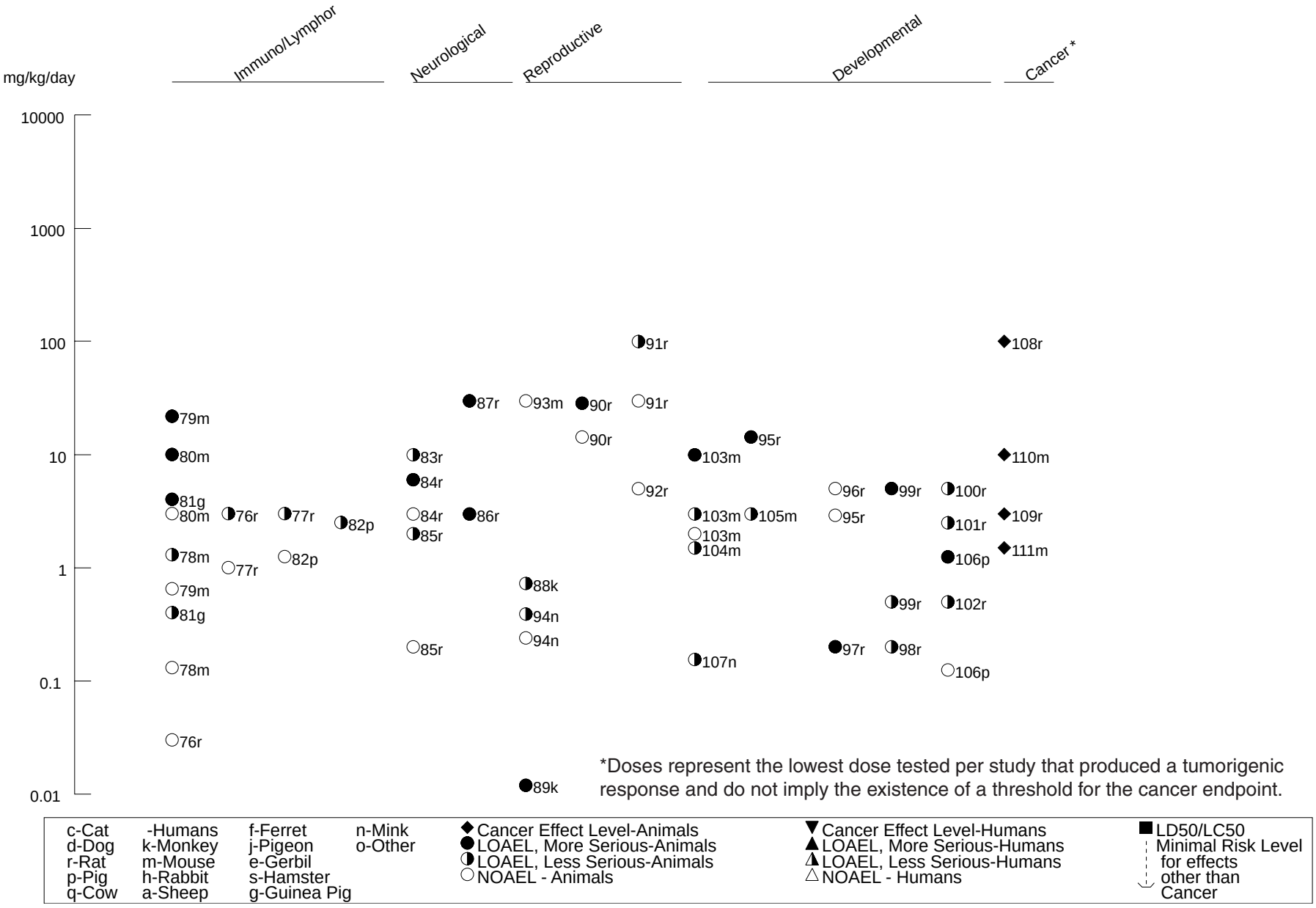


Figure 5-2. Levels of Significant Exposure to Polybrominated Biphenyls- Oral (*Continued*)

Chronic (≥365 days)

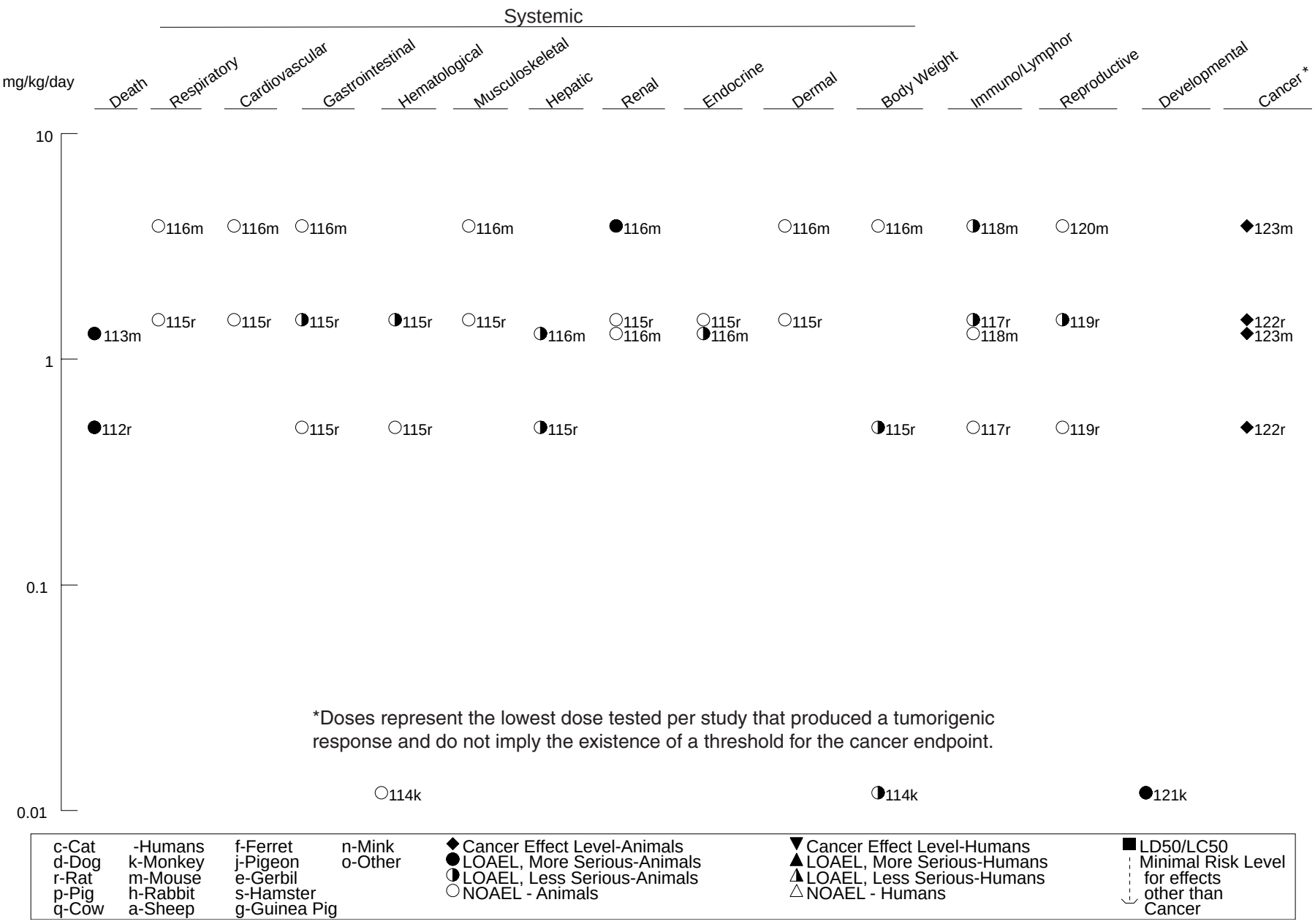


Table 5-3 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)		LOAEL Serious (mg/kg)		Reference Chemical Form
	ACUTE EXPOSURE								
	Death								
1	Rat (Wistar)	once (GO)					6200	(44-day LD50)	British Industrial Biological Research Association 1977 PentaBDE
2	Rat Spartan	once (GO)					5000	(4/5 died)	IRCD 1975b PentaBDE
3	Rat (Sprague-Dawley)	once (GO)					5000	(14-day LD50)	Pharmakon Research International Inc. 1984 PentaBDE
	Systemic								
4	Rat (CD)	10 d Gd 6-15 (GO)	Bd Wt	10	100	(30% reduced maternal body weight gain)			Argus Research Laboratories 1985a PentaBDE
5	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Hepatic	76.6 M					Carlson, 1980b OctaBDE
6	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Hepatic	56.4 M					Carlson, 1980b PentaBDE

Table 5-3 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
7	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Endocr		18 (reduced serum T4)		Darnerud and Sinjari 1996 PentaBDE
8	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Hepatic		18 (reduced liver vitamin A)		Hallgren et al. 2001 PentaBDE
			Endocr		18 (reduced serum T4)		
9	Rat Spartan	once (GO)	Bd Wt	5000			IRCD 1975a OctaBDE
10	Rat Spartan	once (GO)	Bd Wt	500			IRCD 1975b PentaBDE
11	Rat (CD)	10 d Gd 6-15 (GO)	Bd Wt	25			Life Science Research Israel Ltd. (1987) OctaBDE
12	Rat (CD)	10 d Gd 6-15 (GO)	Bd Wt	25	50 (40% reduced maternal body weight gain)		WIL Research Laboratories 1986 OctaBDE

Table 5-3 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)	Reference Chemical Form
13	Rat (Long-Evans)	4 d 1x/d (GO)	Endocr	10	30	(reduced serum T4)		Zhou et al. 2001 PentaBDE
14	Rat (Long-Evans)	4 d 1x/d (GO)	Endocr	3	10	(reduced serum T4)		Zhou et al. 2001 OctaBDE
15	Mouse (C57BL/6N)	14 d 1x/d (GO)	Endocr		18	(reduced serum T4)		Darnerud and Sinjari 1996 PentaBDE
16	Mouse C57BL/6J	once (GO)	Hepatic	500				Fowles et al. 1994 PentaBDE
			Endocr	100	500	(reduced serum T4)		
17	Mouse (C57BL/6N)	14d 1x/d (GO)	Hepatic	72				Fowles et al. 1994 PentaBDE
			Endocr		18	(reduced serum T4)		
			Bd Wt	72				

Table 5-3 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)	Reference Chemical Form
18	Mouse (C57BL/6N)	14 d 1x/d (GO)	Hepatic	18	36	(reduced liver vitamin A)		Hallgren et al. 2001 PentaBDE
			Endocr		18	(reduced serum T4)		
	Immuno/ Lymphoret							
19	Rat (Sprague-Dawley)	14 d 1x/d (GO)		36				Darnerud and Thuvander 1998 PentaBDE
20	Mouse (C57BL/6N)	14 d 1x/d (GO)		18	36	(reduced in vitro production of IgG in mitogen- stimulated splenocytes)		Darnerud and Thuvander 1998 PentaBDE
21	Mouse (C57BL/6N)	once (GO)		500				Fowles et al. 1994 PentaBDE
22	Mouse (C57BL/6N)	14d 1x/d (GO)		36	72	(reduced antibody response to sheep red blood cells, decreased thymus weight)		Fowles et al. 1994 PentaBDE

Table 5-3 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Developmental						
23	Rat (CD)	10 d Gd 6-15 (GO)		200			Argus Research Laboratories 1985a PentaBDE
24	Rat (CD)	10 d Gd 6-15 (GO)		10	25 (increased resorptions and reduced fetal body weight)		Argus Research Laboratories 1985b OctaBDE
25	Rat (CD)	10 d Gd 6-15 (GO)		2.5	10 (minimal increased post-implantation loss)		Life Science Research Israel Ltd. (1987) OctaBDE
26	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
27	Rat (Long-Evans)	14 d Gd 6-Gd 20 (GO)		1[b]	10 (reduced serum T4 in fetuses)		Zhou et al. 2002 PentaBDE
28	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

Table 5-3 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	INTERMEDIATE EXPOSURE						
	Systemic						
29	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	1.77 M			Carlson 1980a PentaBDE
30	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	14.1 M			Carlson 1980a PentaBDE
31	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	2.4 M			Carlson, 1980a OctaBDE
32	Rat (Sprague-Dawley)	90 d (GO)	Hepatic	19.2 M			Carlson, 1980a OctaBDE
33	Rat (CD)	28 d (F)	Hepatic		9 (increased liver weight and enlarged parenchymal cells)		IRDC 1976 PentaBDE
			Renal	90			
			Endocr	90			
			Bd Wt	90			

Table 5-3 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
34	Rat (CD)	28 d (F)	Hepatic		9 (increased liver weight and enlarged parenchymal cells)		IRDC 1976 OctaBDE
			Renal	90			
			Endocr	90			
			Bd Wt	90			

Table 5-3 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
35	Rat (CD)	13 wk (F)	Resp	750 F			IRDC 1977 OctaBDE
			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)		

Table 5-3 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
36	Rat (Sprague-Dawley)	90d (F)	Resp	100			WIL Research Laboratories 1984 PentaBDE
			Cardio	100			
			Gastro	100			
			Hemato	100			
			Musc/skel	100			
			Hepatic		2[c] (minimal LOAEL for 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)		

Table 5-3 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
37	Rat (Long-Evans)	36 d Gd 6-Pnd 21 (GO)	Endocr	10	30 (reduced maternal serum T4)		Zhou et al. 2002 PentaBDE
	Immuno/ Lymphoret						
38	Rat (CD)	13 wk (F)		750 F			IRDC 1977 OctaBDE
39	Rat (Sprague-Dawley)	90d (F)		100			WIL Research Laboratories 1984 PentaBDE
	Reproductive						
40	Rat (CD)	13 wk (F)		600 M[d] 750 F			IRDC 1977 OctaBDE
41	Rat (Sprague-Dawley)	90d (F)		100 M 100 F			WIL Research Laboratories 1984 PentaBDE

[a] Key to figure

Table 5-3 Levels of Significant Exposure to Lower Brominated Diphenyl Ethers - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Developmental						
42	Rat (Long-Evans)	36 d Gd 6-Pnd 21 (GO)		1	10 (reduced serum T4 in fetuses and offspring on Gd 20 and Pnd 4 and 14)		Zhou et al. 2002 PentaBDE

a The number corresponds to entries in Figure 5- 3.

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.03 mg/kg/day. The MRL was derived by dividing the NOAEL by an uncertainty factor of 30 (10 for extrapolation from animals to humans, 3 for human variability because effects were observed in a sensitive subgroup).

c Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.007 mg/kg/day. The MRL was derived by dividing the LOAEL by an uncertainty factor of 300 (3 for converting a minimal LOAEL to an NOAEL, 10 for extrapolation from animals to humans, 10 for human variability)

d Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 5-3. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); (DW) = drinking water; endocr = endocrine; (F)= feed; F = female; Gd = gestation day; (G) = gavage; gastro = gastrointestinal; (GO) = gavage in oil; hemato = hematological; hr = hour(s); LD50 = lethal dose; 50% kill; LOAEL = lowest observed adverse effect level;min = minute(s); M = male; Musc/skel = musculoskeletal; NOAEL = no observed adverse effect level; NS = not specified; pnd = post natal day; Resp = respiratory; T4 = thyroxine ; wk = week(s); x = time(s)

Figure 5-3. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers- Oral

Acute (≤14 days)

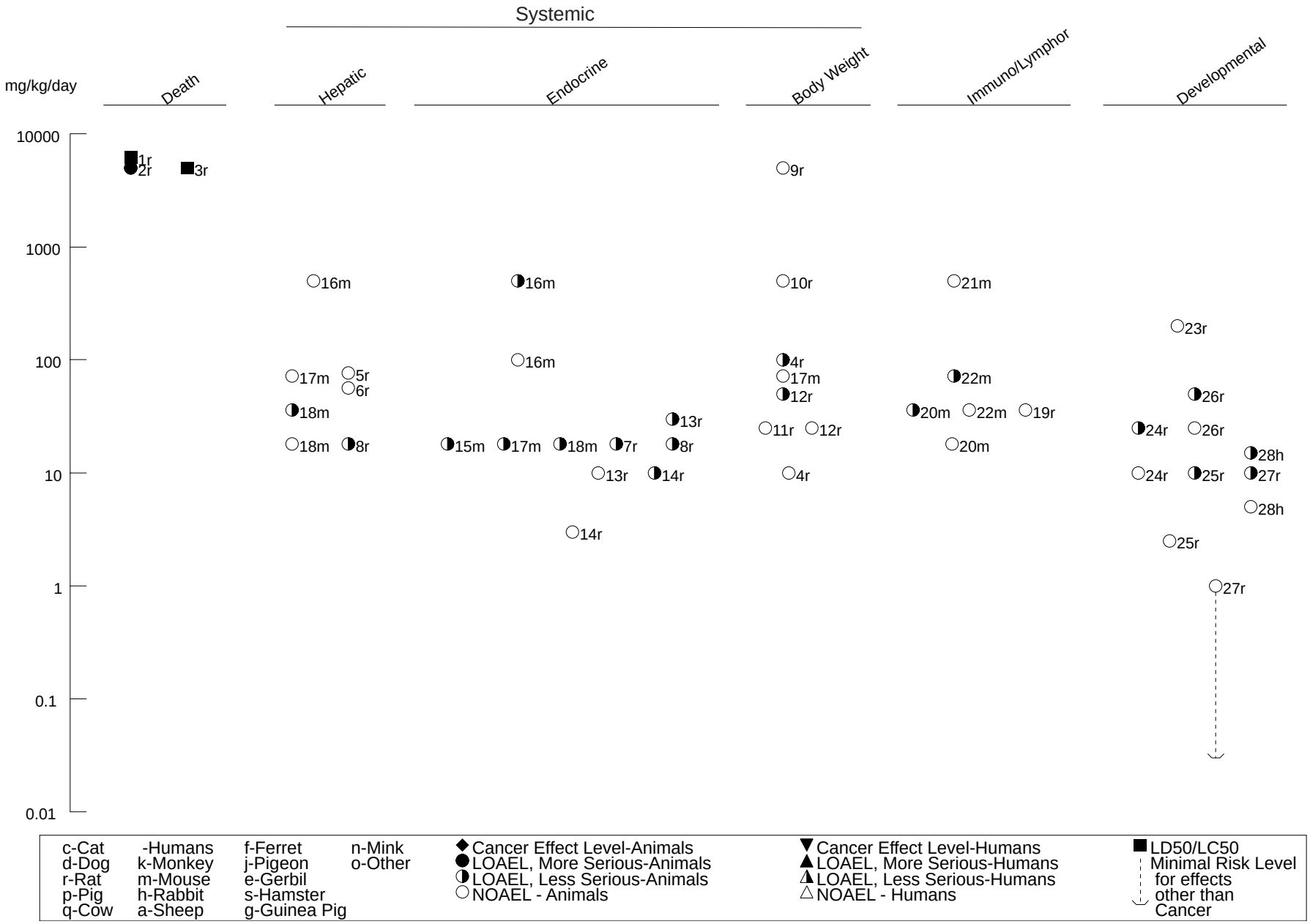


Figure 5-3. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers- Oral (*Continued*)

Intermediate (15-364 days)

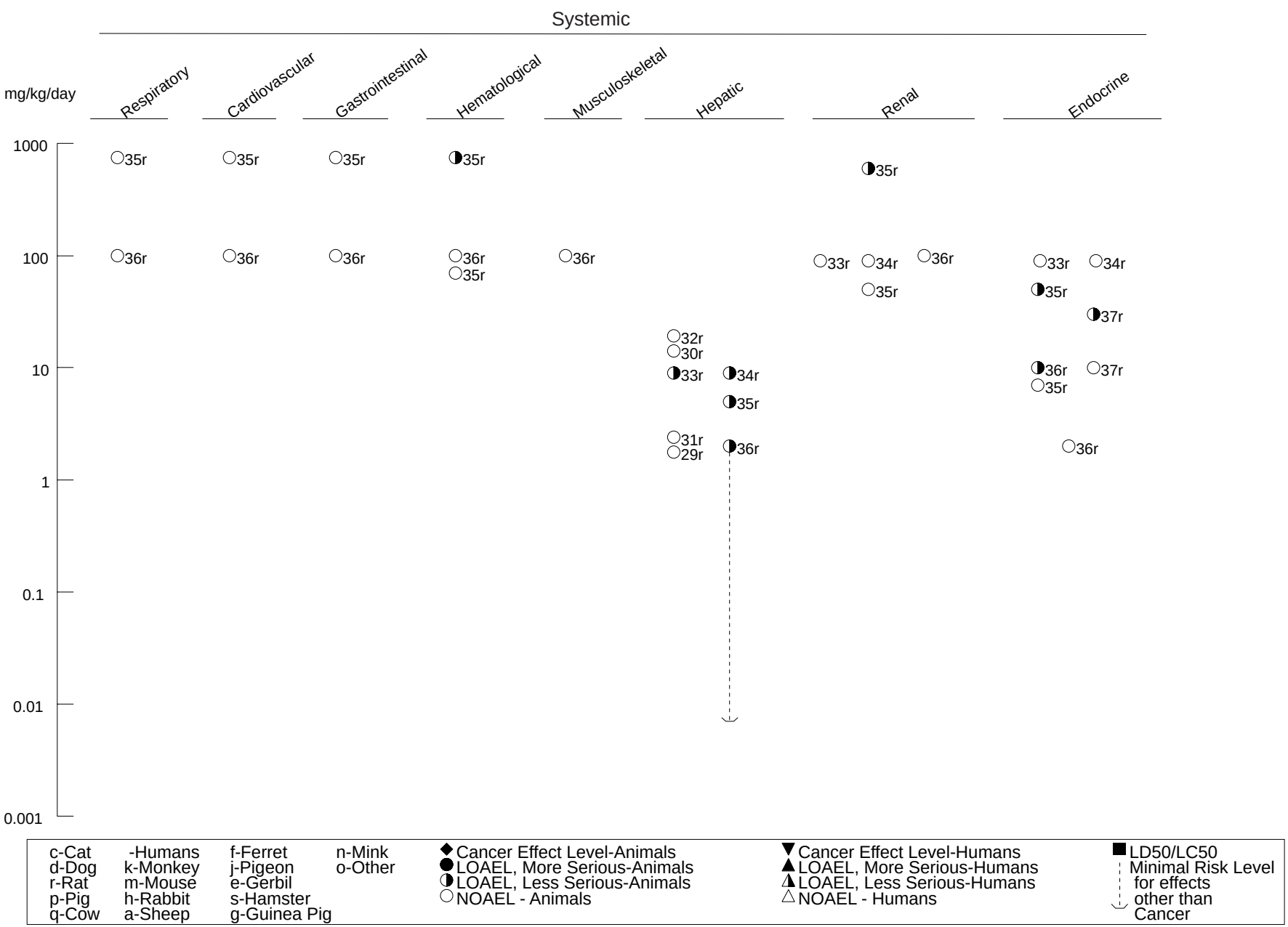


Figure 5-3. Levels of Significant Exposure to Lower Brominated Diphenyl Ethers- Oral (*Continued*)

Intermediate (15-364 days)

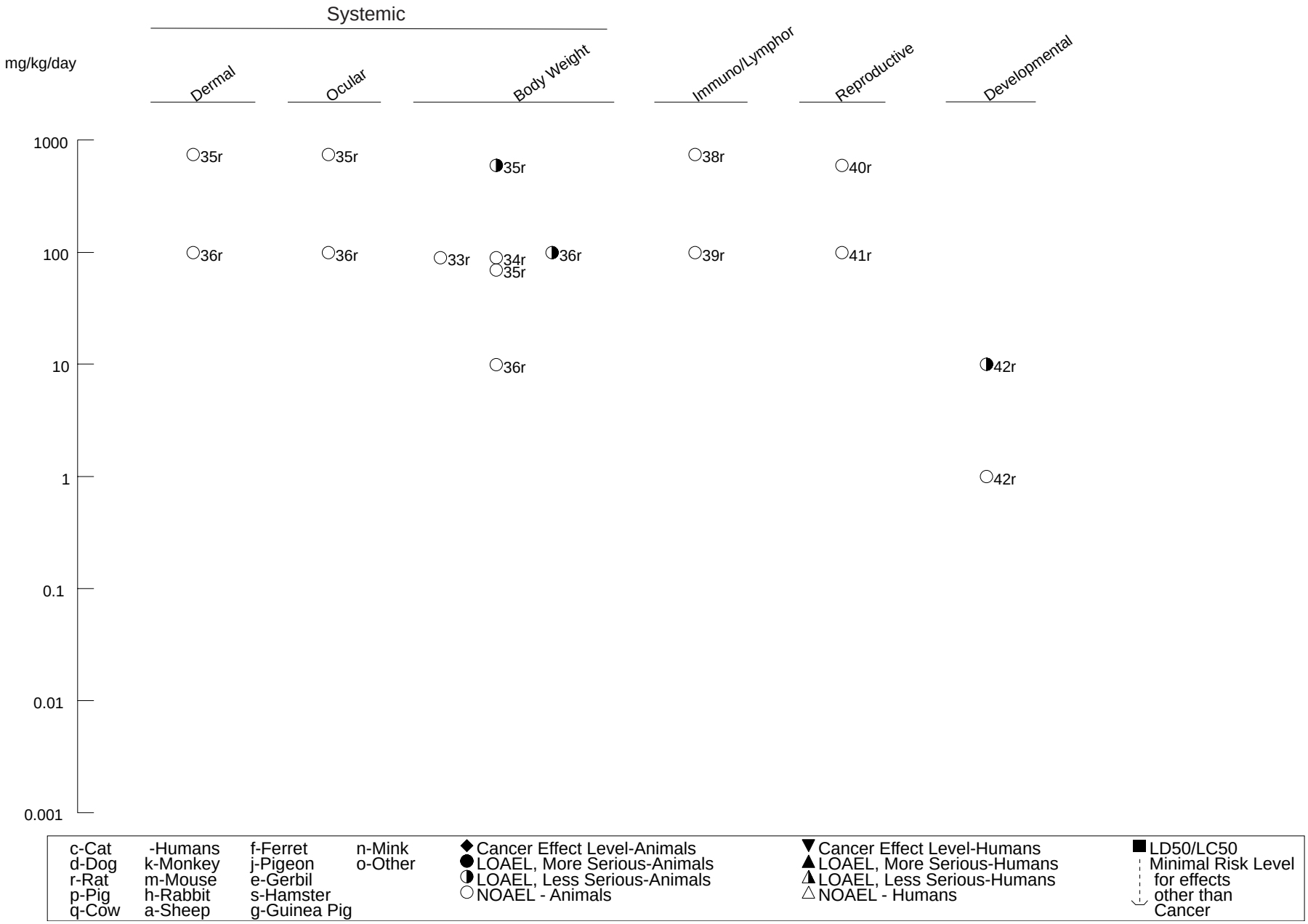


Table 5-4 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE						
	Systemic						
1	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Hepatic	95.9 M			Carlson 1980b DecaBDE
2	Rat Spartan	once (GO)	Bd Wt	5000			IRCD 1974 DecaBDE
3	Rat (Fischer- 344)	14d 1x/d (F)	Bd Wt	16000			NTP 1986 94-97% decaBDE
4	Rat (Long-Evans)	4 d 1x/d (GO)	Endocr	100			Zhou et al. 2001 DecaBDE
5	Mouse (B6C3F1)	14d 1x/d (F)	Bd Wt	19000			NTP 1986 94-97% decaBDE

Table 5-4 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	INTERMEDIATE EXPOSURE						
	Systemic						
6	Rat (CD)	28 d (F)	Hepatic	90			IRDC 1976 DecaBDE
			Renal	90			
			Endocr	90			
			Bd Wt	90			

Table 5-4 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
7	Rat (Fischer- 344)	13 wk (F)	Resp	8000			NTP 1986 94-97% decaBDE
			Cardio	8000			
			Gastro	8000			
			Hemato	8000			
			Musc/skel	8000			
			Hepatic	8000			
			Renal	8000			
			Endocr	8000			
			Bd Wt	8000			

Table 5-4 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
8	Mouse (B6C3F1)	13 wk (F)	Resp	9500			NTP 1986 94-97% decaBDE
			Cardio	9500			
			Gastro	9500			
			Hemato	9500			
			Musc/skel	9500			
			Hepatic	9500			
			Renal	9500			
			Endocr	9500			
			Bd Wt	9500			
	Immuno/ Lymphoret						
9	Rat (Fischer- 344)	13 wk (F)		8000			NTP 1986 94-97% decaBDE

Table 5-4 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
10	Mouse (B6C3F1)	13 wk (F)		9500			NTP 1986 94-97% decaBDE
	Reproductive						
11	Rat (Fischer- 344)	13 wk (F)		8000			NTP 1986 94-97% decaBDE
12	Mouse (B6C3F1)	13 wk (F)		9500			NTP 1986 94-97% decaBDE
	Developmental						
13	Rat (Sprague-Dawley)	20 d Gd 0-19 (GO)		1000[b] F			Hardy et al. 2002 99% decaBDE

Table 5-4 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	CHRONIC EXPOSURE						
	Systemic						
14	Rat (Sprague-Dawley)	2 yr (F)	Resp	1			Kociba et al. 1975; Norris et al. 1975b 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 5-4 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
15	Rat (Fischer- 344)	103 wk (F)	Resp	2550 F			NTP 1986 94-97% decaBDE
			Cardio	2550 F			
			Gastro	1120 M	2240 M		
			Hemato	2550 F			
			Musc/skel	2550 F			
			Hepatic	1200 M	1120 M		
					2250 F (pre-neoplastic nodules)		
			Renal	2550 F			
			Endocr	2550 F			
			Bd Wt	2550 F			

Table 5-4 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
16	Mouse (B6C3F1)	103 wk (F)	Resp	7780 F			NTP 1986 94-97% decaBDE
			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						
17	Rat (Sprague-Dawley)	2 yr (F)		1			Kociba et al. 1975; Norris et al. 1975b 77% decaBDE, 22% nonaBDE

Table 5-4 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
18	Rat (Fischer- 344)	103 wk (F)			2240 M (splenic fibrosis and lymphoid hyperplasia) 1200 F (splenic hematopoiesis)		NTP 1986 94-97% decaBDE
19	Mouse (B6C3F1)	103 wk (F)		7780 F			NTP 1986 94-97% decaBDE
	Reproductive						
20	Rat (Sprague-Dawley)	2 yr (F)		1 M 1 F			Kociba et al. 1975; Norris et al. 1975b 77% decaBDE, 22% nonaBDE
21	Rat (Fischer- 344)	103 wk (F)		2240 M 2550 F			NTP 1986 94-97% decaBDE

Table 5-4 Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
22	Mouse (B6C3F1)	103 wk (F)		6650 M 7780 F			NTP 1986 94-97% decaBDE
	Cancer						
23	Rat (Fischer- 344)	103 wk (F)				1120 M (CEL: liver neoplastic nodules)	NTP 1986 94-97% decaBDE
24	Mouse (B6C3F1)	103 wk (F)				3200 M (CEL: hepatocellular adenomas and carcinomas)	NTP 1986 94-97% decaBDE

a The number corresponds to entries in Figure 5 4.

b Used to derive an intermediate-duration (15-364 days) oral minimal risk level (MRL) of 10 mg/kg/day for decaBDE. The MRL was derived by dividing the 1,000 mg/kg/day NOAEL by an uncertainty factor of 100 (10 for species to species extrapolation and 10 for human variability).

c Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 5-4. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); (DW) = drinking water; endocr = endocrine; (F)= feed; F = female; Gd = gestation day; (G) = gavage; gastro = gastrointestinal; (GO) = gavage in oil; hemato = hematological; hr = hour(s); LOAEL = lowest observed adverse effect level; M = male; min = minute(s); Musc/skel = musculoskeletal; NOAEL = no observed adverse effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s); yr = year(s)

Figure 5-4. Levels of Significant Exposure to Decabromodiphenyl Ether - Oral

Acute (≤14 days)

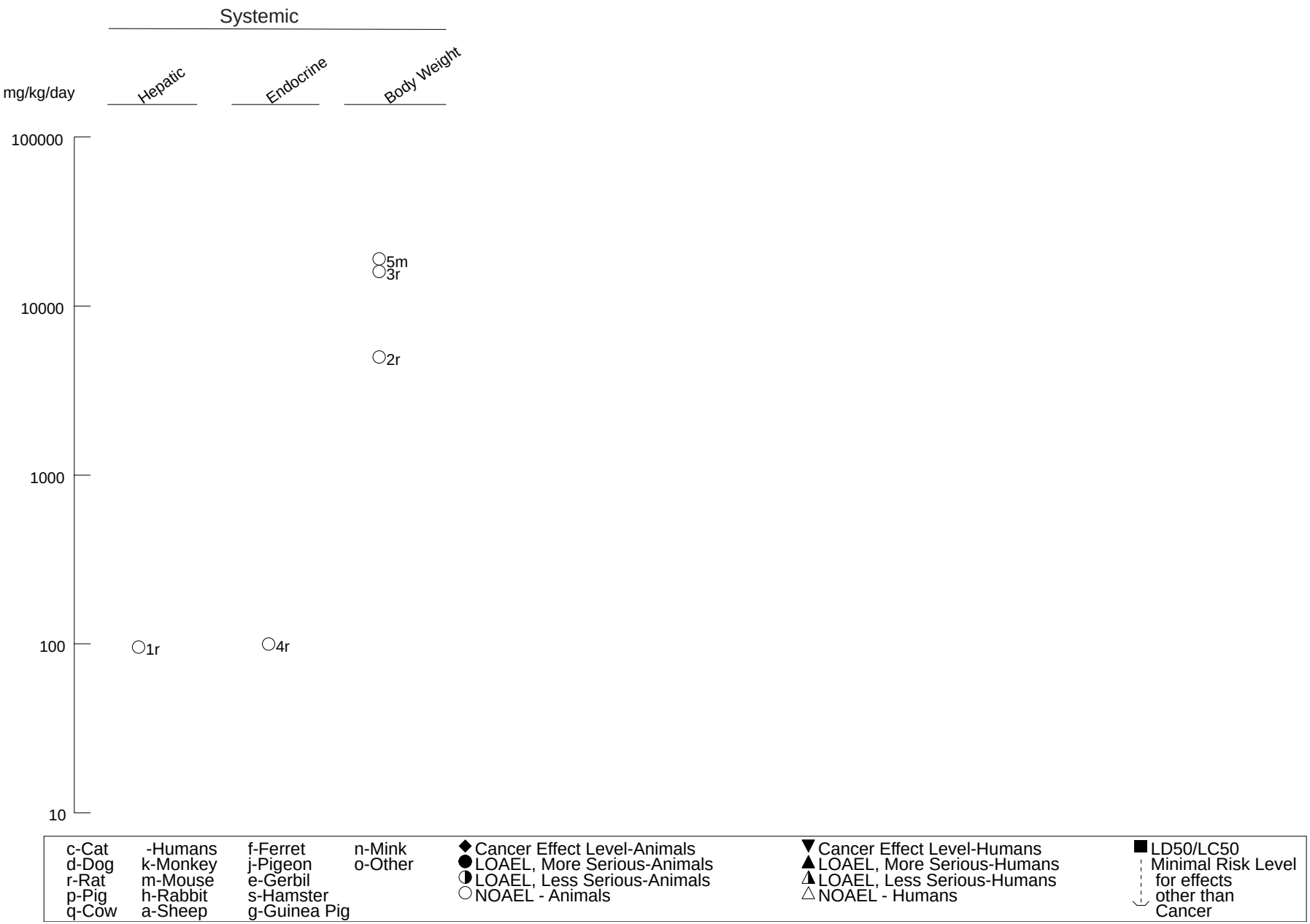


Figure 5-4. Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (*Continued*)

Intermediate (15-364 days)

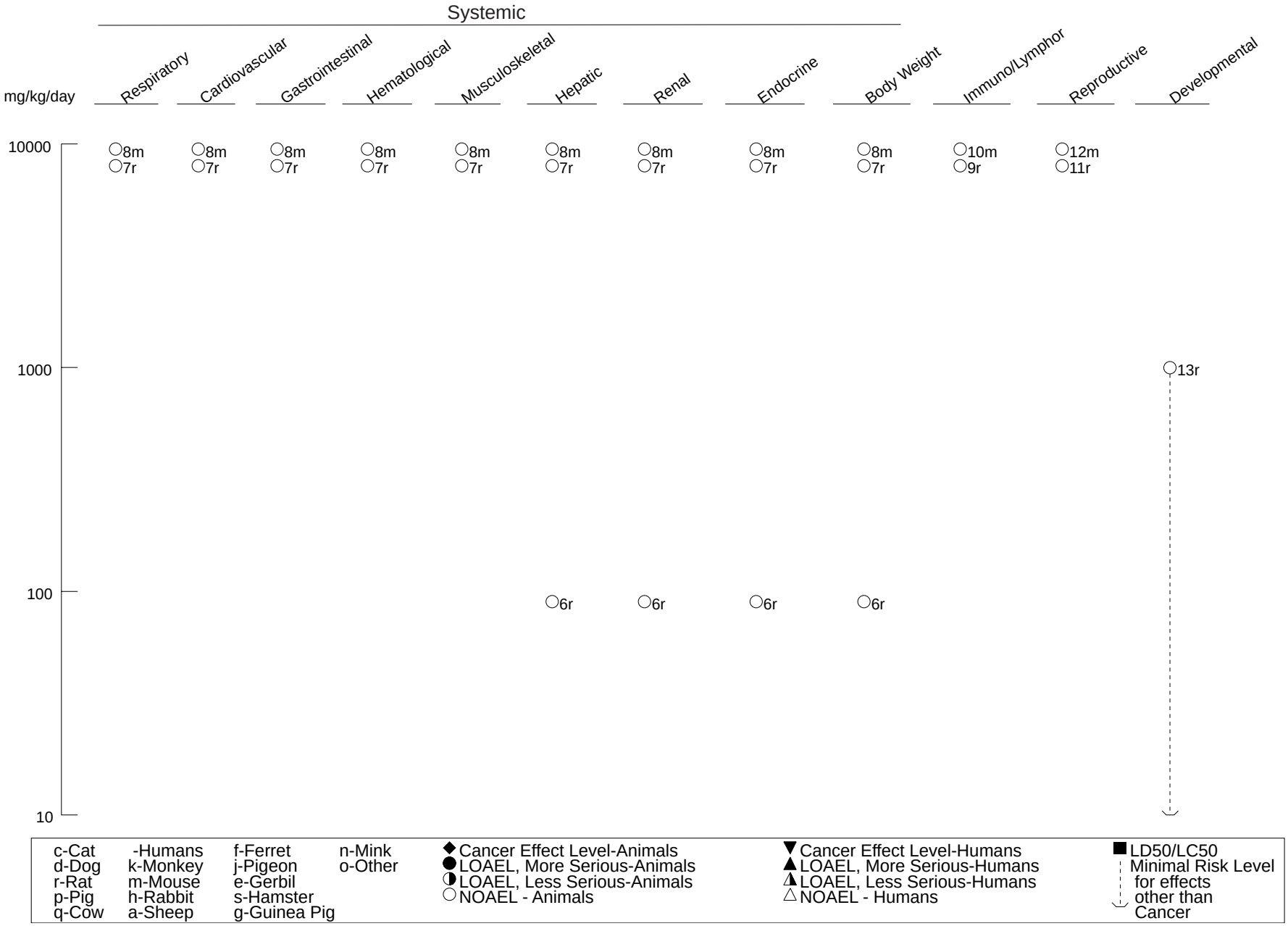
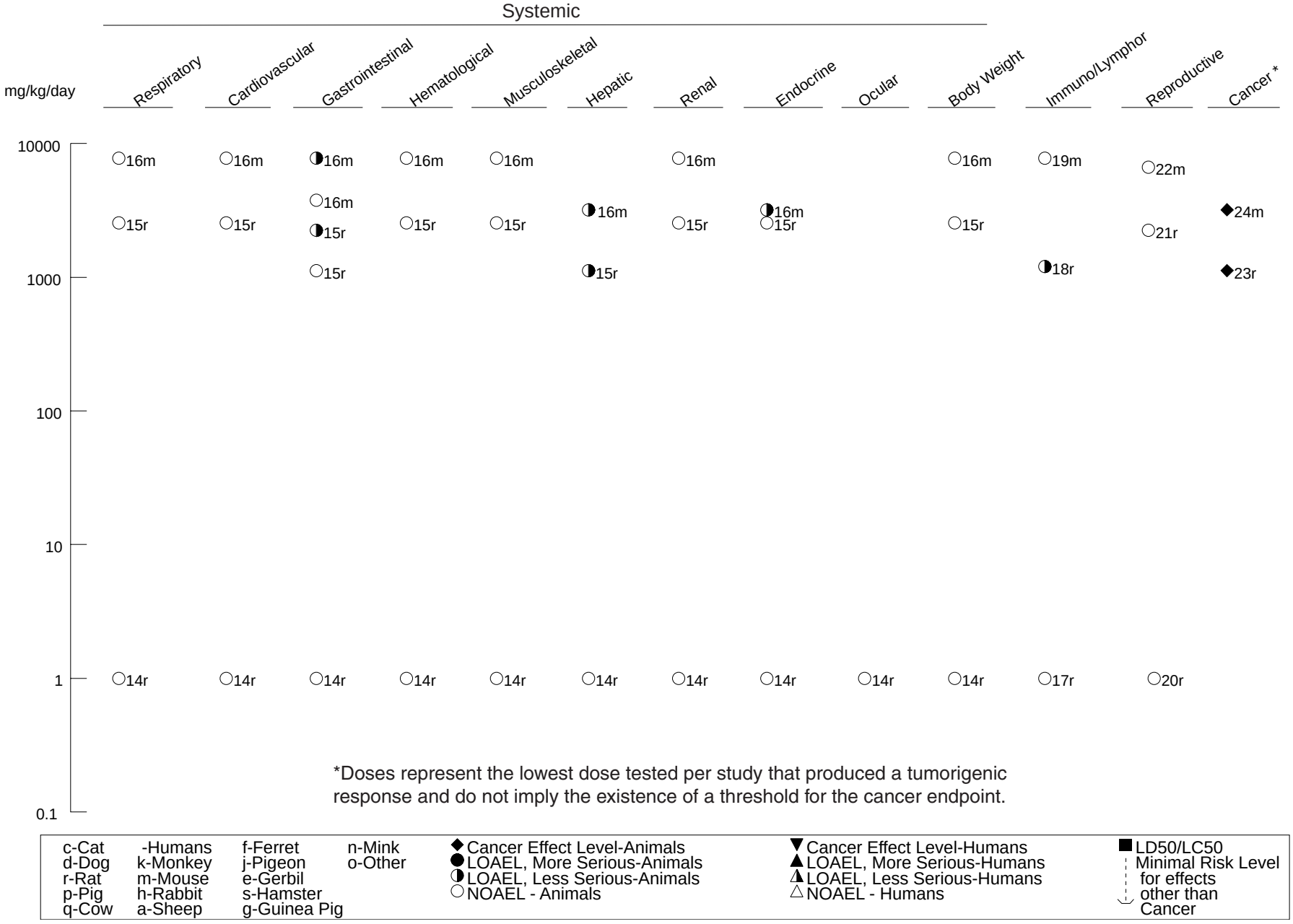


Figure 5-4. Levels of Significant Exposure to Decabromodiphenyl Ether - Oral (*Continued*)

Chronic (≥365 days)



general statement indicated that the rats had hunchback posture, rough coat, and sunken eyes, were lethargic, and appeared dehydrated and emaciated. No deaths occurred in rats administered octabromobiphenyl mixture in a single dose ≤1,000 mg/kg with 4 weeks of observation (Lee et al. 1975a), 2,000 mg/kg with 2 weeks of observation (Norris et al. 1975a), 17,000 mg/kg with 1 week of observation (Lee et al. 1975a; Waritz et al. 1977), or 3,000 mg/kg/day on 2 consecutive days with 4 weeks of observation (Lee et al. 1975a). The 17,000 mg/kg dose was the highest that was feasible to administer, apparently due to gavage volume because it had to be administered as divided doses given in a 4-hour period. Dietary administration of octabromobiphenyl mixture in estimated dosages of ≤70 mg/kg/day for 2 weeks was not lethal in rats, but there was no posttreatment observation period (Lee et al. 1975b; Waritz et al. 1977). In the only study of a decabromobiphenyl mixture, a single dose as high as 5,000 mg/kg caused no deaths in rats observed for 14 days (Millischer et al. 1980). In mice, dietary administration of FireMaster BP-6 for 2 weeks produced death (cause not reported) at estimated doses of 130 mg/kg/day, but not ≤36 mg/kg/day (Cagen et al. 1977; Fraker 1980; Fraker and Aust 1978). Information on acute oral lethality in species other than rats and mice was not located.

In intermediate-duration studies with rats, no deaths were induced by dietary administration of FireMaster BP-6 at estimated dosages of ≤5 mg/kg/day for ≤82 days (Darjono et al. 1983) or ≤10 mg/kg/day for 30 days (Akoso et al. 1982a). No deaths were observed in rats fed ≤50 mg/kg/day of an unspecified PBB mixture for 30 or 60 days (Sleight and Sanger 1976). Twice weekly gavage with 100 mg/kg FireMaster FF-1 in corn oil for two 3-week dosing periods, separated by ≈6 weeks, was not lethal in rats observed for 2 years (Kimbrough et al. 1981). Twenty-two gavage doses of 100 mg/kg FireMaster FF-1 in corn oil (5 days/week for 4.5 weeks) produced 38 and 100% mortality in male and female rats, respectively; the average times to death were 46.7 and 60.3 days, respectively (Gupta and Moore 1979). Similar treatment with 30 mg/kg/day FireMaster FF-1 was not lethal in rats observed for ≈5 months. Based on these gavage data, the calculated LD_{50} in rats observed for ≈60 days posttreatment (i.e., 90-day LD_{50}) was 149 and 65 mg/kg/day for male and female rats, respectively (Gupta and Moore 1979). This study did not specifically address the cause of death, but emaciated appearance and gross loss of subcutaneous and visceral adipose tissue indicate wasting was a contributing factor. Rats that were treated with FireMaster FF-1 in corn oil by gavage on 5 days/week for 25 weeks exhibited dose-related decreased survival at ≥0.3 mg/kg/day (cause of death not discussed), but not at 0.1 mg/kg/day (NTP 1983). The decreased survival was only apparent when the rats were observed for a lifetime (≈15–22 months posttreatment) and consistent only in males. Survival was also decreased in male but not female rats given ≥0.5 mg/kg/day FireMaster FF-1 in the diet for up to 104 weeks (Chhabra et al. 1993; NTP 1992). The decreased survival appeared to be related to increased incidences of mononuclear cell leukemia. No deaths were observed in

rats treated with octabromobiphenyl mixture in the diet at estimated dosages of ≤71 mg/kg/day for 4 weeks and observed for ≤18 weeks (Lee et al. 1975b; Waritz et al. 1977). Rats treated with ≤1 mg/kg/day dietary octabromobiphenyl mixture for 8 months did not die, but there were some deaths (number and cause not reported) in rats treated with higher dietary dosages (8–800 mg/kg/day) for 30 days (Norris et al. 1975a). Insufficient information is available to determine if the deaths were treatment-related, since incidences and other pertinent information were not reported.

Survival data for intermediate-duration exposure to PBBs are less extensive for species other than rat, but indicate that guinea pigs and mink are particularly susceptible. High mortality occurred in guinea pigs fed estimated dosages of 2 mg/kg/day FireMaster BP-6 for 45 days (Vos and van Genderen 1973, 1974) or ≥4 mg/kg/day of an unspecified PBB mixture for 30 days (Sleight and Sanger 1976); dosages of ≤0.4 mg/kg/day of either mixture were not lethal. The Litchfield and Wilcoxon procedure was used to calculate dietary LD_{50} values of 0.47 and 0.61 mg/kg/day (estimated dosages) for male and female mink, respectively, exposed to FireMaster FF-1 for life (63–294 days) (Aulerich and Ringer 1979; Ringer et al. 1981). Dosages ≤0.18 mg/kg/day did not significantly increase mortality in the mink. Dietary administration of FireMaster BP-6 in an estimated dosage of 21.7 mg/kg/day for 12 weeks caused some deaths in mice (number not reported), leading to sacrifice of other test animals (Martino et al. 1981; Wilson-Martino et al. 1980). Mean survival time decreased significantly in female mice treated with 10 mg/kg/day of FireMaster FF-1 in corn oil by gavage on 5 days/week for 25 weeks, but not ≤3 mg/kg/day (NTP 1983). Decreased survival was only apparent when the mice were observed for ≤24 months posttreatment (lifetime observation) and not observed in similarly treated males. Survival was also decreased in female mice given ≥1.3 mg/kg/day FireMaster FF-1 in the diet for up to 105 weeks; decreased survival occurred in similarly treated male mice at 3.9 mg/kg/day (Chhabra et al. 1993; NTP 1992). The cause of death was not discussed in the NTP (1983, 1992) mouse studies, but hepatocellular tumors increased significantly in both sexes at dosages that decreased survival.

No deaths occurred in two swine that ingested estimated dosages of ≤8 mg/kg/day for 16 weeks; one pig was observed for 102 days following exposure and the other for 14 weeks following exposure (Ku et al. 1978). An adult male monkey died after consuming 0.73 mg/kg/day FireMaster FF-1 in the diet for 25 weeks (Allen et al. 1978; Lambrecht et al. 1978). The death was attributed to severe gastrointestinal changes, including ulcerative colitis. The only other animal in this study was a juvenile female who survived 50 weeks of a dietary dosage of 1.43 mg/kg/day. In another study, one juvenile female monkey that consumed 18 mg/kg/day FireMaster FF-1 in the diet died after 137 days of continuous exposure (Allen et al. 1978). Although only one or two monkeys were tested in these studies, effects characteristic

of PBB poisoning (e.g., dermal changes, body weight loss) indicate that the deaths were exposure-related. Pregnant cows given 67 mg/kg/day FireMaster BP-6 in capsules for 60 days (dosing began ≥10 days after pregnancy diagnosis) were sacrificed between days 33 and 66 because of impending death (Moorhead et al. 1977). Clinical signs developed progressively and included anorexia, emaciation, and depressed general condition. No mortality occurred in cows treated with ≤0.65 mg/kg/day and observed for 1 or 140 days following the end of treatment.

The LD_{50} value and reliable LOAEL values for death in each species in the acute- and intermediate-duration categories are recorded in Table 5-2 and plotted in Figure 5-2.

Polybrominated Diphenyl Ethers. 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% octaDBE) and observed for the following 14 days (IRDC 1974; Norris et al. 1975b). 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).

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% octaDBE) for 2 years (Kociba et al. 1975; Norris et al. 1975b), 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).

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, observed 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).

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).

The LD_{50} and LOAEL values for death in the acute-duration BDE studies in rats are recorded in Tables 5-3 (lower BDEs) and 5-4 (decaBDE) and plotted in Figures 5-3 (lower BDEs) and 5-4 (decaBDE).

5.2.2.2 Systemic Effects

The systemic effects in humans and animals following oral exposure to PBBs and 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 5-2 (PBBs), 5-3 (lower PDBEs), or 5-4 (decaBDE) and plotted in Figures 5-2 (PBBs), 5-3 (lower PDBEs), or 5-4 (decaBDE).

Respiratory Effects.

Polybrominated Biphenyls. No studies were located regarding respiratory effects in humans after oral exposure to PBBs.

The preponderance of data does not indicate that PBBs are respiratory system toxicants in animals, even at doses sufficient to cause death. No exposure-related histological changes were observed in the lungs or trachea of rats that were administered FireMaster FF-1 in a single dose of 200 or 1,000 mg/kg and observed for 2–23 months posttreatment (Kimbrough et al. 1978b, 1981). Rats and mice exposed to ≤30 mg/kg/day FireMaster FF-1 for 2 weeks also showed no histological alterations in the lung, trachea, or nasal turbinates (Gupta et al. 1981). Information on acute-duration respiratory effects in other species was not located.

In intermediate- and chronic-duration studies with rats, histology of the lung, trachea, or nasal turbinate was not altered by FireMaster FF-1 or FireMaster BP-6 dosages of ≤30 mg/kg/day by gavage for 30 days (Gupta et al. 1981), ≤10 mg/kg/day in the diet for 30 days (Akoso et al. 1982a; Sleight et al. 1978), ≤10 mg/kg/day by gavage for 25 weeks (NTP 1983), or ≤1.5 mg/kg/day in the diet for up to 104 weeks (NTP 1992). Rat lung histology also was not affected by exposure to 50 mg/kg/day of an unspecified PBB mixture in the feed for 30 days (Sleight and Sanger 1976). In studies with mice, FireMaster FF-1 produced no histopathological changes in the lungs, trachea, or nasal turbinates following gavage

exposure to ≤10 mg/kg/day for 25 weeks (NTP 1983) or ≤30 mg/kg/day for 30 days (Gupta et al. 1981), or dietary exposure to ≤3.9 mg/kg/day for up to 105 weeks (NTP 1992). Guinea pig lung histology was unaffected by exposures of ≤20 mg/kg/day of an unspecified PBB mixture in the feed for 30 days (Sleight and Sanger 1976). Relative lung weights increased in mink that died following exposure to ≤2.4 mg/kg/day FireMaster FF-1 for 313 days, but it is unclear if this effect is adverse because the animals had lost weight and histopathology was not reported (Aulerich and Ringer 1979; Ringer et al. 1981). Effects in six pregnant cows given 67 mg/kg/day of FireMaster BP-6 in capsules for up to 60 days included increased respiratory rate and occasional nasal discharge (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed gross pneumonia (one cow), microscopic lesions of early purulent bronchopneumonia (two cows), and petechial hemorrhages of the tracheal mucosa (one cow). No histological changes were observed in the trachea or lungs treated with ≤0.65 mg/kg/day and observed for 1–140 days following the end of treatment. Information on respiratory effects of octabromobiphenyl mixture or other PBB mixtures in animals was not located.

Polybrominated Diphenyl Ethers. 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 respiratory 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 respiratory 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% octaDBDE) for 2 years (Kociba et al. 1975; Norris et al. 1975b), or in rats and mice exposed to decaBDE at estimated doses of ≤2,550 and ≤7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986).

No histopathological changes in the respiratory tract were found in dietary studies of rats exposed to ≤750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977) or ≤100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Cardiovascular Effects.

Polybrominated Biphenyls. No studies were located regarding cardiovascular effects in humans after oral exposure to PBBs.

Animal data do not generally indicate cardiovascular toxicity of PBBs even at lethal doses, but cardiovascular function was not evaluated in most studies. No exposure-related histological changes in the heart were observed in rats administered FireMaster FF-1 in a single dose of 200 or ≤1,000 mg/kg and observed for 2–23 months posttreatment (Kimbrough et al. 1978a, 1981), or ≤30 mg/kg/day for 2 weeks (Gupta et al. 1981; Kimbrough et al. 1978b, 1981). Mice exposed to ≤30 mg/kg/day FireMaster FF-1 for 2 weeks also showed no histological alterations in the heart (Gupta et al. 1981). In intermediate-and chronic-duration studies with rats, FireMaster FF-1 or FireMaster BP-6 dosages of ≤30 mg/kg/day by gavage for 30 days (Gupta et al. 1981), ≤10 mg/kg/day in the diet for 30 days (Akoso et al. 1982a; Sleight et al. 1978), ≤10 mg/kg/day by gavage for 25 weeks (NTP 1983), or ≤1.5 mg/kg/day in the diet for up to 104 weeks (NTP 1992) did not alter heart weight or histology. Rat heart histology also was unaffected by exposure to 50 mg/kg/day of an unspecified PBB mixture for 30 days (Sleight and Sanger 1976). Rats exposed to ≤5 mg/kg/day FireMaster BP-6 for 30 days exhibited no exposure-related changes in blood pressure, but histology or other cardiovascular end points were not evaluated (McCormack et al. 1978). Rats exposed to octabromobiphenyl mixture in dosages of ≤1 mg/kg/day for 8 months or ≤800 mg/kg/day for 30 days showed no changes in heart weight, but histology or function was not evaluated (Norris et al. 1975a). In studies with mice, FireMaster FF-1 produced no changes in heart weight or histology following gavage exposure to ≤10 mg/kg/day for 25 days (Gupta et al. 1981), ≤30 mg/kg/day for 30 days (NTP 1983), or dietary exposure to ≤3.9 mg/kg/day for up to 105 weeks (Chhabra et al. 1993; NTP 1992). No effects on heart relative weight or histology were reported in mink that died following exposure to ≤2.4 mg/kg/day FireMaster FF-1 for 313 days (Aulerich and Ringer 1979; Ringer et al. 1981). Relative heart weights were increased in swine exposed to ≤8 mg/kg/day of an unspecified PBB mixture for 16 weeks, but gross pathology was normal and histology was not evaluated (Ku et al. 1978). Necropsy of a monkey that died following ingestion of 0.73 mg/kg/day FireMaster FF-1 for 25 weeks showed an enlarged heart, but histology was not evaluated and a similar effect was not reported in two other monkeys exposed to higher dosages (Allen et al. 1978; Lambrecht et al. 1978). Mean heart rate was 32% lower than the pre-exposure value in pregnant cows that were treated with 67 mg/kg/day of FireMaster BP-6 in capsules for 10 days (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed petechial and ecchymotic hemorrhages of the myocardium and endocardium in two of six cows. No cardiovascular effects were observed in cows given ≤0.65 mg/kg/day and observed for 1–140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding cardiovascular effects in humans after oral exposure to PBDEs.

Effects of PBDEs on cardiovascular function have not been studied in orally exposed animals. No histopathological changes in the heart 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 cardiac histopathology in rats that were fed ≤1.0 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaDBDE) for 2 years (Norris et al. 1975b), or in rats and mice exposed to decaBDE in estimated doses of ≤2,550 and ≤7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986).

No histopathological changes in the heart were found in dietary studies of rats exposed to ≤750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977) or ≤100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Gastrointestinal Effects.

Polybrominated Biphenyls. No symptoms of gastrointestinal effects were reported by residents of quarantined Michigan farms in an epidemiological study conducted by the U.S. Center for Disease Control and the Michigan Department of Public Health (Landrigan et al. 1979). In a medical history survey conducted by the Environmental Science Laboratory of the Mount Sinai School of Medicine, no statistically significant difference was observed between the prevalence rates of gastrointestinal symptoms for 933 Michigan residents who were likely to have ingested PBB-contaminated food and the rates for a control group of 229 Wisconsin farm residents (Anderson et al. 1978c). The Michigan residents were examined ≈3 years after the contamination episode occurred. No other studies were located regarding gastrointestinal effects in humans after oral exposure to PBBs.

Gastric lesions have developed in various animals that ingested PBBs, particularly after prolonged exposure to FireMaster FF-1 or FireMaster BP-6. No exposure-related histological changes in the gastrointestinal tract or esophagus were observed in rats administered FireMaster FF-1 in a single dose ≤1,000 mg/kg and observed for 2–23 months posttreatment (Kimbrough et al. 1978b, 1981). Rats or mice exposed to ≤30 mg/kg/day FireMaster FF-1 for 2 weeks also showed no histological alterations in the gastrointestinal tract (esophagus not examined) (Gupta et al. 1981). In intermediate-duration studies, the gastrointestinal tract of rats exposed to FireMaster BP-6 or FireMaster FF-1 by gavage or diet at ≤50 mg/kg/day for 4–4.5 weeks showed no histopathological changes (esophagus not examined) (Akoso et al. 1982a; Gupta and Moore 1979; Gupta et al. 1981; Sleight and Sanger 1976; Sleight et al. 1978). Histological examination of the gastrointestinal tract of rats administered FireMaster FF-1 by gavage for 25 weeks showed significantly increased incidences of gastric ulcers at ≥1 mg/kg/day and hyperplastic

gastropathy at ≥3 mg/kg/day after lifetime observation (23 months). These gastric effects were not observed in rats examined at the end of the gavage treatment period, although similar changes (forestomach hyperplasia, inflammation, and ulceration) occurred in rats exposed to 1.5 mg/kg/day FireMaster FF-1 in the diet for up to 104 weeks (Chhabra et al. 1993; NTP 1992). In the only study of a decabromobiphenyl mixture, rats were fed estimated dosages as high as 100 mg/kg/day for 13 weeks (Millischer et al. 1980). A comprehensive histology evaluation was performed in this study, but the liver is the only tissue specifically mentioned as having been examined. Due to the total number of tissues examined (21) and route of exposure, it is probable that the gastrointestinal tract was examined but not discussed because no histopathologic changes were observed.

Gastrointestinal tract histology was normal in mice exposed to FireMaster FF-1 dosages of ≤10 mg/kg/day by gavage for 25 weeks or ≤3.9 mg/kg/day in the diet for up to 105 weeks (NTP 1983, 1992). FireMaster FF-1 produced no histological changes in the gastrointestinal tract of mice exposed to ≤30 mg/kg/day for 30 days (Gupta et al. 1981). Gross pathologic examination of swine administered an unspecified PBB mixture for 16 weeks showed that the glandular portion of the stomach "appeared somewhat hyperplastic" (additional details were not reported, and histology was not evaluated) at 8 mg/kg/day, but not at 1 mg/kg/day (Ku et al. 1978). Biopsies of two monkeys performed following their ingestion of 0.73 or 1.43 mg/kg/day FireMaster FF-1 for 12 weeks showed proliferation of gastric mucosal cells, focal areas of infiltration of chronic inflammatory cells, and isolated penetrations of the gastric mucosa into the underlying submucosa (Allen et al. 1978; Lambrecht et al. 1978). Necropsies performed after 25 or 50 weeks of exposure also showed hyperplastic gastroenteritis and, in the low-dose monkey (that died of "severe gastrointestinal changes"), severe ulcerative colitis. Hyperplastic gastroenteritis was described in another monkey exposed to a higher dosage (18 mg/kg/day) of FireMaster FF-1 for 137 days (Allen et al. 1978). Gastrointestinal effects in six pregnant cows that were administered 67 mg/kg/day of FireMaster BP-6 in capsules for up to 60 days included diarrhea, dehydration (possibly a result of the diarrhea), and occasional constipation (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed edema and hemorrhage of the colon and rectum mucosa, although histology was normal in the esophagus, rumen, omasum, and reticulum. No histological changes were observed in the gastrointestinal tract of cows with ≤0.65 mg/kg/day and observed 1 or 140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding gastrointestinal effects in humans after oral exposure to PBDEs.

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% octaDBDE) for 2 years (Kociba et al. 1975; Norris et al. 1975b). 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).

No histopathological changes in the gastrointestinal tract were found in dietary studies of rats exposed to ≤750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977) or ≤100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Hematological Effects.

Polybrominated Biphenyls. No studies were located regarding hematological effects in humans after oral exposure to PBBs.

In animals, hematologic changes indicative of possible anemia are common findings in animals resulting from longer-term exposure to PBBs. Comprehensive hematological examinations in rats and mice administered ≤30 mg/kg/day FireMaster FF-1 for 2 weeks showed no exposure-related changes (Gupta et al. 1981). No additional information on hematology in animals following acute-duration exposure to PBBs was located. In intermediate-duration studies, no consistent hematological changes were found in rats exposed to ≤10 mg/kg/day FireMaster BP-6 for 30 days (Akoso et al. 1982a; Sleight et al. 1978). Some hematologic effects occurred in rats at higher dosages or longer durations. Exposure to 30 mg/kg/day FireMaster FF-1 for 4.5 weeks significantly reduced hemoglobin concentration, packed cell volume (PCV), and platelet count in rats evaluated up to ≈60 days postexposure (Gupta and Moore 1979). In another study in which rats were administered the same dosages of FireMaster FF-1 (≤30 mg/kg/day) for 30 days, longer postexposure (up to 90 days) evaluation revealed transient responses (Gupta et al. 1981). Transitory and slight but significant ($p<0.05$) decreases in red blood cell count, hemoglobin concentration, and PCV values were found; they returned to control levels by 60-days post-dosing. No consistent hematological changes were observed in rats administered ≤50 mg/kg/day of an unspecified PBB mixture for 30 days (Sleight and Sanger 1976) or ≤10 mg/kg/day FireMaster FF-1 for 6 months (Luster et al. 1980). Rats exposed to FireMaster FF-1 for 25 weeks showed no hematological changes at 0.1 mg/kg/day, but had dose-related, significantly decreased hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and PCV at ≥0.3 mg/kg/day, and increased total leukocytes at

≥1 mg/kg/day; there were no effects on erythrocyte or platelet counts (NTP 1983). Mice similarly treated for 25 weeks had decreased erythrocyte count and MCV at ≥0.3 mg/kg/day and decreased platelets and lymphocytes at ≥1 mg/kg/day, but no hematological effects were noted at 0.1 mg/kg/day (NTP 1983). No hematologic alterations were found in mice exposed to FireMaster FF-1 at dosages of ≤10 mg/kg/day for 6 months or ≤30 mg/kg/day for 30 days (Gupta et al. 1981; Luster et al. 1980).

Hematologic evaluation of swine treated with an unspecified PBB mixture for 16 weeks showed significantly decreased hemoglobin and hematocrit values in two of four animals exposed to 8 mg/kg/day at week 6, after which values returned to normal or near-normal within 2 weeks (Ku et al. 1978). Decreased PCV and serum protein developed in monkeys exposed to FireMaster FF-1 in dosages of ≥0.73 mg/kg/day for ≥25 weeks (two animals); additional hematologic effects observed in one monkey exposed to 18 mg/kg/day for 137 days were decreased erythrocyte and white blood cell counts (Allen et al. 1978; Lambrecht et al. 1978). No hematological changes were measured in cows treated with ≤0.65 mg/kg/day FireMaster BP-6 in capsules for 60 days, and observed for up to 140 days following the end of treatment (Moorhead et al. 1977). Similar treatment with 67 mg/kg/day did not cause abnormal hematologic indices in four of six cows; changes in the other two animals (e.g., leukocytosis, increased PCV) have uncertain toxicologic significance because the animals at this dose were sacrificed between days 33 and 66 because of impending death due to poor health.

Studies of hematologic effects of octabromobiphenyl mixture, performed only in rats, showed significantly decreased red blood cell count and PCV following 800 mg/kg/day for 30 days, but no hematological changes resulting from ≤1 mg/kg/day for 8 months (Norris et al. 1975a). In the only study of a decabromobiphenyl mixture, dietary administration of 100 mg/kg/day for 13 weeks caused no hematologic changes in rats (Millischer et al. 1980). Erythrocyte and leucocyte counts, differential leukocyte count, and hematocrit and hemoglobin levels were measured.

Polybrominated Diphenyl Ethers. No studies were located regarding hematological effects in humans after oral exposure to PBDEs.

OctaBDE caused red blood cell effects in an intermediate-duration dietary studies in rats in which erythrocytes, hematocrit, and hemoglobin were reduced following exposure to 750 mg/kg/day for 13 weeks (no effects at ≤70 mg/kg/day) (IRDC 1977).

DecaBDE and pentaBDE did not induce hematological effects in animals. 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, 1975b), ≤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% octaDBE) for 2 years (Kociba et al. 1975; Norris et al. 1975b) or ≤100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Musculoskeletal Effects.

Polybrominated Biphenyls. Symptoms of musculoskeletal effects, described as "joint pain" and "swelling in joints," were frequently cited health complaints in two epidemiological studies of groups of Michigan residents who were likely to have ingested PBB-contaminated food (Anderson et al. 1978c; Landrigan et al. 1979). Although one study demonstrated a statistically significant difference between the prevalence rate for these types of symptoms in Michigan residents compared with nonexposed residents of Wisconsin farms (Anderson et al. 1978c), neither study demonstrated a positive association between serum PBB levels and the prevalence rates for symptoms of musculoskeletal effects.

There are no pathology data indicating that PBBs produce effects in musculoskeletal tissues of animals. No exposure-related histological changes in muscle or bone marrow were observed in rats that were administered a single 1,000 mg/kg dose of FireMaster FF-1 and observed for 2 years (Kimbrough et al. 1981). Rats and mice exposed to ≤30 mg/kg/day FireMaster FF-1 for 2 weeks showed no histological alterations in muscle or sternum (Gupta et al. 1981). In intermediate-and chronic-duration studies, rats and/or mice exposed to FireMaster FF-1 or FireMaster BP-6 dosages of ≤30 mg/kg/day by gavage for 30 days (Gupta et al. 1981), ≤10 mg/kg/day in the diet for 30 days (Akoso et al. 1982a; Sleight et al. 1978), ≤10 mg/kg/day by gavage for 25 weeks (NTP 1983), or ≤3.9 mg/kg/day in the diet for up to 105 weeks (NTP 1992) showed no histopathological changes in muscle or bone. A dosage of an unspecified PBB mixture as high as 50 mg/kg/day for 30 days produced no histopathological changes in rat muscle (Sleight and Sanger 1976). Excess porphyrins were detected in bone and/or teeth by fluorescence under ultraviolet light in some of the rat studies (Gupta and Moore 1979; NTP 1983), but this appears to be a consequence of altered porphyrin metabolism (Hill 1985). No histological alterations were observed in sternebrae bone marrow of pregnant cows given FireMaster BP-6 in capsules for up to 60 days (Moorhead et al. 1977). Cows treated with 67 mg/kg/day were necropsied following sacrifice

between days 33 and 66 because of impending death due to poor health, and those treated with nonlethal lower dosages of ≤0.65 mg/kg/day were examined 1 or 140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding musculoskeletal effects in humans after oral exposure to PBDEs.

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% octaDBE) for 2 years (Kociba et al. 1975; Norris et al. 1975b), 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). A study of pentaBDE found no musculoskeletal changes in rats exposed dietary doses of ≤100 mg/kg/day for 90 days (WIL Research Laboratories 1984). No information was located on possible musculoskeletal effects of octaBDE.

Hepatic Effects.

Polybrominated Biphenyls. Results from several studies of humans exposed to PBBs do not demonstrate, in general, a conclusive association between adverse effects on the liver and oral exposure to PBBs. In a study in which serum was collected in 1974, 1977, 1978, and 1979 from 89, 240, 220, and 200 individuals, respectively, who were predominately residents of quarantined Michigan farms, no consistent statistically significant correlations were found between serum PBB levels and levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) or serum bilirubin (Kreiss et al. 1982). The prevalence rates of Michigan residents with abnormally high levels of SGPT (12.7% prevalence rate), SGOT (12.7%), or lactate dehydrogenase (8.6%) were statistically significantly higher than comparable rates for residents of Wisconsin farms (2.7, 2.0, and 3.3%) (Anderson et al. 1979). A contingency table analysis indicated that the prevalence of abnormal SGPT values in Michigan residents with serum PBB levels ≤1 ppb (8%) was lower than the prevalence rate for residents with serum PBB levels ≥1 ppb (14%), but correlation coefficients for serum PBB levels and serum liver enzyme levels were uniformly low ($r<0.1$) (Anderson et al. 1979). Physical examinations of Michigan residents (37 men and 9 women) with known exposure to PBBs and a history of incapacitating health care complaints revealed that 72% of the subjects displayed mildly enlarged livers, which were associated with elevations in serum liver enzymes (SGOT and SGPT) predominately less than 2-fold above normal values (Stross et al. 1979). Mildly enlarged livers, confirmed by liver scanning, were observed in 4 of 23 (17%) Michigan residents with known PBB exposure and incapacitating health complaints and in 2 of 28 (7%) workers involved in the manufacture and distribution of PBBs,

respectively; however, these workers had histories of either substantial alcohol intake or exposure to multiple chemicals (Stross et al. 1981). Results of a caffeine breath test, discussed in Section 5.8.2, suggest that PBBs may have induced hepatic microsomal enzymes in exposed Michigan residents (Lambert et al. 1990).

Hepatic effects of PBBs are documented in various animal species although rats have been the species tested most extensively. The changes appear to be similar among species and reversible when mild. Characteristic hepatic effects include proliferation of the smooth endoplasmic reticulum, microsomal enzyme induction, increased serum levels of liver-associated enzymes indicative of possible hepatocellular damage, liver enlargement, hepatocyte vacuolation and fat deposition, fibrosis, and necrosis. PBBs also cause alterations in levels of cholesterol and other lipids in liver and serum, levels of vitamin A in liver and urine, and levels of porphyrins in liver, other tissues, and urine. These changes could be secondary to liver damage or due to direct effects on lipid, vitamin A, and porphyrin metabolism, which occurs primarily in the liver. Induction of microsomal enzymes by PBBs is a sensitive effect generally regarded as an adaptive response of the liver rather than as a manifestation of hepatotoxicity *per se* (Guzelian 1985). Although not necessarily adverse, induction of microsomal enzymes could alter the rate or pathways of metabolism of other xenobiotic or endogenous substances and increase activation of promutagens and procarcinogens or increase detoxification pathways. In addition, the induction of some microsomal enzyme activities is an indicator of exposure to PBBs and related compounds (AhR agonists), which elicit a well known pattern of toxic responses (see Chapters 3 and 4). PBB-related liver enlargement is usually associated with hepatocyte enlargement and an increase in smooth endoplasmic reticulum and/or increased microsomal enzymatic activity; therefore, it is not considered an adverse effect unless accompanied by other biochemical changes and/or histological alterations.

Rats administered FireMaster FF-1 in a single 1,000 mg/kg dose and observed for 2–23 months posttreatment or a lethal dose of 1,000 mg/kg/day for 2 weeks developed enlarged livers with fatty and necrotic changes leading to fibrosis (Gupta and Moore 1979; Kimbrough et al. 1978b, 1981). Lower single doses of FireMaster FF-1 caused vacuolation and some biochemical changes (e.g., increased serum cholesterol and phospholipids, decreased serum retinol) at 500 mg/kg (Bernert et al. 1983; Kimbrough et al. 1980), and hepatic porphyrin accumulation with no histologic changes at 200 mg/kg (Kimbrough et al. 1981). Repeated exposure to lower dosages of ≥3 mg/kg/day FireMaster FF-1 for 2 weeks (Gupta et al. 1981) or 5 mg/kg/day FireMaster BP-6 for 10 days (Raber and Carter 1986) caused hepatocyte enlargement and some fatty and single-cell necrotic changes in weanling and young rats. A limited

amount of data suggest that octabromobiphenyl mixture-induced hepatic effects in rats are milder than for FireMaster mixtures at similar dosages. Fatty changes appear to be the most severe hepatic histopathologic effect of octabromobiphenyl observed following a single 1,000 mg/kg dose or doses of 3,000 mg/kg/day for 2 days and 6.53 mg/kg/day (but not 0.66 mg/kg/day) for 2 weeks (Lee et al. 1975a, 1975b; Waritz et al. 1977). In studies with mice, a single dose of 36 mg/kg FireMaster BP-6 increased liver weight (histology not evaluated) and had no consistent effects on disposition of injected ouabain or indocyanine green, indicating that hepatic function was not compromised (Cagen et al. 1977). Sporadic increases in the clearance of ouabain and indocyanine green were attributed to increased liver size. Exposure to 130 mg/kg/day FireMaster BP-6, for 11 days caused focal areas of coagulative necrosis (Corbett et al. 1975) and ≥3 mg/kg/day FireMaster FF-1 for 2 weeks caused scattered necrosis in mice (Gupta et al. 1981).

In intermediate-duration studies with rats, dosages ≥0.05 mg/kg/day FireMaster BP-6 for 20 days induced hepatic microsomal enzymes but histology was not evaluated (Babish et al. 1978). Dose-related hepatocyte swelling and vacuolation were induced by ≥0.1 mg/kg/day FireMaster BP-6 for 30 days (Akoso et al. 1982a), lipid accumulation, porphyrin levels, and atypical foci were increased by ≥0.3 mg/kg/day FireMaster FF-1 for 25 weeks (lethal dose) (NTP 1983), and bile duct hyperplasia was induced by 0.5 mg/kg/day FireMaster BP-6 for 82 days (Darjono et al. 1983). Rats exposed to higher, but not necessarily lethal, dosages of FireMaster FF-1 or FireMaster BP-6 for 1–3 months showed progression of these effects, including marked degenerative changes and porphyrin accumulation in these and other studies (Gupta and Moore 1979; Gupta et al. 1981; Kasza et al. 1978a; McCormack et al. 1978; Sleight and Sanger 1976; Sleight et al. 1978). In the only chronic study, incidences of hepatocellular hypertrophy, cytoplasmic vacuolation, atypical foci, and oval cell hyperplasia were increased in rats fed ≥0.5 mg/kg/day FireMaster FF-1 for up to 104 weeks (Chhabra et al. 1993; NTP 1992). Compared to this adult-only exposure, combined perinatal and adult exposure resulted in increased incidences of oval cell hyperplasia at 0.5 mg/kg/day and hypertrophy, cytoplasmic vacuolation, and bile duct fibrosis at 1.5 mg/kg/day. Studies of octabromobiphenyl mixture in rats have shown hepatic effects (e.g., hypertrophy and hyperplasia of centrilobular cells, vacuolation, and other fatty degenerative changes) at dosages ≥6.53 mg/kg/day for 4 weeks (Lee et al. 1975b; Norris et al. 1975a; Waritz et al. 1977), but normal liver histology at ≤1 mg/kg/day for 8 months (Norris et al. 1975a). A 13-week dietary study with decabromobiphenyl mixture found that hepatic effects in rats, including vacuolization and distension of centrilobular hepatocytes often accompanied by slightly increased lipid, did not occur at dosages <100 mg/kg/day (Millischer et al. 1980). Information on hepatic effects of octabromobiphenyl mixture and decabromobiphenyl in species other than the rat was not located.

In mice, exposure to FireMaster FF-1 for 25 weeks increased liver weight, porphyrin content, and SGOT at ≥0.3 mg/kg/day and hepatocyte swelling occurred at ≥1 mg/kg/day (NTP 1983). Hepatic effects in mice exposed to ≥1.3 mg/kg/day FireMaster FF-1 for up to 105 weeks included hepatocyte hypertrophy, vacuolization, and necrosis; bile duct hyperplasia also developed (NTP 1992). Dosages ≥3 mg/kg/day for 4–6 weeks, but not 0.3 mg/kg/day, also induced hepatocyte necrosis and/or vacuolation in mice (Gupta et al. 1981; Loose et al. 1981; NTP 1983). Fatty changes and centrilobular necrosis developed in pregnant swine fed ≥1.25 mg/kg/day, but not 0.125 mg/kg/day, FireMaster BP-6 for 12 weeks during the second half of gestation through lactation (Werner and Sleight 1981). This adverse effect level cannot be corroborated in nonpregnant swine exposed to ≤8 mg/kg/day of unspecified PBBs for 16 weeks due to lack of liver histology evaluations, although relative liver weight increased at ≥1 mg/kg/day and no gross changes were observed (Ku et al. 1978). Guinea pigs appear to be particularly susceptible to hepatic effects of PBBs (unspecified) as indicated by ultrastructural vacuolation and formation of myelin bodies in hepatocytes following exposure to ≥0.04 mg/kg/day for 30 days; liver weights were increased at 0.4 mg/kg/day and histological vacuolation and severe centrilobular fatty change were observed at a lethal dose of 4 mg/kg/day (Sleight and Sanger 1976). Mink that ingested ≥0.24 mg/kg/day FireMaster FF-1 for ≤313 days showed increased liver weight and fatty infiltration (Aulerich and Ringer 1979; Ringer et al. 1981). In monkeys, lethal FireMaster FF-1 dosages ≥0.73 mg/kg/day for 25–50 weeks caused hepatocyte enlargement with increased lipid droplets, bile duct hyperplasia, increased SGPT, and decreased serum cholesterol (Allen et al. 1978; Lambrecht et al. 1978). Effects in six pregnant cows given 67 mg/kg/day of FireMaster BP-6 in capsules for up to 60 days included increased serum lactic dehydrogenase (LDH) and SGOT (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed increased liver weight and pathologic liver changes including friable appearance, glycogen depletion in hepatocytes, sinusoidal dilation, and scattered areas of early fatty degeneration. In general, the hepatic effects observed in cows are less pronounced than in other species at lethal doses. No adverse hepatic effects were observed in cows treated with ≤0.65 mg/kg/day and examined 1 or 140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding hepatic effects in humans after oral exposure to PBDEs.

No information was located on adverse hepatic effects of acute-duration oral exposure to PBDEs in animals. Intermediate- and chronic-duration studies in rodents indicate that the liver is a target of toxicity of PBDEs. As discussed below, repeated dietary exposure to commercial PBDE products typically

caused liver enlargement with or without degenerative changes, and effects were generally dose-related in incidence and severity, more frequent and pronounced in males than females, and more severe and induced by lower doses with octaBDE and pentaBDE than decaBDE. Regarding decaBDE, the low purity former mixture (77.4% pure containing 21.8% nonaBDE and 0.8% octaBDE) was much more hepatotoxic than the current day commercial product (≈99% pure). In particular, hepatic effects included increased liver weights at ≥80 mg/kg/day with centrilobular cytoplasmic enlargement and vacuolation at 800 mg/kg/day in male rats exposed to 77% decaBDE for 30 days (Norris et al. 1973, 1975a, 1975b), whereas exposure to 94–97% decaBDE for 13 weeks caused no liver pathology in rats and mice exposed to estimated doses as high as 2,000–8,000 and 2,375–9,500 mg/kg/day, respectively (NTP 1986). The NOAEL for hepatic effects from the NTP (1986) 13-week study (8,000 mg/kg/day) was used as the basis for the intermediate-duration MRL for oral exposure to decaBDE, as indicated in a footnote to Table 5-4 and discussed in Chapter 4 and Appendix A. 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 heptatic 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 the 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. 1975b). Unlike the decaBDE used in the NTP (1986) study, the composition of the mixture tested by Kociba et al. (1975) is substantially different than that of current-day commercial decaBDE products (≈99%).

OctaBDE caused increased liver weight and histopathological changes such as hepatocellular enlargement and vacuolation in rats exposed to doses as low as 5mg/kg/day (lowest tested dose) for 4–13 weeks (IRDC 1976, 1977). Hepatic effects in rats exposed to octaBDE for 13 weeks included increases in absolute and/or relative liver weight at ≥5–7 mg/kg/day and liver lesions in 40% of males at 5 mg/kg/day and 100% of both sexes at ≥50 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 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 mg/kg/day for 13 weeks (IRDC 1977).

PentaBDE induced liver effects in rats exposed to ≥9 mg/kg/day for 28 days (increased liver weight and enlargement of centrolobular and midzonal liver parenchymal cells) (IRDC 1976) and ≥2 mg/kg/day for 90 days (hepatocytomegaly) (WIL Research Laboratories 1984). The hepatomegaly in the 90-day study was dose-related with respect to severity (some affected hepatocytes at higher doses had vacuoles that likely contained lipid) and 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 and 100 mg/kg/day pentaBDE for 90 days 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). The 2 mg/kg/day LOAEL for hepatic effects of pentaBDE was used as the basis for the intermediate-duration MRL for oral exposure to tetra- to heptaBDEs as indicated in the footnote to Table 5-3 and discussed in Chapter 4 and Appendix A. Liver vitamin A concentrations were increased in rats and mice exposed to a commercial pentaBDE mixture (Bromkal 70-5 DE) by gavage in doses of 18 and 36 mg/kg/day, respectively, for 90 days (Hallgren et al. 2001).

Hepatic microsomal enzyme induction is a well-documented effect for the pentaBDE and octaBDE commercial products, but has not been observed for decaBDE (Carlson 1980b; Zhou et al. 2001). 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-nitro-phenyl 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, but not considered to be adverse due to the lack of any accompanying hepatic histological abnormalities. Rats that were treated with equimolar (0.1 mmol/kg/day) gavage doses of deca-, octa-, or pentaBDE (95.9, 76.6, or 56.4 mg/kg/day, respectively) for 14 days had octa- and pentaBDE-induced increases in liver weight and microsomal enzyme activity (e.g., increased EPN detoxification, *p*-nitroanisole demethylation, uridinediphosphate-glucuronyltransferase [UDPGT] activity, and benzo[a]pyrene hydroxylase activity); exposure to decaBDE only increased liver weight (Carlson 1980b). DecaBDE also had no effect on hepatic UDPGT, ethoxyresorufin-o-deethylase (EROD), or pentoxyresorufin-o-deethylase (PROD) activities in weanling rats that were treated with ≤100 mg/kg/day by gavage for 4 days (Zhou et al. 2001). Similar exposure to 0.3–300 mg/kg/day pentaBDE caused significantly increased EROD and PROD at ≥10 mg/kg/day and UDPGT at ≥30 mg/kg/day, and octaBDE induced PROD at ≥10 mg/kg/day and EROD and UDPGT at ≥30 mg/kg/day; neither of these PBDEs caused induction at ≤1 mg/kg/day.

Hepatic EROD, PROD, and methoxyresorufin-o-deethylase (MROD) were increased in mice exposed to ≥18 mg/kg/day (lowest tested dose) of pentaBDE by gavage for 14 days, although UDPGT was unchanged at ≤36 mg/kg/day (Fowles et al. 1994; Hallgren et al. 2001). Rats that were similarly treated with pentaBDE for 14 days had increased activities of EROD, MROD, and PROD at ≥18 mg/kg/day (lowest tested dose) and increased UDPGT at 36 mg/kg/day (Hallgren et al. 2001). PentaBDE also increased hepatic microsomal enzyme activity in maternally-exposed rats and their offspring (Zhou et al. 2002). Exposure to 1, 10, or 30 mg/kg/day by gavage from gestation day (GD) 6 through postnatal day (PND) 21 caused significantly increased hepatic EROD and PROD at ≥10 mg/kg/day in dams (GD 20 and PND 22) and offspring (GD 20 and PNDs 4, 14, and 36), as well as increased UDPGT at 30 mg/kg/day in dams (GD 20 and PND 22) and offspring (GD 20 and PNDs 4 and 14).

Renal Effects.

Polybrominated Biphenyls. No statistically significant correlations were found between serum PBB levels and serum levels of blood urea nitrogen (BUN) or creatinine in a study of residents of quarantined Michigan farms after the 1973 PBB contamination episode (Kreiss et al. 1982). No other studies were located with information pertinent to renal effects in humans after oral exposure to PBBs.

Studies with animals have shown some renal effects following prolonged exposure to PBBs, but findings are generally inconsistent, and the functional significance is uncertain. No exposure-related histological changes in kidneys or bladder were observed in rats administered FireMaster FF-1 in a single dose of 200 or 1,000 mg/kg (Kimbrough et al. 1978b, 1981) and observed for 2–23 months posttreatment (Kimbrough et al. 1978a, 1981), or ≤30 mg/kg/day for 2 weeks (Gupta et al. 1981). Gross examination of rats exposed to 1,000 mg/kg/day for 2 weeks showed darkened kidneys (Gupta and Moore 1979). Other renal information was not reported, but the dosage was lethal. Urinalysis was normal in rats following exposure to ≤30 mg/kg/day for 2 weeks (Gupta et al. 1981); urinalysis was not evaluated in the other rat studies. Bladder histology, examined in some of the rat studies, was also reported to be normal (Gupta et al. 1981; Kimbrough et al. 1981). Kidney histology was not altered in rats exposed to ≤71 mg/kg/day octabromobiphenyl mixture for 2 weeks (Lee et al. 1975b; Waritz et al. 1977). Mice exposed to ≤30 mg/kg/day FireMaster FF-1 for 2 weeks showed no abnormal kidney or bladder histology or urinalysis findings (Gupta et al. 1981). Information on acute-duration renal effects in other species was not located.

In intermediate-duration studies with rats, dietary exposure to FireMaster BP-6 for 30 days produced no PBB-related alterations in urinalysis indices or BUN at 5 mg/kg/day (highest tested dose) or kidney

histology at ≤10 mg/kg/day (Akoso et al. 1982a; Sleight et al. 1978). However, 5 mg/kg/day FireMaster BP-6 in the diet for 3 months caused progressive obsolescence of glomeruli in rats (Bowman's membrane was shrunken and glomerular tufts were shrunken, inactive, or had been largely replaced by scar tissue), although relative kidney weight, BUN, and renal function tests (clearance of inulin, *p*-aminohippurate, or fractional sodium excretion) were normal (McCormack et al. 1978). Also, *in vitro* accumulation of *p*-aminohippurate and N-methylnicotinamide, and ammoniagenesis and gluconeogenesis were not affected in renal cortical slices from these treated rats. Administration of FireMaster FF-1 by gavage for 25 weeks caused no renal effects at 0.1 mg/kg/day, but produced chronic progressive nephropathy at ≥1 mg/kg/day, and more serious histopathology at 10 mg/kg/day (NTP 1983). Renal pathology at the 10 mg/kg/day dosage included atrophy and edema of glomerular tufts with marked dilation of Bowman's capsule and dilation of some renal tubules, with either serous fluid or proteinaceous casts in both cortical and medullary regions, and no changes in BUN (NTP 1983). Chronic administration of FireMaster FF-1 in the diet for up to 104 weeks, however, failed to produce any treatment-related histopathologic changes at dosages as high as 1.5 mg/kg/day (NTP 1992). The reason for the inconsistency between this finding and the results of the NTP (1983) study is unclear, but the different methods of oral treatment could be a factor.

Intermediate-duration gavage exposure to a higher FireMaster FF-1 dose of 30 mg/kg/day for 4.5 weeks caused dilation of Bowman's capsule with serous fluid in rats observed for ≈60 days posttreatment (Gupta and Moore 1979); however, rats that were similarly treated (≤30 mg/kg/day for 30 days) but observed longer (90 days posttreatment) had normal kidney histology, urinalysis values, and BUN (Gupta et al. 1981). Rats administered 50 mg/kg/day of an unspecified PBB mixture in the diet for 30 days with no posttreatment observation had increased BUN but no changes in urinalysis values or kidney histology (Sleight and Sanger 1976). In studies with octabromobiphenyl mixture in rats, dietary exposure to ≥8 mg/kg/day for 30 days caused hyaline degenerative cytoplasmic changes in kidneys with normal urinalysis values (Norris et al. 1975a). This finding is inconsistent with a report of normal kidney histology in rats exposed to ≤71 mg/kg/day octabromobiphenyl mixture in the diet for 4 weeks (urinalysis not constructed) (Lee et al. 1975b; Waritz et al. 1977); the reason for the discrepancy cannot be discerned from the reports. Kidney histology and urinalysis findings were also normal in rats administered ≤1 mg/kg/day octabromobiphenyl mixture for 8 months (Norris et al. 1975a). In the only study of a decabromobiphenyl mixture, urinalysis was normal in rats fed 100 mg/kg/day for 13 weeks (Millischer et al. 1980). Comprehensive histology evaluations were performed at this and lower dosages in this study, but the liver is the only tissue specifically mentioned as having been examined. Due to the total number

tissues examined (21) and the route of exposure, it is probable that kidneys were examined but were not discussed because no histopathologic changes were found.

In gavage studies with mice, FireMaster FF-1 produced no renal histopathologic changes following exposure to ≤10 mg/kg/day for 25 weeks (BUN was normal) (NTP 1983) or ≤30 mg/kg/day for 30 days (normal BUN and urinalysis) (Gupta et al. 1981). Dietary exposure to 3.9 mg/kg/day FireMaster FF-1 for up to 105 weeks, however, caused an increased incidence of chronic progressive nephropathy in mice; this effect was not found at 1.3 mg/kg/day (NTP 1992). Kidney histological alterations were not reported in mink exposed to ≤2.4 mg/kg/day FireMaster FF-1 for 313 days (Aulerich and Ringer 1979; Ringer et al. 1981). Swine exposed to unspecified PBBs for 16 weeks had increased relative kidney weight at ≥1 mg/kg/day, but the adversity of this change is unclear since no gross renal pathology was observed (≤8 mg/kg/day) and histology was not evaluated (Ku et al. 1978). Monkeys that ingested 18 mg/kg/day FireMaster FF-1 for 137 days developed hyperplasia of the bladder epithelium, but histological changes in the kidneys were not reported (Allen et al. 1978). Urine alterations in six pregnant cows given 67 mg/kg/day of FireMaster BP-6 in capsules for up to 60 days included increased protein concentration and BUN, and decreased pH and specific gravity (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed enlarged, distended, and discolored kidneys, extreme dilation of the collecting ducts and convoluted tubules, degenerative changes in the tubular epithelium, and congestion with scattered microscopic hemorrhages in the medulla. The renal effects in cows appear to be more severe than those generally observed in other species at lethal doses. No urinalysis alterations or changes in kidney histology were observed in other cows treated with ≤0.65 mg/kg/day and examined 1 or 140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding renal effects in humans after oral exposure to PBDEs.

Dietary studies of PBDEs in animals observed kidney effects that are mainly attributable to octaBDE. Renal effects induced by octaBDE included noninflammatory kidney changes in male rats exposed to 600 mg/kg/day for 13 weeks (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). Another study of octaBDE found no histopathological changes in the kidneys of rats exposed to ≤90 mg/kg/day for 90 days (IRDC 1976). Studies of pentaBDE found no renal histopathology in rats exposed dietary doses of ≤90 mg/kg/day for 28 days (IRDC 1976) or ≤100 mg/kg/day for 90 days (WIL Research Laboratories 1984).

Studies of low purity (≈77%) commercial decaBDE mixtures found kidney pathology (hyaline degenerative cytoplasmic changes) in male rats exposed to 800 mg/kg/day for 30 days (Norris et al. 1973, 1975a, 1975b), but not in rats exposed to ≤90 mg/kg/day for 90 days (IRDC 1976) or ≤1.0 mg/kg/day for 2 years (Kociba et al. 1975; Norris et al. 1975b). 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 renal histopathological changes were induced by 94–97% pure commercial decaBDE in rats exposed to ≤8,000 mg/kg/day for 13 weeks 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).

Endocrine Effects.

Polybrominated Biphenyls. No studies were located regarding endocrine effects in humans after oral exposure to PBBs.

Thyroid effects have been observed in animals treated with PBBs by gavage or diet in acute-, intermediate-, and chronic-duration studies. Characteristic changes include decreases in serum levels of serum thyroxine (T_4) and serum triiodothyronine (T_3) hormones, thyroid enlargement, and effects in the follicular cells including reduced size, hyperplasia with columnar appearance and papillary projections, and accumulation of colloid droplets. In the only acute study that investigated thyroid end points more sensitive than histology, rats administered an unspecified PBB mixture for 10 days showed serum T_4 levels (T_3 not evaluated) that were significantly reduced ($p \leq 0.05$) at ≥3 mg/kg/day, but not at 1 mg/kg/day (Allen-Rowlands et al. 1981). The reduction in T_4 levels was both dose- and time-dependent as shown by 20-day results discussed below with intermediate-duration studies. Based on the NOAEL for decreased serum T_4, an acute oral MRL of 0.01 mg/kg/day was calculated as indicated in the footnote to Table 5-2 and discussed in Chapter 3 and Appendix A. A single ≤286 mg/kg dose of an unspecified PBB mixture caused no change in 4-hour thyroidal ^{131}I uptake and incorporation into thyroglobulin in rats (Allen-Rowlands et al. 1981). No thyroid histological alterations were observed in rats in acute-duration studies with FireMaster FF-1, even with a single dose ≤1,000 mg/kg and up to 2 years posttreatment observation (Kimbrough et al. 1978b, 1981) or dosages of ≤1,000 mg/kg/day for 2 weeks (Gupta and Moore 1979; Gupta et al. 1981). The only information on thyroid effects of acute exposure to octabromobiphenyl mixture is a lack of histological changes in rats administered ≤71 mg/kg/day for 2 weeks (Lee et al. 1975b; Waritz et al. 1977). The only information on acute-duration thyroid effects of PBBs in species

other than rat is the normal histologic integrity of the thyroid in mice at FireMaster FF-1 dosages of ≤30 mg/kg/day for 2 weeks (Gupta et al. 1981).

In intermediate-duration studies with rats, serum levels of T_3 or T_4 decreased at FireMaster dosages as low as 0.3 mg/kg/day FireMaster FF-1 for 25 weeks (NTP 1983), 0.45 mg/kg/day FireMaster BP-6 for 7 months (Byrne et al. 1987), 5 mg/kg/day FireMaster BP-6 for 30 days (Akoso et al. 1982b) or 1 mg/kg/day of an unspecified PBB mixture for 20 days (Allen-Rowlands et al. 1981). In the latter study, 8–11 rats were evaluated after exposure to 1, 3, or 6 mg/kg/day for 20 days. Other thyroid effects in these rats included significantly increased absolute thyroid weight at ≥3 mg/kg/day (not evaluated at 1 mg/kg/day), and increased plasma TSH levels, increased 5-hour thyroid uptake of ^{131}I and deceased incorporation of ^{131}I into monoiodotyrosine (MIT) at 6 mg/kg/day (Allen-Rowlands et al. 1981). No effects on incorporation of ^{131}I into diiodotyrosine (DIT), T_3, or T_4 were observed. Serum T_4 levels were also reduced at ≥1 mg/kg/day in rats exposed for 20 days and evaluated after being placed on restricted food intake for ≥2 months following treatment (Allen-Rowlands et al. 1981). Rats administered 2.5 mg/kg/day of an unspecified hexabromobiphenyl mixture for 7 months showed no significant changes in serum T_3, but serum T_4 was not evaluated (Sepkovic and Byrne 1984). Thyroid ultrastructural changes were produced in rats by FireMaster BP-6 dosages as low as 0.05 mg/kg/day for 30–35 days (Akoso et al. 1982b; Kasza et al. 1978a), and histologic changes of the thyroid were observed at ≥5 mg/kg/day FireMaster BP-6 for 30 days (Sleight et al. 1978) and ≥30 mg/kg/day FireMaster FF-1 for 4.5 weeks (Gupta and Moore 1979). In the study that evaluated thyroid effects at the lowest dose, rats were administered estimated doses of 0.05, 0.5, or 5 mg/kg/day FireMaster BP-6 in the diet for 30 days (Akoso et al. 1982b). Effects were dose-dependent and included increased number and decreased size of follicles (especially at the peripheral location) at ≥0.05 mg/kg/day, follicles with epithelial tall columnar appearance and some papillary projections in the lumen at ≥0.5 mg/kg/day, and extensive follicular changes (hyperplasia and hypertrophy of follicular cells, prominent, and numerous papillary projections), increased relative thyroid weight, and decreased serum T_3 and T_4 at 5 mg/kg/day. Chronic exposure to ≤1.5 mg/kg/day FireMaster FF-1 for up to 104 weeks caused no thyroid histological alterations, but ultrastructure and serum thyroid hormones were not assayed (NTP 1992).

In the only intermediate-duration rat study of octabromobiphenyl mixture that assessed thyroid hormones, a dose of 2.5 mg/kg/day for 7 months produced no significant changes in serum T_3, but serum T_4 was not evaluated (Sepkovic and Byrne 1984). The histologic integrity of the thyroid was normal in rats fed octabromobiphenyl mixture at dosages as high as 1 mg/kg/day for 8 months (Norris et al. 1975a), 2.5 mg/kg/day for 7 months (Sepkovic and Byrne 1984), and 71 mg/kg/day for 4 weeks (Lee et al. 1975b;

Waritz et al. 1977), although ≥8 mg/kg/day for 30 days induced dose-related thyroid hyperplasia (Norris et al. 1975a). An explanation for the discrepancy in the octabromobiphenyl mixture NOAELs of ≤71 mg/kg/day and LOAELs of ≥8 mg/kg/day is not apparent, particularly since treatment durations were similar, methods of treatment (diet) and animal strain and sex (male) were the same, and only the NOAEL study appears to have observed the animals (for 2–18 weeks) posttreatment.

Effects on the adrenal gland also have been observed in animals exposed to PBBs. As found for thyroid as discussed above, acute-duration exposure to FireMaster FF-1 produced no changes in rat adrenal histology following a single dose as high as 1,000 mg/kg (Gupta et al. 1981; Kimbrough et al. 1978b, 1981). Dosages of 1,000 mg/kg/day FireMaster FF-1 for 2 weeks caused gross adrenal damage (darkened glands) in rats, but ≤30 mg/kg/day caused no gross or histologic damage (Gupta and Moore 1979; Gupta et al. 1981). The only information on acute-duration adrenal effects of PBBs in species other than rat is normal adrenal histology in mice at FireMaster FF-1 dosages of ≤30 mg/kg/day for 2 weeks (Gupta et al. 1981). No acute-duration studies of PBBs measure serum corticosteroid levels. In intermediate-duration studies, serum corticosterone B, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHS) decreased in rats fed ≥0.25 mg/kg/day FireMaster BP-6 for 5–7 months, but not 0.05 mg/kg/day (Byrne et al. 1988). Serum corticosterone levels and adrenal weight did not change in rats exposed to ≤6 mg/kg/day of an unspecified PBB mixture for a shorter duration of 20 days (other adrenal hormones were not evaluated) (Castracane et al. 1982). Adrenal histology was not evaluated in these studies, but no treatment-related alterations were observed in rats in other intermediate-duration studies with FireMaster BP-6 or FireMaster FF-1 (Akoso et al. 1982b; NTP 1983; Sleight and Sanger 1976; Sleight et al. 1978), except at lethal dosages (Gupta and Moore 1979), or in a chronic study with FireMaster FF-1 (NTP 1992). Necropsies of rats treated with 100–1,000 mg/kg/day FireMaster FF-1 for 4.5 weeks showed darkened adrenals (Gupta and Moore 1979). In the only rat study of octabromobiphenyl mixture that examined the adrenal gland, 2.5 mg/kg/day for 7 months produced no changes in relative adrenal weight; histology or serum corticosteroids were not evaluated (Sepkovic and Byrne 1984). Intermediate- or chronic-duration studies with FireMaster FF-1 in mice showed no adrenal histological effects at ≤3.9 mg/kg/day for up to 105 weeks (NTP 1992), ≤10 mg/kg/day for 25 weeks (NTP 1983), or ≤30 mg/kg/day for 30 days (Gupta et al. 1981).

Polybrominated Diphenyl Ethers. Plasma levels of the congener 2,2',4,4'-tetraBDE (BDE 47) and various other persistent organohalogen compounds (non-PBDEs), as well as hormone levels (free and total T_3 and T_4, thyroid stimulating hormone [TSH], free testosterone, follicle-stimulating hormone, lutenizing hormone, and prolactin), were analyzed in 110 men who consumed varying amounts of fatty

fish (0–32 meals per month) from the Baltic Sea (Hagmar et al. 2001). There was a weak negative correlation between BDE 47 and plasma TSH after age adjustment, but the congener could not explain more than 10% of the variance in TSH (r^2=0.10, p<0.001). The fact that BDE 47 could only explain 10% of the variance in TSH is not surprising due to the occurrence of PCBs and other likely similarly acting compounds in the Baltic fisherman.

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 5.2.2.7, 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). Dose-related increases in thyroid hyperplasia were reported for male Sprague-Dawley rats exposed to 80 and 800 mg/kg/day for 30 days (Norris et al. 1973, 1975b), 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, 1975b) (i.e., 77.4% decaBDE, 21.8% nonaBDE, and 0.8% octaBDE, compared to the ≥94% decaBDE composition used in the NTP studies).

Thyroid function was not tested in any of the intermediate- and chronic-duration studies of decaBDE, although an acute study in rats (Zhou et al. 2001), summarized in the following paragraph, found that a >98% pure decaBDE product (DE-83R) caused no serum changes in thyroid hormones. Studies of commercial octa- and pentaBDE mixtures and individual constituent congeners of these mixtures, also summarized below, indicate that the rat and mouse thyroid is particularly sensitive to the lower brominated BDEs. The human relevance of the animal thyroid data is unclear due to evidence indicating that the main thyroid effect, decreased serum T_4, occurs by a mechanism that is not clearly relevant to humans (see Section 5.5.2 Mechanisms of Toxicity).

Thyroid hormone levels were determined in weanling (28-day-old) female Long-Evans that were treated by gavage for 4 days with commercial mixtures of decaBDE (DE-83R) or octaBDE (DE-79) in doses of 0.3, 1, 3, 10, 30, 60, or 100 mg/kg/day, or pentaBDE (DE-71) in doses of 0.3, 1, 3, 10, 30, 100, or

300 mg/kg/day (Zhou et al. 2001). The animals were sacrificed on the day after the last exposure and evaluated for changes in serum levels of total T_4, total T_3, and TSH. DecaBDE caused no changes in levels of any of the thyroid hormones. OctaBDE induced a dose-related reduction in serum T_4 levels with statistically significant ($p<0.05$) decreases occurring at ≥10 mg/kg/day and a 70% maximum decrease compared to controls at the highest dose of 100 mg/kg/day. Serum total T_3 levels were significantly reduced at ≥60 mg/kg/day with a maximum reduction of 25% at 100 mg/kg/day. PentaBDE caused dose-related reductions in serum T_4 levels with significant decreases occurring at ≥30 mg/kg/day and an 80% maximum decrease compared to controls at the highest dose of 300 mg/kg/day. Serum total T_3 levels were significantly reduced at ≥100 mg/kg/day with a maximum reduction of 30% at 300 mg/kg/day. Neither octaBDE nor pentaBDE caused exposure-related changes in serum TSH concentrations. Benchmark dose (BMD) analysis of the octaBDE data found that the BMD and BMDL (95% lower confidence limit on the BMD) resulting in a 20% reduction in thyroid hormones (LED_{20}) were 9.25 and 5.29 mg/kg/day, respectively, for serum T_4 and 53.38 and 11.98 mg/kg/day, respectively, for serum T_3. For pentaBDE, the respective BMD and BMDL resulting in 20% reduced hormone levels were 12.74 and 6.95 mg/kg/day for serum T_4 and 32.94 and 8.56 mg/kg/day for serum T_3.

Serum levels of thyroid hormones were not evaluated in any other study of octaBDE. Thyroid hyperplasia was equivocally increased in male CD rats that were exposed to 90 mg/kg/day of octaBDE (unspecified mixture) in the diet for 28 days (IRDC 1976); incidences of slight or moderate hyperplasia were 0/5, 0/5, and 3/5 at 0, 9, and 90 mg/kg/day, respectively. CD 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 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 and were concluded to be likely compound-related. Most of the follicles in the thyroids of 4/35 males at 600 mg/kg/day and 1/35 females at 750 mg/kg/day had epithelium that was tall columnar rather than the normal cuboidal type. This effect was considered to be a very slight but probably compound-related histological change. The thyroid glands were considered within the range of normal morphology at 8 weeks postexposure in the 600/750 mg/kg/day rats as well as in the lower dose groups (IRDC 1977).

The 4-day study in weanling rats summarized above (Zhou et al. 2001) is not the only study of the commercial pentaBDE mixture DE-71 that found reduced thyroid hormone levels in maternally-exposed rats and their offspring (Zhou et al. 2002). Long-Evans rats were administered 1, 10, or 30 mg/kg/day of pentaBDE (DE-71) in corn oil by gavage from GD 6 through PND 21. Total concentrations of serum T_4 and T_3 were evaluated in dams on GD 20 and PND 22 and offspring were evaluated on GD 20 and PNDs

4, 14, 36, and 90. Serum T_4 was significantly ($p<0.05$) reduced compared to controls in dams at 30 mg/kg/day on GD 20 and PND 22 (48 and 44% reduced, respectively), and in offspring at ≥10 mg/kg/day on GD 20 (≥15% reduced) and PNDs 4 and 14 (50 and 64% reduced at 10 and 30 mg/kg/day, respectively). The effect on serum T_4 concentrations in the offspring returned to control levels by PND 36. The NOAEL for the effect on serum T_4 levels (1 mg/kg/day) was used as the basis for the acute-duration MRL for oral exposure to tetra- to heptaBDEs as indicated in the footnote to Table 5-3 and discussed in Chapter 4 and Appendix A. The BMD and BMDL resulting in a 20% reduction in serum T_4 levels were reported to be 2.36 and 0.94 mg/kg/day, respectively. There were no exposure-related changes in serum T_3 levels in the dams or offspring, or any significant effects of treatment on litter size, sex ratio, or nonneurodevelopmental measures of offspring viability and growth as discussed in Section 5.2.2.6 (Developmental Effects).

Reduced serum T_4 levels additionally occurred in adult offspring of rats that were exposed to commercial pentaBDE mixture DE-71 during gestation and lactation in two studies incompletely reported as abstracts (Taylor et al. 2002, 2003). In one study, rats were orally dosed with 0, 1, 10, or 30 mg/kg/day of DE-71 in corn oil from GD 6 through PND 21 (Taylor et al. 2002). Evaluation of the offspring at various ages (not specified) showed effects that included reduced serum T_4 levels; specific data were not reported, although the BMD and BMDL for reduced T_4 were 2.3 and 0.9 mg/kg/day, respectively. In the other study, rats were similarly administered 1-100 mg/kg/day of DE-71 on GD 6 to PND 21 (Taylor et al. 2003). Measurement of serum levels of thyroid hormones in offspring showed dose-related decreases in T_4 at 5, 30, and 100 mg/kg/day on PND 5 (73.3, 49.3, and 43.5% of controls) and PND 14 (75.6, 33.6, and 29.9% of controls). The serum T_4 decreases in both studies were accompanied by increases in hepatic metabolism (EROD, PROD, UDPGT, and glucuronidation). Other effects included neuro-behavioral and reproductive developmental changes as summarized in Sections 5.2.2.4 (Neurological Effects) and 5.2.2.5 (Reproductive Effects).

Commercial pentaBDE mixture DE-71 was tested under EPA's Endocrine Disruptor Screening Program (EDSP) using male and female rat pubertal protocols for detecting thyroid active agents (Laws et al. 2003; Stoker et al. 2003). These studies are incompletely reported as abstracts. End points in both studies included pubertal development (time to preputial separation in males and vaginal opening in females), body and reproductive tissue weights, thyroid function (serum levels of T_4, T_3, and TSH), and liver microsomal enzyme induction (EROD, PROD, and UDPGT activity). Both sexes showed delays in reproductive development that were accompanied by changes in thyroid hormone levels and hepatic metabolism. In the study with males, Wistar rats were gavaged with 0, 3, 30, or 60 mg/kg/day doses in

corn oil for 5 days on PNDs 24–28, or for 31 days on PNDs 23–53 (Stoker et al. 2003). Pubertal development was delayed as shown by significant increases in the age of preputial separation at 30 and 60 mg/kg/day (2.0- and 2.3-day delay, respectively). Seminal vesical and ventral prostate weights were reduced at 60 mg/kg/day with no significant changes in testis or epididymal weights or serum testosterone levels. Thyroid hormone measurements showed reductions in serum T_4 at 30 and 60 mg/kg/day (74 and 81% lower than controls) after 5 days of exposure, serum T_4 at 3, 30, and 60 mg/kg/day (20, 80, and 86% lower) after 31 days, and serum T_3 at 30 and 60 mg/kg/day (25 and 20% lower) after 31 days, and increased serum TSH at 30 and 60 mg/kg/day (64 and 113% higher than controls) after 31 days. Hepatic effects included increased relative liver weight and microsomal EROD, PROD, and UDPGT activity at 30 and 60 mg/kg/day at both time points. In the study with females, Wistar rats were gavaged with DE-71 in corn oil in doses of 0, 3, 30, or 60 mg/kg/day for 5 days on PNDs 22–26, or for 20 days on PNDs 22–41 (Laws et al. 2003). The 60 mg/kg/day dose caused a small but statistically significant delay in the age of vaginal opening (1.8-day delay compared to controls). Other effects included a significant decrease in serum T_4 at ≥30 mg/kg/day at both time points, a linear trend for increased TSH (1.6-fold increase at 60 mg/kg/day) after 20 days of exposure, and increased relative liver weight and microsomal EROD, PROD, and UDPGT activity at ≥30 mg/kg/day after at both time points. The results of both studies indicate that the changes in thyroid hormone levels were related to induction of liver metabolic enzymes.

End points assessed in a comprehensive 90-day feeding study of a commercial pentaBDE (DE-71) mixture in male and female Sprague-Dawley rats included serum T_3 and T_4 levels (TSH not measured) and thyroid histology (WIL Research Laboratories 1984). Effects observed in both sexes included significantly reduced plasma T_4 levels at ≥10 mg/kg/day and increased follicular cell hyperplasia at 100 mg/kg/day. Incidences of follicular cell hyperplasia in the 0, 2, 10, and 100 mg/kg/day dose groups of this study were 0/10, 2/10, 2/10, and 5/10 in males and 0/10, 0/10, 1/10, and 4/10 in females. 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. Thyroid hyperplasia was equivocally increased in male CD rats that were exposed to 90 mg/kg/day of pentaBDE (unspecified mixture) in the diet for 28 days (IRDC 1976). Incidences of slight or moderate hyperplasia in the 0, 9, or 90 mg/kg/day dose groups of this study were 0/5, 1/5, and 3/5 males, respectively; no increases were seen in females.

Serum total T_4 concentrations were significantly reduced in female C57BL/6J mice that were given a single gavage dose of commercial pentaBDE mixture DE-71 as low as 0.8 mg/kg (lowest tested dose)

(Fowles et al. 1994). Reductions in serum T_4 were found in four of five dose groups (i.e., 0.8, 4, 20, and 500 mg/kg, but not 100 mg/kg), but did not occur in a dose-dependent manner. This lack of dose-dependency might be explained by the number of animals per group, which was relatively small (n=6). Another single-dose gavage study, this time in rats (Hakk et al. 2002), reported transient increases in total T4 plasma levels, but the small (n=3) number of animals per group and the lack of reported tests of statistical significance make the interpretation of the findings unclear. Evaluation of mice exposed to 18, 36, or 72 mg/kg/day of pentaBDE by daily gavage for 14 days showed significantly reduced total and free T_4 levels at ≥18 mg/kg/day (Fowles et al. 1994). Serum T_3 and TSH levels and thyroid histology were not evaluated in these single-dose and 14-day mouse studies. Serum levels of total and free T_4 were also reduced in female Sprague-Dawley rats and female B6C3F1 mice that were exposed to ≥18 mg/kg/day of the commercial pentaBDE mixtures Bromkal 70 or Bromkal 70-5 DE, or the congener 2,2',4,4'-tetraBDE (BDE 47), by daily gavage for 14 days (Darnerud and Sinjari 1996; Hallgren and Darnerud 1998; Hallgren et al. 1998, 2001). Exposure to the mixtures or congener did not cause decreases in serum T_4 at doses ≤6 mg/kg/day, or any changes in serum TSH or thyroid histology at any tested level (≤36 mg/kg/day) in either species. The decreases in serum T_4 were associated with reduced *ex vivo* binding of T_4 to the plasma thyroid hormone transporter protein transthyretin, as well as with induction of hepatic microsomal enzymes (EROD, MROD, PROD, and UDPGT) (Hallgren and Darnerud 1998). 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 a commercial pentaBDE mixture (DE-71) in doses of 18, 36, or 72 mg/kg/day by daily gavage for 14 days (Fowles et al. 1994). As reported in a study abstract (Kuriyama and Chahoud 2003), serum levels of testosterone and LH were unchanged in adult male offspring of Wistar rats that were maternally exposed to a single 60 or 300 µg/kg oral dose of the penta congener BDE 99 on day 6 of gestation. Although there were no effects on these male reproductive hormones, spermatid and sperm numbers were reduced as summarized in Section 5.2.2.6 Developmental Effects.

Dermal Effects.

Polybrominated Biphenyls. Limited human data from an epidemiological study provide suggestive evidence that oral exposure to PBBs may produce skin disorders in humans, but do not provide information regarding dose-response relationships. Symptoms of skin disorders (rashes, acne, increased sensitivity to the sun, darkening or thickening of the skin, discoloration or deformity of fingernails or toenails, slow healing of cuts) were reported with greater frequency in a group of 406 Michigan residents probably exposed to PBBs than in a group of 153 likely unexposed residents, but no association was evident between serum PBB levels and prevalence of skin disorders (Anderson et al. 1978c). In a medical

history survey study conducted in 1976, symptoms of skin disorders (peeling and scaling, erythema, hair loss, increased nail growth, increased sweating) experienced during the previous 3 years were reported at higher prevalence rates in a group of 321 Michigan residents from quarantined farms and in a group of 177 nonquarantined farm residents than in a group of 149 nonexposed Wisconsin residents (Chanda et al. 1982). Physical examination of the combined group of Michigan residents revealed alopecia in 4% of the subjects compared to no occurrence of alopecia in the control group.

In animals, repeated exposures to PBBs produced characteristic dermal changes in certain species, particularly monkeys, but generally not in haired rodents. No exposure-related histological changes were observed in the skin, salivary glands, or eyes of rats administered a single dose of 200 mg/kg FireMaster FF-1 and observed for 18–22 months (Kimbrough et al. 1981). Rats and mice exposed to ≤30 mg/kg/day FireMaster FF-1 for 2 weeks showed no histological alterations in pinnae, ear canals, or salivary glands, but examination of skin was not performed (Gupta et al. 1981). In intermediate- and chronic-duration studies, rats and/or mice exposed to FireMaster FF-1 or FireMaster BP-6 dosages of ≤30 mg/kg/day by gavage for 30 days (Gupta et al. 1981), ≤10 mg/kg/day in the diet for 30 days (Akoso et al. 1982a), ≤10 mg/kg/day by gavage for 25 weeks (NTP 1983), or ≤3.9 mg/kg/day in the diet for up to 105 weeks (Chhabra et al. 1993; NTP 1992) showed no histopathological changes in skin, pinnae, ear canals, or salivary glands. Xerophthalmia (extreme dryness of the conjunctiva, with keratinization of epithelium following chronic conjunctivitis) was observed in rats after 82 days of dietary exposure to 5 mg/kg/day FireMaster BP-6 (Darjono et al. 1983). Alopecia, loss of eyelashes, generalized subcutaneous edema, dry scaly skin, and periorbital edema developed in monkeys exposed to FireMaster FF-1 in dosages of ≥0.73 mg/kg/day for ≥25 weeks (two animals) or 18 mg/kg/day for 137 days (one animal) (Allen et al. 1978; Lambrecht et al. 1978). Histological examination, performed only in the monkey exposed to 18 mg/kg/day, showed atrophy and squamous metaplasia of sebaceous glands and keratinization of hair follicles (Allen et al. 1978). Dermatosis on the ventral surface was a clinical sign in two of four swine administered 8 mg/kg/day FireMaster FF-1 for 16 weeks (Ku et al. 1978). No additional information was reported on the dermatosis (a nonspecific term used to denote any cutaneous lesion or group of lesions), and histologic examinations were not completed.

Polybrominated Diphenyl Ethers. No studies were located regarding dermal effects in humans after oral exposure to PBDEs.

Histopathological examinations showed no dermal changes in rats following dietary exposure to ≤750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977) or ≤100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Ocular Effects.

Polybrominated Biphenyls. No studies were located regarding ocular effects in humans after oral exposure to PBBs.

Occasional eye discharge was observed in pregnant cows that were administered 67 mg/kg/day of FireMaster BP-6 in capsules for up to 60 days (Moorhead et al. 1977). Necropsy following sacrifice between days 33 and 66 because of impending death due to poor health showed hyperkeratosis of the eyelids and squamous metaplasia with keratin cysts in the tarsal glands in five of six animals. No ocular effects were observed in other cows treated with ≤0.65 mg/kg/day and examined 1 or 140 days following the end of treatment. Histological changes were not observed in the eyes of rats exposed to FireMaster FF-1 for 2 weeks (Gupta et al. 1981), or in rats and mice treated by gavage (NTP 1983) or fed FireMaster FF-1 for up to 105 weeks (Chhabra et al. 1993; NTP 1992).

Polybrominated Diphenyl Ethers. 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 ≤1.0 mg/kg/day of 77.4% decaBDE (containing 21.8% nonaBDE and 0.8% octaDBE) for 2 years (Kociba et al. 1975; Norris et al. 1975b), ≤750 mg/kg/day of octaBDE for 13 weeks (IRDC 1977), or ≤100 mg/kg/day of pentaBDE for 90 days (WIL Research Laboratories 1984).

Body Weight Effects.

Polybrominated Biphenyls. No studies were located regarding body weight effects in humans after oral exposure to PBBs.

Reduced body weight was observed in various species following acute oral administration of relatively high doses of PBBs; this effect is most evident with repeated exposure. In general, decreases in food and water intake are not sufficient to account for decreases in body weight. Effects on body weight can be quite pronounced following intermediate- and chronic-duration exposure, constituting a wasting

syndrome manifested by weight loss and depletion of body fat. In acute-duration studies with rats, a single 1,000 mg/kg dose of FireMaster FF-1 caused decreased weight gain during the following 2 years (Kimbrough et al. 1981), and a single 800 mg/kg dose of FireMaster BP-6 during pregnancy caused maternal weight loss (Beaudoin 1977). Single FireMaster doses of 400 (BP-6) or 500 (FF-1) mg/kg/day did not affect body weight in rats (Beaudoin 1977; Kimbrough et al. 1980). Administration of 1,000 mg/kg/day FireMaster FF-1 or 130 mg/kg/day FireMaster BP-6 for 2 weeks produced decreased weight gain or weight loss in rats and mice, respectively (Corbett et al. 1978; Fraker 1980; Fraker and Aust 1978; Gupta and Moore 1979), but 5 mg/kg/day FireMaster BP-6 for 10 days had no effect on body weight in rats (Raber and Carter 1986). A single ≤2,000 mg/kg dose or two daily 3,000 mg/kg doses of octabromobiphenyl mixture had no effect on body weight gain in rats observed for the following 14–28 days (Lee et al. 1975a; Norris et al. 1975a). No changes in body weight were produced in rats exposed to ≤71 mg/kg/day octabromobiphenyl mixture for 2 weeks (Lee et al. 1975b; Waritz et al. 1977). In intermediate-duration studies, decreased body weight gain and/or weight loss has been observed in rats at dosages as low as 3 mg/kg/day FireMaster FF-1 for 6 months (Luster et al. 1980; NTP 1983), 5 mg/kg/day FireMaster BP-6 for 1–3 months (McCormack et al. 1978; Sleight et al. 1978), and 30–50 mg/kg/day FireMaster FF-1 for 4.5–5 weeks (Gupta and Moore 1979; Sleight and Sanger 1976). FireMaster FF-1 dosages ≥100 mg/kg/day for 4.5 weeks (lethal doses) caused weight loss and emaciation in rats (Gupta and Moore 1979). Final body weights were decreased ≥11–28% in rats exposed to 0.5 or 1.5 mg/kg/day FireMaster FF-1 for up to 104 weeks (NTP 1992). No body weight changes were observed in rats fed a decabromobiphenyl mixture at dosages as high as 100 mg/kg/day for 13 weeks (Millischer et al. 1980).

In mice exposed to FireMaster FF-1, estimated dosages of 10 mg/kg/day for 25 weeks (NTP 1992) and 21.7 mg/kg/day for 6 weeks (Loose et al. 1981) decreased the rate of weight gain. Chronic exposure to ≤3.9 mg/kg/day FireMaster FF-1 for up to 105 weeks, however, did not produce adverse effects on mouse body weight (NTP 1992). Guinea pig, mink, and monkey seem to be particularly sensitive species, as indicated by pronounced weight loss in guinea pigs from ingestion of 4 mg/kg/day of unspecified PBBs for 30 days (Sleight and Sanger 1976), decreased weight gain in mink at FireMaster FF-1 dosages as low as 0.39 mg/kg/day with weight loss at 1.86 mg/kg/day (Aulerich and Ringer 1979; Ringer et al. 1981), and weight loss in monkeys at FireMaster FF-1 dosages as low as 0.73 mg/kg/day for 25–50 weeks (Allen et al. 1978; Lambrecht et al. 1978). Monkeys that ingested an estimated FireMaster FF-1 dosage of 0.012 mg/kg/day for 66 weeks lost weight (Lambrecht et al. 1978). Food intake and body weight gain were reduced in pregnant cows after 4 and 20 days administration of 67 mg/kg/day FireMaster BP-6 in capsules (Moorhead et al. 1977). This dosage was lethal because death was impending between days 33

and 66 (treatment duration was 60 days). There were no effects on food intake or body weight in cows treated with ≤0.65 mg/kg/day and observed 1 or 140 days following the end of treatment.

Polybrominated Diphenyl Ethers. No studies were located regarding body weight effects in humans after oral exposure to PBDEs.

DecaBDE had no effect on body weight gain in rats and mice that were exposed to dietary doses of ≤16,000 and ≤19,000 mg/kg/day, respectively for 14 days, ≤8,000 and ≤9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986), or ≤2,550 and ≤7,780 mg/kg/day, respectively, for 103 weeks (NTP 1986). Dietary ingestion of 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaDBE) 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, 1975b).

OctaBDE did not affect body weight in rats exposed to dietary doses of ≤90 mg/kg/day for 28 days (IRDC 1976), although exposure to ≥600 mg/kg/day for 13 weeks caused ≥12% decreases in weight gain (IRDC 1977). There were no pentaBDE-related body weight changes in rats exposed to ≤90 mg/kg/day for 28 days (IRDC 1976) or mice exposed to ≤72 mg/kg/day pentaBDE for 14 days (Fowles et al. 1994), although the rate of weight gain was reduced in rats exposed to ≤100 mg/kg/day pentaBDE for 90 days (WIL Research Laboratories 1984).

5.2.2.3 Immunological and Lymphoreticular Effects

Polybrominated Biphenyls. Numerous reports have been published regarding the immunological competence of individuals exposed to PBBs in the Michigan feed contamination episode. Due to the relatively high number of published reports and to the fact that often different groups of investigators appear to have examined the same cohort, only representative studies are discussed below.

Immunological parameters were compared between a group of 45 adult Michigan dairy farmers and their families who were exposed for periods ranging from 3 months to 4 years and two groups of control individuals not known to have been exposed to PBBs (Bekesi et al. 1978, 1979). In 27 of the 45 Michigan subjects, the peripheral blood lymphocytes responded within a normal range to phytohemagglutinin (PHA) and PWM mitogen-induced lymphoblastogenesis, but had reduced reactivity in mixed leukocyte cultures relative to controls. In the remaining 18 Michigan subjects, the response to PHA and PWM and the reactivity to mixed leukocyte cultures was significantly reduced ($p<0.00001$)

relative to controls. Assays for membrane markers of peripheral blood lymphocytes showed significant reductions in markers in the Michigan subpopulation with abnormal lymphoblastogenesis. Both Michigan subpopulations had a significant increase in the number of lymphocytes without detectable surface markers, relative to controls. The number of markers for monocytes was not significantly different among the groups studied. There were no significant differences in serum PBB levels between the two Michigan subsets. No consistent correlation could be demonstrated between lymphocyte function and PBB plasma concentration.

Reexamination of a group of 40 Michigan farmers 5 years after the first examination (Bekesi et al. 1985; Roboz et al. 1985) showed that the number of T-lymphocytes and the lymphocyte response to stimulation with PHA were altered to the same extent reported 5 years earlier (Bekesi et al. 1978).

In a similar study, Michigan subjects were classified into three groups according to their serum PBB levels: high (>300 ppb), low (<1–11 ppb), and unexposed (controls) (Silva et al. 1979). The percentage of subjects that complained of recurrent infection was similar in the two exposed groups (about 20%). The total leukocyte count, percentage of lymphocytes, and percentage of subpopulations of T- and B-lymphocytes were similar among the three groups. Mean spontaneous lymphocyte transformation and lymphocyte mitogenic responsiveness to stimulation with three different mitogens were not significantly different among the three groups. Furthermore, there was no correlation between a poor mitogenic response and low numbers of T-lymphocytes (Silver et al. 1979).

It was also reported at this time that Michigan farm residents with the highest exposure to PBB had significantly elevated levels of IgM, IgA, and IgG relative to Wisconsin dairy farm residents (Bekesi et al. 1985). Cluster analysis of several immunological parameters performed for husbands and wives showed, according to the investigators, significant correlations for surface markers, lymphocyte functions, and IgG values (no correlation coefficient was >0.337). This finding was interpreted as supporting a common dietary source for the immune dysfunction rather than a genetic predisposition (Bekesi et al. 1985).

In yet another report, Michigan farmers reported a higher rate of infections (11%) than a group of chemical workers exposed to PBB (3%) (Stross et al. 1981), however, average PBB levels in serum, bile, and fat were higher in the chemical workers than in the farmers. When the patients were divided according to their PBB fat level into high, moderate, and low, there was an equal distribution of abnormal physical, laboratory, and diagnostic findings among the groups.

The immunological effects of the commercial PBB mixtures FireMaster FF-1 and FireMaster BP-6 have been examined in rats, mice, guinea pigs, dogs, and pigs, but in many cases, the most sensitive immunological end points were not examined. In all but two studies, the animals were exposed for an intermediate duration, and many studies administered the PBBs by gavage (exceptions noted below). Additionally, most studies were conducted in rats, a species that may be a poor model for investigating dioxin-like effects on the adult immune system. Identification of the most sensitive species is further complicated by the fact that not all studies examined the same end points, although limited data suggest that guinea pigs may be particularly sensitive. Immunological effects in animals, attributed to exposure to PBBs *in utero* or through lactation, are discussed in Section 5.2.2.6.

Limited data exist regarding immunological effects of PBBs in animals following acute oral exposure. No histopathological alterations were observed in the spleen and thymus of rats treated with a single dose of 1,000 mg/kg FireMaster FF-1 and observed for 2 years (Kimbrough et al. 1978b). A similar lack of effects in the thymus, spleen, and lymph nodes was reported in rats and mice treated for 2 weeks with up to 30 mg/kg/day FireMaster FF-1 (Gupta et al. 1981). However, mice treated with ≈130 mg/kg FireMaster BP-6 in the diet for 14 days were incapable of mounting an antibody-mediated response following immunization with sheep red blood cells (SRBC) (Fraker 1980; Fraker and Aust 1978). This treatment also reduced absolute thymus weight by 88% and caused high lethality in mice.

Numerous intermediate-duration studies have examined the immunological effects of PBBs in rats. For example, treatment of rats with FireMaster FF-1 for 25 weeks significantly increased absolute and relative spleen weight at ≥1 mg/kg/day and significantly decreased absolute and relative thymus weight at ≥0.3 mg/kg/day (NTP 1983). Nevertheless, no histopathological alterations were observed in these organs and in lymph nodes with doses of up to 10 mg/kg/day (NTP 1983). Similar results were reported in rats treated with 30 mg/kg/day FireMaster FF-1 for 4–5 weeks (Gupta and Moore 1979; Gupta et al. 1981), but a dose of 100 mg/kg/day caused thymic atrophy and necrosis of lymphoblasts (Gupta and Moore 1979). A much smaller dose, 0.5 mg/kg/day FireMaster BP-6 in the diet for 150 days, reportedly caused moderate lymphoid depletion in thymus and spleen (Rezabek et al. 1989). In the only chronic rat study, splenic fibrosis developed following exposure to 1.5 mg/kg/day, but not 0.5 mg/kg/day, FireMaster FF-1 in the diet for up to 104 weeks (NTP 1992). Treatment of rats for 5 weeks with 30 mg/kg/day FireMaster FF-1 significantly reduced the *in vitro* lymphocytic response to stimulation with two out of three mitogens and thymus and spleen weight (Luster et al. 1978). Relative thymus weight was reduced at 3 mg/kg/day; however, treatment with the test material did not alter the production of antibodies 4 days after immunization with SRBC. The same group of investigators reported significantly decreased

lymphoproliferative responses to mitogens or allogenic cells in rats following treatment with 3 mg/kg/day FireMaster FF-1 for 6 months (Luster et al. 1980); a dose of 1 mg/kg/day was without effect. It must be mentioned, however, that in the studies conducted by Luster and co-workers, doses ≥3 mg/kg/day FireMaster, reduced body weight by ≥15% in the animals, suggesting that PBBs can affect the immune system, but only at dose levels that produce overt toxicity.

Mice treated for 30 days with FireMaster BP-6 in the diet at levels of approximately ≥1.3 mg/kg/day had a significantly reduced antibody-mediated response to SRBC ($p<0.001$) (Fraker 1980; Fraker and Aust 1978). Absolute thymus weight was significantly reduced ($p<0.01$) relative to controls with all dose levels tested (0.13, 1.3, 13 mg/kg/day). Delayed-type hypersensitivity was not altered by PBB treatment. Corticosterone levels in plasma were elevated in treated mice relative to controls, but not elevated enough to be responsible for the immunological findings. No histopathological effects were observed in the thymus, spleen, or lymph nodes of mice treated with 30 mg/kg/day FireMaster BP-6 for 4–5 weeks, but relative thymus weight was temporarily decreased (Gupta et al. 1981). Other studies in mice reported increased lethality ($p<0.05$) after bacterial inoculation in groups treated with 10 mg/kg/day FireMaster FF-1 for 6 months (Luster et al. 1980) and increased lethality ($p<0.05$) due to challenge with *Salmonella thyphosa* lipopolysaccharide after 3 or 6 weeks of dietary exposure to ≈21.7 mg/kg/day FireMaster FF-1 (Loose et al. 1981). No histopathological changes were observed in the spleen, thymus, and lymph nodes of mice treated with up to 10 mg/kg/day FireMaster FF-1 for 25 weeks (NTP 1983), although 3.9 mg/kg/day for up to 105 weeks caused increased splenic hematopoiesis (NTP 1992).

Guinea pigs administered 0.4 mg/kg/day FireMaster BP-6 in the diet for 45 days exhibited a significant reduction ($p<0.01$) in tetanus-antitoxin titers following injection of tetanus toxoid (Vos and van Genderen 1973, 1974). A dose 5 times higher caused marked thymus atrophy, splenic effects (marked depletion of the follicles and periarteriolar lymphocyte sheaths), and lethality. Pregnant sows fed a diet that provided approximately 2.5 mg/kg/day FireMaster BP-6 for a total of 12 weeks (including part of gestation and lactation) showed a significantly reduced ($p<0.05$) lymphocyte response to stimulation with PHA and PWM mitogens relative to controls (Howard et al. 1980); a dose of 1.25 mg/kg/day was without effect. However, PBB treatment did not affect bactericidal activity of whole blood towards *Escherichia coli* and *Staphylococcus aureus*.

Two cows gavaged with daily doses of 67 mg/kg/day of an unspecified PBB mixture for 38 consecutive days showed minimal alterations in tests of humoral and cell immunity relative to a group of 54 unexposed animals (Kateley et al. 1982). The concentration of PBBs in tissues from these two cows

reached 1,000 mg/kg, and they became moribund and were later sacrificed. A similar lack of significant immunological effects was reported in the same study for 58 cows from contaminated farms in Michigan that had PBB body burdens ranging from 0.02 to 24 mg/kg for at least 2 years (Kateley et al. 1982). Cows that received gavage doses of ≤0.65 mg/kg/day FireMaster PB-6 for 60 days showed no histopathologic alterations in the thymus or spleen (Moorhead et al. 1977). However, doses of 67 mg/kg induced thymic involution and atrophy, and were nearly lethal.

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 5-2 and plotted in Figure 5-2.

Polybrominated Diphenyl Ethers. No studies were located regarding immunological effects in humans after oral exposure to PBDEs. No effects on pokeweed mitogen-stimulated DNA proliferation or IgG immunoglobulin synthesis were found in human lymphocytes exposed to 2,2',4,4'-tetraBDE (BDE 47) or 2,2',3,4,4'-pentaBDE (BDE 85) *in vitro* (Fernlof et al. 1997).

Limited information is available on effects of acute-duration exposure to pentaBDE on immunologic function in animals. A single gavage dose of 0.8–500 mg/kg of pentaBDE (DE-71) did not effect the plaque-forming splenic cell (PFC) antibody response to injected SRBC in mice (Fowles et al. 1994). Mice that were given 18, 36, or 72 mg/kg/day doses of pentaBDE (DE-71) by gavage for 14 days had significantly reduced antibody response to SRBC (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 natural killer cell (NKC) activity to murine YAC-1 target cells; NKC activity was not evaluated in the single dose study. A 14-day study of another pentaBDE mixture (Bromkal 70-5 DE) was conducted in which mice and rats were administered 18 or 36 mg/kg/day by 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. The effects in both 14-day studies of commercial pentaBDE mixtures occurred only at the highest dose and are possibly due to contamination with PBDDs and PBDFs. Mice that were similarly tested with 18 mg/kg/day of the congener 2,2’,4,4’-tetraBDE (BDE 47) for 14 days had significantly reduced numbers of total splenocytes as well as CD4+, CD8+, and CD45R+ cells in spleen (Darnerud and Thuvander 1998). This was the only dose level of a single congener used in the mice. Rats were not evaluated.

Histopathological examinations of spleen, thymus, lymph node and/or bone marrow tissues showed no effects of repeated dietary administration in intermediate-duration studies in rats exposed to ≤8,000 mg/kg/day decaBDE for 13 weeks (NTP 1986), in mice exposed to ≤9,500 mg/kg/day decaBDE for 13 weeks, in rats exposed to ≤750 mg/kg/day octaBDE for 13 weeks (IRDC 1977), or in rats exposed to ≤100 mg/kg/day pentaBDE for 90 days (WIL Research Laboratories 1984). Chronic ingestion of decaBDE caused lesions in the spleen of rats exposed to ≥1,200 mg/kg/day (splenic hematopoiesis in males) or 2,240 mg/kg/day (splenic fibrosis and lymphoid hyperplasia in females) for 103 weeks (NTP 1986).

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 5-3 and plotted in Figure 5-3.

5.2.2.4 Neurological Effects

Polybrominated Biphenyls. Neurological symptoms were reported frequently by Michigan residents during a 3–4-year period following the 1973 PBB contamination episode, but positive associations between serum PBB levels and frequency of neurological symptoms were not found in several studies. In an epidemiological study conducted by the U.S. Center for Disease Control and the Michigan Department of Public Health, fatigue was reported more frequently by several putatively exposed groups including 2,148 residents of farms quarantined for PBB contamination (36.4% prevalence rate), 1,421 recipients of food from contaminated farms (32.4%), 252 chemical workers involved in PBB manufacturing or distribution (22.0%), and 331 residents of farms with low levels of PBB contamination (41.4%), than by a small (60 persons) unexposed control group (15.8%); however, no positive association was apparent between serum levels of PBB and prevalence rates for any reported symptom (Landrigan et al. 1979). Neurological symptoms, including marked tiredness and decrements in the capacity for intellectual and physical work, also were reported with greater frequencies in groups of farmers and residents of Michigan likely to have consumed farm products contaminated with PBB, than in groups of unexposed Wisconsin farmers; however, serum PBB levels were not positively associated with prevalence rates for any symptom including neurological symptoms, nor with performance on neurobehavioral tests for a subset of this population (Anderson et al. 1978c, 1979; Valciukas et al. 1978, 1979). In a 1976 medical history questionnaire study of 342 Michigan children likely to have been exposed to PBBs and 72 unexposed children from Wisconsin, the number of subjectively reported symptoms of ill health, including several symptoms of neurological effects, did not increase with increasing serum PBB levels (assayed in 1976),

but rather decreased; general neurological examinations did not reveal a pattern of abnormality among the Michigan children (Barr 1980). Subjectively reported symptoms of neurological effects including weakness, fatigue, difficulty in concentrating, and irritability were prevalent in a group of 23 farmers involved in the Michigan PBB contamination episode, but tests of intelligence, memory, and nerve conduction velocity failed to demonstrate abnormalities. In addition, a group of 28 workers involved in the manufacture or distribution of PBB displayed higher average serum PBB levels than the farmers (48 ppb versus 14 ppb), but did not report a prevalence of symptoms of neurological effects (Stross et al. 1981). In a study of 21 Michigan residents who consumed PBB-contaminated food and had lingering medical complaints and 21 volunteer control subjects with putative low-dose exposure to PBB, no positive association was observed between PBB levels in fat tissue and performance in a battery of neuropsychological tests (Brown and Nixon 1979). In general, the findings of the epidemiological and clinical studies of people exposed to PBBs in Michigan are inconclusive; they do not clearly demonstrate or eliminate the possibility of an association between PBB oral exposure and the occurrence of neurological effects.

Limited data indicate that orally (gavage) administered PBBs can produce neurological effects in rats. FireMaster FF-1 at 10 mg/kg/day (3 days/week) for 8 weeks did not alter the performance of rats in tests of operant behavior, but decreased motor activity, grip strength, and startle responsiveness observed in rats following administration of ≤10 mg/kg/day for 6 months or 30 mg/kg/day for 4 weeks (Tilson and Cabe 1979). Motor activity changes were also observed in rats administered doses of FireMaster FF-1 as low as 1 mg/kg/day for 4 weeks (Geller et al. 1979). In this experiment, neither learning nor performance of a simple discrimination task was affected by 1, 3, or 6 mg/kg/day dosage levels, but increased motor activity was observed at 1 mg/kg/day. No changes were apparent at 3 mg/kg/day and decreased motor activity was apparent at 6 mg/kg/day, compared with controls. Weakness of the hind limb was noted in rats treated with 10 mg/kg/day FireMaster FF-1 for 6 months compared with control rats (Cabe and Tilson 1978). Histological examination of brain and/or spinal nerve tissue found no FireMaster FF-1-related alterations in rats or mice administered up to 10 mg/kg/day for 25 weeks (NTP 1983) or 3.9 mg/kg/day for up to 105 weeks (NTP 1992).

Neurodevelopmental effects were assessed in offspring of mice that were treated with 3 or 10 mg/kg/day doses of FireMaster (FF-1) in corn oil by gavage on every other day during gestation and until weaning of the offspring at 21 days of age (Tilson 1992). Acoustic startle response, negative geotaxis, motor activity, and body weight were measured in 8 pups/sex/dose at 30, 60, and 120 days of age. Tests for avoidance learning and neurochemistry were performed on one pup/sex/dose at 30 days of age and on the remaining

animals at 120 days of age. Reductions in acoustic startle responsiveness and negative geotaxis latency were observed at 10 and ≥3 mg/kg/day, respectively, in both sexes at 30 and 60 days of age. Motor activity was decreased in 10 mg/kg/day females at 120 days of age. The learning tests showed increased avoidance response latencies at 30 and 120 days of age in both sexes at ≥3 mg/kg/day, but no effect on acquisition or retention. Neurochemical measurements included serotonin and metabolites, dopamine and metabolites, and norepinephrine in the cortex, hippocampus, and striation; the only effect observed was a decrease in dopamine concentration in the striation of both males and females at 120 days of age.

Postnatal neurodevelopmental effects were also evaluated in offspring of rats that received 0.2 or 2 mg/kg/day doses of FireMaster BP-6 dissolved in peanut butter from day 6 of gestation through day 24 postpartum and observed until postnatal day 60 (Henck et al. 1994). Multivariate analysis of variance of neurodevelopmental end points showed significant PBB-related effects for acquisition of forward locomotion, cliff avoidance, cage emergence, and open-field activity in male and female offspring of the rats exposed to 2 mg/kg/day. The most prominent behavioral effects were delays in acquisition of forward locomotion and suppressed open-field activity. Other effects in the offspring included reduced crown-rump length and body weight at birth and reduced postnatal body weight as summarized in Section 5.2.2.6 (Developmental Effects).

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 5-2 and plotted in Figure 5-2.

Polybrominated Diphenyl Ethers. No studies were located regarding neurological effects in humans after oral exposure to PBDEs.

A limited amount of information is available on neurological effects of PBDE mixtures in animals. None of the commercial decaBDE, octaBDE, and pentaBDE products have been screened for neurotoxicity using comprehensive test batteries that typically include functional observational, motor activity, and neuropathology evaluations.

There were no indications of neurotoxicity for commercial decaBDE, as assessed by overt clinical signs and nervous system histopathology, in rats and mice in lifetime feeding studies of the 94–97% pure mixture (NTP 1986). These animals were exposed to high dietary levels of the decaBDE product (estimated doses as high as 2,550 mg/kg/day in rats and 7,780 mg/kg/day in mice) for 103 weeks. There also 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, or ≤8,000 and ≤9,500 mg/kg/day, respectively, for 13 weeks (NTP 1986). Histological examinations of the nervous system were not performed in these 14-day and 13-week studies. 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.

Three studies, reported in limited detail as abstracts, assessed neurobehavioral development in offspring of rats that were perinatally exposed to commercial pentaBDE mixture DE-71 in corn oil by gavage on GD 6 through PND 21, in dose ranges of 1–30 mg/kg/day (Taylor et al. 2002), 1–100 mg/kg/day (Taylor et al. 2003), or 5–100 mg/kg/day (MacPhail et al. 2003). Behavioral end points included motor activity (MacPhail et al. 2003; Taylor et al. 2002, 2003), auditory startle (Taylor et al. 2002, 2003), and fear conditioning (Taylor et al. 2003). Evaluation of adult offspring showed no alterations in motor or sensory development as assessed by overall levels of motor activity, within-session habituation of activity, or auditory startle response, although tests of fear conditioning revealed decreases in cue-based (but not context-based) performance. The lack of motor activity changes in the DE-71 mixture-exposed rats differs from attenuating effects on habituation of motor activity observed in mice exposed to individual PBDE congeners, as summarized below.

Neurobehavioral effects of postnatal or perinatal exposure to individual PBDE congeners have been evaluated in a number of mouse studies. As detailed below, these studies collectively indicate that the developing nervous system is a target of particular congeners, as shown by mild performance impairments in tests of spontaneous motor behavior and learning and memory in adult mice. The aberrations in spontaneous behavior generally appeared to persist and worsen with age, and were induced during a defined critical phase of neonatal brain development.

In a series of congener studies using similar experimental designs, single doses of 2,2',4,4'-tetraBDE (BDE 47), 2,2',4,4',5-pentaBDE (BDE 99), 2,2',4,4',5,5'-hexaBDE (BDE 153), or 2,2',3,3',4,4',5,5',6,6'-decaBDE (BDE 209) were postnatally administered to male NMRI mice in a 20% fat emulsion vehicle by gavage (Eriksson et al. 1999, 2001a, 2002a; Viberg et al. 2001a, 2001b, 2002a, 2002b, 2003a). Evaluations included spontaneous motor behavior at 2, 4, and/or 6 months of age (BDEs 47, 99, 153, and 209), and Morris swim maze performance at 5 or 6 months of age (BDEs 47, 99, and 153). The spontaneous behavior test measured open-field locomotion (horizontal movements), rearing (vertical movements), and total activity (all types of vibrations within a test cage). The measurements were

performed during three consecutive 20-minute periods to assess habituation, defined as a decrease in the motor activities in response to diminishing novelty of the test chamber over 60 minutes. The water maze test assessed spatial learning ability and memory by evaluating the ability to locate a submerged platform. Cognition was tested by providing visual cues to find and remember the location of a platform submerged in a pool of water. The decrease in time needed to locate the platform over a total of 20 trials in a 4-day acquisition period was used as a measure of learning ability. On day 5, the platform was relocated and the decrease in time needed to find the relocated platform over five trials was used to assess relearning ability.

In the study with 2,2',3,3',4,4',5,5',6,6'-decaBDE (BDE 209), the mice were dosed once with 0, 2.22, or 20.1 mg/kg on PNDs 3 or 19, or 0, 1.34, 13.4 or 20.1 mg/kg on PND 10, and evaluated for spontaneous behavior at 2, 4, and 6 months of age (Viberg et al. 2001b, 2003a). A non-habituating behavior profile was observed in the mice exposed on PND 3, but not in those exposed on PND 10 or 19. In particular, following exposure on PND 3, aberrations in spontaneous motor behavior were manifested at 6 months of age in the mice exposed to 2.22 mg/kg, and at 2, 4, and 6 months of age in those exposed to 20.1 mg/kg. The aberrations were more pronounced in the older animals, indicating that the effects worsened with age. The findings are consistent with tissue distribution data in this study, suggesting that brain uptake of ^{14}C-BDE 209 was more efficient in the younger animals and that the amount of radioactivity reaching the brain increased with time (see Section 5.4.2). Additional information on the behavioral results of this study, including quantitative data, was not reported.

In the study with 2,2',4,4',5,5'-hexaBDE (BDE 153), mice were dosed once with 0, 0.45, 0.9, or 9.0 mg/kg on PND 10, and evaluated for spontaneous behavior at 2, 4, and 6 months of age, and swim maze performance at 6 months of age (Viberg et al. 2001b, 2002a). Non-habituating behavior occurred in mice exposed to 0.45 mg/kg at 6 months of age, and 0.9 and 9.0 mg/kg at 2, 4, and 6 months of age. The effect worsened with age and was most pronounced at 9.0 mg/kg. The swim maze test found that all mice improved their ability to locate the platform during the acquisition period, although those exposed to ≥0.9 mg/kg had significantly longer latencies to locate the platform on days 2 and 3 during the acquisition period. Additional information on the behavioral results of this study, including quantitative data, was not reported.

Mice were also exposed once to 2,2',4,4'-tetraBDE (BDE 47) in doses of 0, 0.7, or 10.5 mg/kg, or 2,2',4,4',5-pentaBDE (BDE 99) in doses of 0, 0.8, or 12.0 mg/kg, on PND 10 (Eriksson et al. 1998, 2002b). Spontaneous behavior was tested at 2 and 4 months of age in all dose groups, and swim maze

performance was tested at 5 months of age in the groups given the high dose of each congener. A disruption of habituation was observed in mice exposed to both congeners. During the first of the three consecutive 20-minute test periods, at both 2 and 4 months, animals treated with 10.5 mg/kg BDE 47 and ≥0.8 mg/kg BDE 99 were significantly less active than controls as shown by dose-related decreases in all three activity measures. During the second period, the activities in all treated groups were comparable to controls at 2 and 4 months. During the third period, exposure to 10.5 mg/kg BDE 47 and ≥0.8 mg/kg BDE 99 caused significantly more activity than controls at 2 and 4 months as shown by dose-related increases in the test measures. The investigators 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 PCB congeners. The same non-habituating behavior at 2 and 4 months of age (hypoactivity followed by hyperactivity) seen in mice exposed to an 8 mg/kg dose of BDE 99 on PND 10 (Viberg et al. 2002b). The only exposure-related effect in the Morris water maze test was found in the mice exposed to BDE 99 at 12.0 mg/kg; performance in finding a new platform location did not improve as it did in controls and mice exposed to BDE 47(Eriksson et al. 1998, 2001a).

Other reports further characterized effects of postnatal exposure to 2,2',4,4',5-pentaBDE (BDE 99) on spontaneous motor behavior in mice. In a study designed to ascertain if NMRI male mice are susceptible during a critical phase of neonatal brain development (i.e., during the period of rapid brain growth), spontaneous behavior was evaluated in adult mice that were administered a single 8 mg/kg dose of BDE 992 on PNDs 3, 10, or 19 and tested at 4 months of age (Eriksson et al. 1999, 2002b). A non-habituating behavioral pattern similar to that observed in the studies summarized above (i.e., decreased locomotion, rearing, and total activity over time in response to diminished novelty of the test chambers) was seen in the mice treated at either 3 or 10 days of age. There were no significant changes in the three spontaneous activity measures in the mice exposed at 19 days of age, suggesting that there was a critical window for the induction of the behavioral disturbances. In an assessment of sex and/or strain dependency (i.e., whether mice other than NMRI males were affected), male and female C57 Bl/J mice were exposed to BDE 99 as a single gavage dose of 0.4, 0.8, 4.0, 8.0, or 16.0 mg/kg on PND 10, and tested at 2, 5, and 8 months of age (Viberg et al. 2003a). Spontaneous motor behavior was significantly impaired at ≥0.8 mg/kg in both sexes at all three ages. The effect was dose-related and appeared to worsen with age, showing a pattern of response similar to that observed for BDE 99 and other congeners in the various studies with NMRI male mice.

In a study of perinatal exposure to 2,2',4,4',5-pentaBDE (BDE 99), groups of two male and two female CD-1 Swiss female mice were gavaged with 0, 0.6, 6, or 30 mg/kg/day in corn oil from GD 6 to PND 21 (Branchi et al. 2001, 2002). Somatic development (body weight gain, hair growth, day of eyelid and ear opening, day of incisor eruption) and neurobehavioral development (righting reflex, forelimb stick grasping reflex, forelimb placing reflexes, negative geotaxis, screen grasping and climbing, pole grasping, ultrasonic vocalizations, homing test) were assessed during PNDs 2–20. Spontaneous activity, including locomotion (horizontal movement), rearing (vertical movement), and thigmotaxis (time and distance traveled close to walls, an index of emotionality), was assessed on PNDs 22, 34, 60, and 120. There were no clear exposure-related indications of prenatal toxicity as shown by maternal weight gain and pregnancy and litter indices. The testing during PNDs 2–22 suggested a delay in sensorimotor development at 30 mg/kg/day, as indicated by approximately 2 days delayed maturation in screen climbing response. The spontaneous behavior testing 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.

5.2.2.5 Reproductive Effects

Polybrominated Biphenyls. Analysis of semen from 41 male residents of Michigan who lived on PBB-contaminated farms or who had bought food directly from such farms and 11 males who were employed in a PBB manufacturing company revealed no abnormalities in the distribution of sperm counts, sperm motility, or sperm morphology, compared with an analysis of semen from 52 unexposed men (Rosenman et al. 1979). This study was conducted in 1977, ≈4 years after initial contamination of Michigan's food supply, and would not have detected an earlier response that was subsequently reversed. PBBs were detected (detection limit of 0.2 ppb) in the serum of 1 of the 52 unexposed men and in all of the exposed men; however, individual or mean values for PBB levels were not reported.

No relationship was found between serum levels of PBBs and frequency and duration of lactation in a retrospective study of women exposed to PBBs during the Michigan contamination episode (Thomas et al. 2001). A group of 1,020 women with available initial serum PBB levels was identified from the Michigan Department of Community Health PBB registry. Among these participants, 446 had a live-born infant after their initial serum PBB level; characteristics of this cohort included mean age of 25.6±5.0 years, initial serum PBB level of 17.5±99.7 ppb, estimated serum PBB level at delivery of 9.4±61.9 ppb, estimated serum PCB level at delivery of 5.5±5.2 ppb, duration of breast-feeding as main

source of nutrition of 2.6±3.3 months, and total duration of breast feeding of 4.1±5.3 months. The numbers of women who breast fed their first infant after the initial serum PBB level and had previously breast-fed were 293 (65.7%) and 49 (11.0%), respectively. Exposure was treated as a categorical variable by dividing the women into groups of low (reference) exposure (≤1 ppb, n=260, serum levels at or below the detection limit), moderate exposure (>1–≤7 ppb, n=141), and high exposure (>7 ppb, n=45, levels above the 90^{th} percentile). Three outcomes of interest were analyzed: (1) the decision to breast feed (yes/no), (2) the duration (months) of breast-feeding as the main source of nutrition, and (3) the total duration (months) of breast-feeding. None of the three outcomes was significantly associated with serum PBB levels, even after controlling for maternal age, previous history of breast-feeding, body mass index, maternal education, household income, history of smoking in the year before pregnancy, consumption of alcohol during the first trimester of pregnancy, history of thyroid disorder, gestational age of the infant, time to pregnancy, and year of birth.

Effects on reproductive organs and reproductive function have been observed in animals following oral exposure to PBBs. An increased incidence of uterine endometrial polyps was observed in rats, 2 years after they were administered a single gavage of 1,000 mg/kg dose FireMaster FF-1 (Kimbrough et al. 1981). Following weaning and two consecutive normal menstrual cycles in 6 months, serum progesterone was decreased in the same four females that showed this effect prebreeding. In a multiple-generation study in which only F_0 rats were fed ≥5 mg/kg/day FireMaster BP-6 in the diet from GD 8 through postpartum day 28 (weaning), reproductive performance with respect to length of gestation or litter size was not affected in the F_1 or F_2 generations (McCormack et al. 1981). Implantation was completely blocked in two of five and two of three female rats that survived gavage administration of 28.6 or 57.1 mg/kg/day FireMaster BP-6, respectively, on alternate days between GDs 0 and 14 (Beaudoin 1979). Histological examination of reproductive organs in male and female rats and mice revealed no abnormalities following gavage treatment with doses up to 10 mg/kg/day FireMaster FF-1 for 25 weeks or 30 mg/kg/day for 4–5 weeks (Gupta and Moore 1979; Gupta et al. 1981; NTP 1983). Necrosis, hyperplasia, and metaplasia in the epithelial lining of the ductus deferens were observed in male rats that died following 100 mg/kg/day FireMaster FF-1 for 4–5 weeks (Gupta and Moore 1979). Treatment of male and female mink with diets providing up to 0.39 mg/kg/day FireMaster FF-1 for 6–7 months before breeding did not affect reproductive performance with respect to fertility or litter size (Aulerich and Ringer 1979; Ringer et al. 1981). In the only chronic study, histological examination of male and female reproductive organs showed increased cystic endometrial hyperplasia in rats exposed to 1.5 mg/kg/day FireMaster FF-1 for up to 104 weeks, but no changes were observed in mice exposed to ≤3.9 mg/kg/day for up to 105 weeks (NTP 1992). Following 6–7 months of exposure to 0.012 mg/kg/day

FireMaster FF-1 in the diet, four of seven female monkeys displayed a lengthening of the menstrual cycle from 28 to 31 days and decreased serum progesterone; prior to the treatment, they had at least 2 years of normal cycles (Allen et al. 1979; Lambrecht et al. 1978). All seven of these monkeys conceived after one to four matings with control males (controls required one to three breedings), but two displayed prolonged implantation bleeding and another two had fetuses that were aborted or stillborn (see Developmental Effects). Reduced spermatogenesis was observed in a male monkey that died after 25 weeks on a diet providing 0.73 mg/kg/day FireMaster FF-1 (Allen et al. 1978).

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 5-2 and plotted in Figure 5-2.

Polybrominated Diphenyl Ethers. No studies were located regarding reproductive effects in humans after oral exposure to PBDEs.

Information on effects of PBDEs on reproductive function is limited to negative findings in a one-generation study of decaBDE (FR-300 BA) in rats (Dow Chemical Co. 1975; Norris et al. 1975b). This commercial mixture was comprised of 77.4% decaBDE, 21.8% nonaBDE, and 0.8% octaBDE and differs from current decaBDE formulations that contain ≥97% decaBDE. Male and female rats were exposed to 3, 30, or 100 mg/kg/day doses in the diet for 60 days before mating and subsequently during a 15-day period during which they were mated. Both sexes continued to receive the test diet throughout gestation and until the end of the 21-day lactation period. 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.

No histopathological changes were observed in male or female reproductive tissues from rats that were exposed to decaBDE in dietary doses of ≤800 mg/kg/day for 30 days (Norris et al. 1973, 1975b), ≤8,000 mg/kg/day for 13 weeks (NTP 1986), ≤1.0 mg/kg/day (77.4% containing 21.8% nonaBDE and 0.8% octaDBE) for 2 years (Kociba et al. 1975; Norris et al. 1975b), 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); octaBDE in doses of ≤750 mg/kg/day for 13 weeks (IRDC 1977); or pentaBDE in doses of ≤100 mg/kg/day for 90 days (WIL Research Laboratories 1984).

Exposure to lower brominated PBDEs has been reported to cause reproductive effects in developing rats. As summarized in Section 5.2.2.6 Developmental Effects, postnatal exposure to the commercial pentaBDE mixture DE-71 caused delayed onset of puberty in male and female rats (Laws et al. 2003; Stoker et al. 2003). Additionally, testicular and ovarian effects occurred in male and female adult offspring of rats that were gestationally exposed the pentaBDE congener BDE 99 (Kuriyama and Chahoud 2003; Talsness et al. 2003).

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 5-3 and plotted in Figure 5-3.

5.2.2.6 Developmental Effects

Polybrominated Biphenyls. Examination of children (≈100 were identified) presumably exposed *in utero* or in early infancy during the peak of the Michigan PBB contamination episode and whose families lived on farms known to be contaminated with PBBs has not revealed any consistent or marked abnormalities in the children's physical and neuropsychological development. No significant abnormalities were found by physical and neurological examination of 33 of these exposed children when they had a mean age of 37.2 months, compared with a group of 20 age-matched, nonexposed control children (Weil et al. 1981). However, subjective interviews with parents suggested that more exposed children than control children had frequent upper respiratory illnesses such as colds, runny noses, and sore throats (Weil et al. 1981). PBBs were measured in the fat of the infants and in the blood of the mothers. Fat levels of PBBs in 27 of the children ranged from 0.01 to 20.96 ppm; half of the values were below 0.120 ppm, and five of the values were above 1.0 ppm. Maternal blood levels ranged from 0.001 to 0.845 ppm and seven mothers had levels that were not detectable (<0.001 ppm). Seagull (1983) administered 5 of 18 tests in a battery of childhood developmental tests (McCarthy Scales of Children's Abilities) to 19 of these exposed children when their ages ranged from ≈2.5–4 years old and concluded that there was a statistically significant negative correlation for four of the five tests between PBB levels in fat tissue and developmental abilities. Mean fat concentrations of PBBs were 0.50 ppm (range, 0.10–0.74 ppm) and 4.218 ppm (range, 0.116–20.960 ppm) in the low and high exposure groups of this study. Schwartz and Rae (1983) later administered the full battery of neuropsychological developmental tests, as well as I.Q. tests, to the same group of children (minus one child whose family refused to participate in the follow-up study) when their ages ranged from approximately 4 to 6 years old. The exposed children's performances were within the normal range in all areas assessed. There were statistically significant negative

correlations between PBB levels in adipose tissue (measured in the earlier study) and performance on some of the developmental tasks, but the tasks with significant correlations were not the same as those noted in the earlier study by Seagull (1983). The available studies, primarily due to the small data set and the inconsistency of the results, do not conclusively establish or eliminate the possibility that *in utero* and early infancy exposure to PBBs might adversely affect the development of human children. The information suggests that if the Michigan PBB contamination episode produced any effects on child development, they were subtle.

A comparison of 1966–1981 fetal mortality rates for Michigan counties with a high percentage of quarantined farms (6.8–20.4%) with those of Michigan counties with no quarantined farms did not conclusively establish differences in rates or trends after the 1973 contamination episode (Humble and Speizer 1984). This study is difficult to interpret because the exposure status method was imprecise, the collected data included only spontaneous abortions occurring after 20 weeks of gestation (early trimester abortions were not counted), and the two populations displayed different pre-1973 trends for fetal mortality rates.

Results from animal studies indicate that *in utero* exposure to PBBs and exposure to PBBs through mothers' milk can produce embryolethal effects, structural abnormalities, growth retardation, liver effects, and neurological effects in offspring. Developmental toxicity has been observed in studies with hexabromobiphenyl and octabromobiphenyl commercial mixtures, but not with commercial decabromobiphenyl PBBs. Rats have been studied most extensively, but data are also available for mice, swine, minks, and monkeys.

Following gavage administration of 200 mg/kg FireMaster FF-1 to rats on gestation days 7 and 14, decreased pup survival to weaning, decreased body weight throughout the lives of offspring, and increased mortality in offspring after 2 years were observed (Groce and Kimbrough 1984). Single doses of 200 mg/kg FireMaster BP-6 administered to rats on one of several days during pregnancy caused increased fetal resorptions, and 400 or 800 mg/kg produced maternal toxicity (expressed as a decrease in body weight gain) and fetal malformations including cleft palate and diaphragmatic hernia (Beaudoin 1977). Increased fetal resorptions also were observed in rats receiving total doses of ≈14.3 mg/kg/day FireMaster BP-6 by gavage on alternate days from days 0 through 14 of pregnancy (Beaudoin 1979). Body weight gain and levels of vitamin A in the liver were reduced in offspring of rats administered 5 mg/kg/day FireMaster BP-6 in the diet on gestation day 8 until weaning at 4 weeks postpartum (McCormack et al. 1982c). Additional effects in pups weaned onto the same treated diets as the dams

included increased hepatic ALA synthetase activity (the rate-limiting enzyme in porphyrin synthesis) and increased urinary excretion of uro- and coproporphyrins at age 16 weeks. Dietary administration of FireMaster BP-6 in dosages of 42.9 mg/kg/day on GDs 7–15 or 50 mg/kg/day on GDs 7–20 produced decreased body weight, but no other developmental effects, in rat fetuses and pups monitored up to 60 days postpartum (Corbett et al. 1975; Harris et al. 1978b). Increased incidences of fetuses with extra ribs were found in rats fed diets providing ≥86 mg/kg/day of octabromobiphenyl mixture from gestation days 6 through 15 (Waritz et al. 1977); however, no embryotoxic, fetotoxic, or teratogenic effects occurred in rats following gavage administration of ≤1,000 mg/kg/day decabromobiphenyl mixture on GDs 6–15 (Millischer et al. 1980).

Effects in offspring of rats exposed to 0.5 mg/kg/day FireMaster FF-1 for 60 days before breeding until 8 weeks postpartum (4 weeks postweaning) and observed for up to the following 2 years included vacuolization and altered foci in the liver (Chhabra et al. 1993; NTP 1992). Pups of mice that were similarly perinatally exposed to 1.5 mg/kg/day FireMaster FF-1 developed liver cytomegaly and altered foci (Chhabra et al. 1993; NTP 1992). As discussed in Section 5.2.2.8 (Carcinogenic Effects), these mice also developed hepatocellular adenoma and carcinoma; combined perinatal and adult exposure induced higher incidences of liver tumors in mice than adult exposure alone (Chhabra et al. 1993; NTP 1992).

In a multiple-generation study, decreased pup survival to weaning, decreased body weight gain, delayed fur development, delayed eye and vaginal opening, and increased liver weight associated with hepatocyte swelling, vacuolization, and focal necrosis were observed in F_1 generation rats whose only exposure was from the mothers fed a diet providing 5 mg/kg/day FireMaster FF-1 from day 8 of pregnancy until weaning at 28 days postpartum; less severe liver responses were observed in the F_1 offspring of dams treated with 0.5 mg/kg/day (McCormack et al. 1981). Although survival of F_2 and F_3 generations was not affected by the 5 mg/kg/day treatment of the F_0 rat dams, F_2 offspring, but not F_3 offspring, displayed increased liver weights, liver enzyme induction, and hepatic histological alterations compared with controls (McCormack et al. 1981). Dietary administration of 2.5 mg/kg/day FireMaster BP-6 to rats from gestation day 0 through postpartum day 15 produced increased relative liver weights, decreased body weights, and decreased serum levels of the thyroid hormone, T_4, in 15-day-old offspring (Meserve et al. 1992). The pups had received direct stimulation of the pituitary by an injection of corticotropin-releasing factor or direct stimulation of the adrenals by an injection of adrenocorticotropic hormone. Provision of a diet containing 0.5 mg/kg/day FireMaster FF-1 to lactating rats for the 18 days following parturition increased liver weights and elevated levels of hepatic cytochrome P-450 and associated enzymic activities in both dams and pups; a diet providing 0.05 mg/kg/day produced no hepatic effects in dams, but induced

hepatic enzymes in the pups (Moore et al. 1978). According to the investigators (Moore et al. 1978), the results could indicate that nursing pups are more sensitive than their dams to liver enzyme induction, or that due to the different kinetic parameters among the PBB congeners, the pups received a more potent PBB mixture than the dams. Yet, a third possibility is that the suckling pups received a higher dose of PBBs relative to their body weights due to bioconcentration of PBBs in breast milk (Dent 1978). Performance deficits in tests of operant behavior were observed in the 6-month-old offspring of rat dams given gavage doses of 0.2 or 2 mg/kg/day FireMaster BP-6 from day 6 of gestation until weaning (Henck and Rech 1986), but not in 75-day-old offspring of rat dams given gavage doses of 0.5 or 5 mg/kg/day for 4 weeks prior to mating (Geller et al. 1985). Effects found in offspring of rats exposed to 0.2 or 2 mg/kg/day doses of FireMaster BP-6 in the diet from day 6 of gestation through day 24 postpartum, and observed through postnatal day 60, included reduced crown-rump length at birth at ≥0.2 mg/kg/day, reduced birth weight and postnatal body weight gain at 2 mg/kg/day, and suppressed acquisition of forward locomotion, cliff avoidance, cage emergence, and open-field activity at 2 mg/kg/day (Henck et al. 1994).

Dietary administration of 50 mg/kg/day FireMaster BP-6 to mice from gestation days 7 through 18 produced decreased fetal body weight and fetal abnormalities including exencephaly, cleft palate, and hydronephrosis; 5 mg/kg/day did not produce significant developmental effects in this study (Corbett et al. 1975). Early postnatal deaths occurred among offspring of mice given 10 mg/kg/day FireMaster FF-1 on alternate days from gestation day 0 until litters were weaned (Luster et al. 1980). Immunological parameters were unaffected in surviving offspring whose mothers received up to 10 mg/kg/day doses, but decreased hematocrit levels were measured in offspring of mothers receiving doses ≥3 mg/kg/day (Luster et al. 1980). Performance deficits in tests of learning behavior were measured in offspring of female mice that received gavage doses of 3 or 10 mg/kg/day FireMaster FF-1 on alternate days from gestation day 0 through weaning at 21 days of age (Tilson 1992).

Decreased body weight at birth and at 4 weeks after birth were measured in mink kits whose parents were fed diets containing 0.155 mg/kg/day FireMaster FF-1 from 7–8 months prior to mating through 4 weeks postpartum (Aulerich and Ringer 1979; Ringer et al. 1981). Increased relative liver weight, fatty and necrotic hepatic changes, slight hyperplasia in the thyroid, and decreased serum levels of thyroid T_3 and T_4 hormones were observed in 4-week-old offspring of swine fed 2.5 mg/kg/day FireMaster BP-6 in the diet during the second half of gestation and during lactation; 1.25 mg/kg/day produced similar effects on the thyroid, but no necrosis in the liver of 4-week-old nursing pigs (Werner and Sleight 1981). Examination of several parameters of immune function in 4-week-old offspring of sows fed

≤2.5 mg/kg/day FireMaster BP-6 during gestation and lactation provided no conclusive evidence for immunosuppressive effects (Howard et al. 1980). An abortion and a stillbirth occurred among seven female monkeys that were fed 0.012 mg/kg/day FireMaster FF-1 in the diet for 7 months prior to conception and during pregnancy (Allen et al. 1979; Lambrecht et al. 1978). The surviving five infants had reduced birth weight and postnatal body weight gain, but no gross abnormalities. The incidence of dystocia (difficult birthing) was 50% increased among first- and second-generation offspring of cows treated with 0.65 mg/kg/day FireMaster BP-6 by gelatin capsule for 180 or 202 days during late pregnancy (Willett et al. 1982). The same dosage for 60 days caused a 21.6% increased incidence of dystocia, but this increase was not statistically significant (p=0.08). Stillbirths and preweaning deaths were not significantly increased, but all mortality was attributable to dystocia. Incidences of dystocia and calf mortality appeared to be related to higher birth weight, which in turn were correlated with concentrations of PBBs in maternal blood and tissues. Growth and development were not affected in the surviving calves. Of six pregnant cows that were similarly treated with a maternolethal dosage (67 mg/kg/day) of FireMaster BP-6, three aborted after 30–38 days, and three retained dead fetuses (Moorhead et al. 1977).

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 5-2 and plotted in Figure 5-2.

Polybrominated Diphenyl Ethers. No studies were located regarding developmental effects in humans after oral exposure to PBDEs.

Information on the developmental toxicity of PBDEs is available from studies of commercial mixtures of deca-, octa- and pentaBDE (Argus Research Laboratories 1985b; Breslin et al. 1989; Dow Chemical Co. 1975, 1985; Hardy et al. 2001, 2002; Life Science Research Israel Ltd. 1987; Norris et al. 1975b; WIL Research Laboratories 1986). None of the commercial BDE mixtures have been shown to be overtly teratogenic in animals, although neurobehavioral tests, summarized in Section 5.2.2.4 (Neurological Effects), indicate that the developing nervous system is a potential target of some individual lower brominated congeners, including 2,2’,4,4’-tetraBDE (BDE 47), 2,2’,4,4’,5-pentaBDE (BDE 99), and 2,2’,4,4’,5,5’-hexaBDE (BDE 153).

No prenatal developmental toxicity was found in a comprehensive study of commercial decaBDE product (Hardy et al. 2001, 2002). The test material was a composite of current commercial decaBDE mixtures produced by three manufacturers and had a composition of 97.34% decaBDE and 2.66% nona-

and octaBDE congeners. The mixture was administered to groups of 25 mated female Sprague-Dawley rats by gavage in corn oil in daily doses of 0, 100, 300, or 1,000 mg/kg/day on GDs 0 through 19. All females were necropsied following sacrifice on GD 20. Maternal end points included clinical observations, body weight and food consumption, gravid uterine and liver weights, gross lesions, and uterine implantation indices (e.g., numbers of corpora lutea, implantations, and early and late resorptions). Fetal end points included viability, body weights, sex distribution, gross malformations (all fetuses), skeletal/cartilaginous malformations and ossification variations (approximately half of each litter), and visceral malformations (remaining fetuses). There were no treatment-related effects on any of the maternal or fetal end points, indicating that 1,000 mg/kg/day was a NOAEL for developmental toxicity of decaDBE.

A lower purity commercial decaBDE product (77% decaDBE, 22% nonaBDE, 0.8% octaBDE) used in the 1970s was fetotoxic in rats at high dose levels that were not maternally toxic. Developmental effects were investigated in rats that were exposed to doses of 10, 100, or 1,000 mg/kg/day by gavage on GDs 6–15 and examined on GD 21 (Dow Chemical Co. 1985; Norris et al. 1975b). No treatment-related maternal toxicity was observed. 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 ($p<0.05$) increased at ≥10 mg/kg/day compared to controls as indicated by resorption/implantation site percentages [1% (3/288), 9% (12/141), 10% (13/135), and 4% (9/203)] and percentages of litters with resorptions [12% (3/25), 64% (9/14), 57% (8/14), and 39% (7/18)]. The resorptions were considered secondary to unusually low control values and unrelated to treatment because (1) the data do not follow a dose-response relationship across the three dose levels, and (2) the rates in the high dose group are comparable to historical control values. As discussed in Section 5.2.2.5 (Reproductive Effects), a one-generation study of the 77% commercial decaBDE mixture in rats found no effects of parental exposure to ≤100 mg/kg/day from 60 days before mating through the end of the lactation on numbers of live pups at birth and during the lactation period, 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. 1975b).

Developmental toxicity testing of another octaBDE mixture (DE-79) in rats used gavage doses of 2.5, 10, 15, 25, or 50 mg/kg/day on GDs 6–15 (WIL Research Laboratories 1986). No exposure-related maternal or overt developmental effects were observed at ≤15 mg/kg/day. The only statistically significant ($p<0.05$) finding at 25 mg/kg/day was an increased serum bromide level. Effects observed at 50 mg/kg/day included significantly reduced mean maternal body weight gain during the posttreatment

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 13th rib) commonly associated with maternal toxicity.

The developmental toxicity of a fourth octaBDE commercial mixture (Saytex 111) was tested in rabbits exposed to doses of 2, 5, or 15 mg/kg/day on GDs 7–19 and examined on GD 28 (Breslin et al. 1989). The 15 mg/kg/day group showed 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. Slight fetotoxicity accompanied the maternal toxicity at 15 mg/kg/day as indicated by a significantly ($p\leq0.05$) increased incidence of delayed ossification of the sternebrae.

A developmental toxicity study of pentaBDE (Saytex 115) is available in which rats were administered 10, 100, or 200 mg/kg/day doses by gavage on GDs 6–15 and examined on GD 20 (Argus Research Laboratories 1985a). The only exposure-related maternal effect was significantly ($p\leq0.01$) reduced body weight gain at ≥100 mg/kg/day during the dosing period. This effect was dose-dependent and increased in severity with continued dosing but recovered during the postdosage period when differences between exposed and control groups were no longer significant. Maternal body weight gain was 2.8, 20.1, and 29.9% less than controls during GDs 6–16 at 10, 100, and 200 mg/kg/day, respectively. There were no exposure-related indications of developmental toxicity. Average fetal body weight per litter was slightly but not significantly reduced at 200 mg/kg/day (2.6% less than controls, $p>0.05$).

Developmental toxicity was also evaluated in rats that were treated with 1, 10, or 30 mg/kg/day of pentaBDE (DE-71) in corn oil by gavage from GD 6 through PND 21 (Zhou et al. 2002). Dams were sacrificed on GD 20 and PND 22 and offspring were sacrificed on GD 20 and PNDs 4, 14, 36, and 90. There were no exposure-related effects on maternal body weight gain, litter size, sex ratio, or 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. Serum measurements of thyroid T_3 and T_4 hormone levels showed that serum T_4 was significantly reduced in the rat dams at 30 mg/kg/day (GD 20 and PND 22) and offspring at ≥10 mg/kg/day (GD 20 and PNDs 4 and 14), as detailed in Endocrine Effects in Section 5.2.2.2. There were no clear effects on pregnancy and birth indices in mice that were gavaged

with the congener 2,2',4,4',5-pentaBDE (BDE 99) in oral doses of 0.6–30 mg/kg/day from GD 6 to PND 21 (Branchi et al. 2001, 2002).

A limited amount of information is available on neurodevelopmental effects of commercial pentaDBE mixture DE-71 in mice in two studies incompletely reported as abstracts (Taylor et al. 2002, 2003). These studies assessed effects in adult offspring of rats that were orally exposed during gestation and lactation (GD 6 to PND 21). In one study, the maternal rats were dosed with 0, 1, 10, or 30 mg/kg/day and offspring were evaluated at various ages (not specified) for end points that included motor activity development, auditory startle response, and age at eye opening. There were no alterations in any of these indices. The second study also found no changes in motor activity, auditory startle response, or eye opening in the offspring following exposure to 1–100 mg/kg/day, although fear conditioning performance was altered (Taylor et al. 2003). Other effects of DE-71 in both studies included decreased serum T_4 levels in the offspring and dams (see Section 5.2.2.2 Endocrine Effects).

Delays in reproductive development occurred in male and female Wistar rats that were exposed to the pentaBDE commercial mixture DE-71 in studies that are incompletely reported as abstracts (Laws et al. 2003; Stoker et al. 2003). Male rats were gavaged with 0, 3, 30, or 60 mg/kg/day doses of DE-71 in corn oil for 5 days on PNDs 24–28, or for 31 days on PNDs 23–53 (Stoker et al. 2003). Pubertal development was delayed as indicated by significant increases in the age at preputial separation at 30 and 60 mg/kg/day (2.0 and 2.3 days delay, respectively). Seminal vesical and ventral prostate weights were reduced at 60 mg/kg/day, although there were no significant changes in testis or epididymal weights or serum testosterone levels. Female rats were gavaged with DE-71 in corn oil in doses of 0, 3, 30, or 60 mg/kg/day for 5 days on PNDs 22–26, or for 20 days on PNDs 22–41 (Laws et al. 2003). Pubertal development was delayed as indicated by a small but statistically significant delay in the age of vaginal opening (1.8 days delay compared to controls) at 60 mg/kg/day. There were no treatment-related changes in weights of female reproductive tissues, and information on levels of female reproductive hormones was not reported. As summarized in Endocrine Effects in Section 5.2.2.2, the delays in male and female reproductive development were accompanied by changes in serum levels of thyroid hormones (decreased T_3 and T_4, increased TSH). The onset of puberty was also delayed in female offspring (vaginal opening), but not in male offspring (preputial separation), of Long Evans rats that were subcutaneously exposed to 1 or 10 mg/kg/day doses of the congener 2,2',4,4',5-pentaBDE (BDE 99) on days 10–18 of gestation (Lichtensteiger et al. 2003b).

Testicular and ovarian effects were observed in male and female adult offspring of Wistar rats that were maternally exposed to a single 60 or 300 µg/kg oral dose of the pentaBDE congener BDE 99 in peanut oil on day 6 of gestation (Kuriyama and Chahoud 2003; Talsness et al. 2003). Evaluations of the male offspring were performed on PND 140 and included reproductive tissue weights (testis, epididymis, ventral prostate, and seminal vesicle), spermatid and sperm numbers, and serum levels of testosterone and LH (Kuriyama and Chahoud 2003). Effects in the male offspring occurred at 60 and 300 µg/kg, including significantly decreased ($p<0.05$) spermatid number (31 and 34% less than controls), sperm number (29 and 18% less than controls), and daily sperm production (31 and 34% less than controls). The female offspring were necropsied in estrus on approximately postnatal day 90, at which time, ovaries were examined by electron microscopy (Talsness et al. 2003). Ultrastructural alterations were observed in ovaries at both dose levels, particularly degenerative changes that included vacuolization and accumulation of vesicules in the cytoplasm of the stromal cells and necrosis in the serosal epithelial cells. The single congener dose levels in this study are much lower than the LOAEL of 2 mg/kg/day for liver effects in rats exposed to a commercial mixture of pentaBDE in the 90-day study used to derive the intermediate oral MRL.

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 5-3 and plotted in Figure 5-3.

5.2.2.7 Cancer

Polybrominated Biphenyls. No epidemiological studies were located that provided evidence for an association between exposure to PBBs and the occurrence of cancer in humans, although one case report is available concerning gastrointestinal cancer in a Michigan dairy farmer with known exposure to PBBs and other chemicals.

The Michigan Department of Public Health, the U.S. Center for Disease Control, the National Institutes of Health, the Food and Drug Administration, and the EPA established a cohort of Michigan residents with varying levels of PBB exposure to determine the short- and long-term effects (especially cancer) of exposure to PBBs (Landrigan et al. 1979). The epidemiological and clinical data collected during the first 4 years after the Michigan PBB contamination episode indicated that cancer was not a prevalent "symptom" among the cohort at that time. Prevalence rates for cancer in exposed groups ranged from 0.4 to 0.6% compared with 0% in a small control group comprised of residents of farms with low PBB contamination (Landrigan et al. 1979). When the cohort was divided into seven groups based on serum

PBB levels, no trend with concentration was apparent, but the incidence of cancer was the highest in the group with the highest serum PBB levels. Subsequent follow-up examinations of this cohort have not been reported.

In studies conducted by the Environmental Science Laboratory of the Mount Sinai School of Medicine, mean plasma levels of carcinoembryonic antigen (CEA), which has been used as a screening tool for tumor recurrence after cancer therapy, were found to be slightly higher in 1976 in a population of 611 Michigan residents who likely ingested PBB-contaminated food than mean levels in a nonexposed population of Wisconsin farm residents, but the difference was not statistically significant (Anderson et al. 1978b). Cancer was not listed as a condition in the report of results of a symptomatology survey completed by this cohort (Anderson et al. 1979). Reports of follow-up examinations of this cohort have not been reported.

A relationship between serum PBBs and risk of breast cancer was suggested in a nested case-control study of 1,925 women enrolled in the Michigan Department of Public Health registry for persons exposed to PBBs (Henderson et al. 1995). Study participants had lived on or received food from a farm quarantined by the Michigan Department of Agriculture, were recruited from July 1976 to December 1977, and followed up annually from 1978 through 1993. Twenty women who developed breast cancer were age- and race-matched to 290 controls. Median serum PBB concentrations were similar in the cancer cases (3 ppb, range 0.5–16 ppb) and controls (2 ppb, range 0.5–419 ppb). Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for increasing serum PBB levels categorized into tertiles (<2 ppb, 2–3 ppb, ≥4 ppb) and a dichotomous variable (<2 ppb, ≥2 ppb). The estimated risk for breast cancer was slightly elevated for women with serum PBB levels of 2–3 ppb (OR=3.5, 95% CI=0.9–13) and ≥4 ppb (OR=3.1, 95% CI=0.8–12) when compared with the reference group (<2 ppb), or when the dichotomous variable was used in the analysis (OR=3.3, 95% CI=0.9-11.4). The results were essentially the same when the data were adjusted for available risk factors (body mass index and family history of cancer), or when matched sets of cases and controls were stratified into two groups based on date of diagnosis (≥10 years after exposure). The results of this study are inconclusive due to the small number of cases, apparent lack of statistically significant increases (p values were not reported, but confidence intervals were broad with lower limits less than unity, indicating that it is difficult to exclude chance as an explanation for the findings), and lack of information on important breast cancer risk factors (e.g., exposure to other organochlorine chemicals and estrogen receptor status).

Another study of the Michigan PBB registry evaluated associations between levels of serum PBBs and risks of various site-specific cancers (Hoque et al. 1998). Primary cancers (195 malignancies) were identified in 187 persons among 3,899 registrants enrolled in 1976 and followed through 1993. Controls were 696 randomly selected cancer-free individuals who were frequency matched to cases by age (in 5-year strata) and sex in a 4:1 ratio (except above age 70 years when, due to lower numbers, all eligible controls were used). PBB levels in the cases were measured at the time of registry enrollment. Serum PBB concentration ranges was categorized into four groups (not detectable–3 ppb, 4–20 ppb, 21–50 ppb, >50 ppb) defined by the median and the 90^{th} and 95^{th} percentiles. Conditional logistic regression was used to calculate univariate and multivariate (adjusted) ORs by cancer site for the three highest serum PBB categories compared to the reference (≤3 ppb) group. The multivariate ORs were adjusted for family history of cancer, smoking status, alcohol use, age, serum PCB level, and sex. Digestive system cancer (12 cases) and lymphoma (not otherwise specified) (8 cases) showed increasing dose-response relationships for risk as PBB concentrations increased. Digestive system cancer was a grouping that comprised of the following sites: liver (five cases), stomach (five cases), esophagus (one case) and pancreas (one case). Adjusted ORs for digestive system cancer were 1.00 (reference), 8.23 (95% CI=1.27–53.3), 12.3 (0.80–191), and 22.9 (1.34–392) for the ≤3, 4–20, 21–50, and >50 ppb categories, respectively. The corresponding adjusted ORs for PBB level and lymphoma risk were 1.00, 3.85 (0.32–46.2), 19.6 (1.52–253), and 48.9 (4.09–585). The lymphoma ORs were incompletely adjusted due to missing data for serum PCB level and family cancer history in the reference category. Increased risks were also observed for breast cancer in the 4–20 ppb category (nine cases, adjusted OR=2.41, 95% CI=0.92–6.30), cancer at an unknown site in the 4–20 ppb category (four cases, adjusted OR=31.0, 95% CI=1.40–685), and leukemia in the 21–50 ppb category (one case, adjusted OR=4.49, 95% CI=0.92–6.30). The associations found in this study should be viewed as suggestive and preliminary due to the small numbers of cases. The 2.4-fold increased risk of breast cancer for PBB levels between 4 and 20 ppb is consistent with the 3-fold increased risk for breast cancer observed for PBB levels >2 ppb in the Henderson et al. (1995) study of the same cohort summarized above.

A Michigan dairy farmer, who had a history of health complaints after 1976, developed malignant cancer of the esophageal and stomach wall in 1986; the man subsequently died in 1988 (Sherman 1991). Samples of adipose tissue, collected in 1976 and 1987, revealed PBB concentrations of 0.83 and 0.85 ppm, respectively. Also detected in the fat tissue collected in 1987 were polychlorinated biphenyls (PCBs) at 3.57 ppm and chlordane residues at concentrations ranging from 0.018 to 0.039 ppm.

FireMaster FF-1 has induced liver tumors and/or neoplastic nodules in rats and mice following single or repeated administration by gavage in oil vehicle, as well as following chronic dietary administration. In female Sherman rats given a single 1,000 mg/kg dose and observed for 2 years, incidences of hepatocellular carcinomas and liver neoplastic nodules were 41.4% (24/58) and 72.4% (42/58), respectively (Kimbrough et al. 1981). In an earlier study using the same treatment (single 1,000 mg/kg dose), groups of five Sherman rats of each sex were examined at 2, 6, 10, and 14 months following treatment (Kimbrough et al. 1978b). Neoplastic nodules were found in the livers of 22.5% (9/40) of the treated rats observed for at least 10 months (4/5, 2/5, and 3/5 in the 10-month females, 14-month males, and 14-month females, respectively). Liver tumors were not found, but this could be related to the relatively small number of animals (20/sex) and/or short duration of observation (≤14 months). Liver neoplastic nodules without tumors also developed in 31.2% (5/16) of Sherman rats treated with a lower single dose (200 mg/kg) and observed for 18–22 months (Kimbrough et al. 1981). No liver tumors or neoplastic nodules developed in untreated control groups in any of these single dose studies, and treatment-related tumors in sites other than liver were not observed. When administered to pregnant rats once on gestation day 7, a dose of 200 mg/kg induced both hepatocellular carcinomas and liver neoplastic nodules in offspring that were observed for 2 years (Groce and Kimbrough 1984). The incidences of tumors in male offspring (9.6% [4/41] versus 0% [0/42] in controls) and nodules in female offspring (17.6% [9/51) versus 4.2% [2/48]) increased significantly ($p \leq 0.055$). Treatment-related tumors were not observed in nonhepatic tissues of the offspring.

Hepatocellular carcinomas and liver neoplastic nodules also increased in female Sherman rats gavaged with 100 mg/kg FireMaster FF-1 twice a week for two 3-week periods (12 total doses) separated by ≈10 weeks (Kimbrough et al. 1981). Following observation for 2 years, the incidence of carcinomas was 60.7% (17/28 versus 0/25 in controls) and incidence of nodules was 85.7% (24/28 versus 1/25). In repeated dose studies performed by NTP (1983), FireMaster FF-1 was administered via gavage to Fischer-344/N rats and B6C3F1 mice of both sexes at dosages of 0, 0.1, 0.3, 1, 3, or 10 mg/kg/day on 5 consecutive days per week for 25 weeks. Both rats and mice were observed for life (up to 23 and 24 months posttreatment, respectively). Incidences of hepatocellular carcinoma were dose-related and significantly ($p<0.01$) increased in male rats at ≥3 mg/kg/day (0/33 [controls], 2/39, 0/40, 1/33, 7/33, and 7/31 [high-dose]) and female rats at 10 mg/kg/day (7/20 versus 0/20 in controls). The incidence of cholangiocarcinoma was significantly ($p<0.01$) increased in female rats at 10 mg/kg/day (7/20 versus 0/20) and almost significant ($p=0.06$) in males at 10 mg/kg/day (2/31 versus 0/33). Liver neoplastic nodules were dose-related and significantly ($p<0.01$) increased in female rats at ≥3 mg/kg/day. No clear treatment-related effects on incidences of hepatic neoplastic nodules in males, or bile duct hyperplasia,

myelomonocytic (mononuclear cell) leukemia, or foci of pancreas-like tissue in the liver in either sex were observed. In mice, incidences of hepatocellular carcinoma significantly ($p<0.01$) increased at 10 mg/kg/day in males (21/22 versus 12/25 in controls) and females (7/8 versus 0/13). Metastasis to lung also significantly ($p<0.05$) increased in female mice at 10 mg/kg/day. No treatment-related effects on hepatocellular adenomas or hepatoblastomas were observed. Thyroid follicular cell adenoma tended to increase in treated female mice, but data are inconclusive due to low incidences and small numbers of animals.

The carcinogenicity of FireMaster FF-1 was additionally evaluated in Fischer-344/N rats and B6C3F1 mice of both sexes that received adult exposure only, perinatal exposure only, or combined perinatal and adult exposure (NTP 1992). The adult-only exposure involved dietary administration of PBBs (F_1 diets) to ≈8-week-old animals for up to 104 weeks (rats) or 105 weeks (mice). Perinatal-only exposure involved dietary treatment of dams (F_0 diets) for 60 days prior to breeding and throughout gestation and lactation until pups were 8 weeks old. The pups were administered the same treatment as the dams from weaning at week 4 until age 8 weeks, and were subsequently administered the same or different dietary treatments (F_1 diets) for up to 104 weeks (rats) or 105 weeks (mice). This study was designed to compare the carcinogenicity of PBBs given in a conventional bioassay protocol (i.e., the adult-only exposure) with that of PBBs given in a combined perinatal and adult exposure regimen.

Eight F_0:F_1 doses (estimated) were tested in rats among one unexposed control group (0:0 mg/kg/day), two adult-only exposure groups (0:0.5 and 0:1.5 mg/kg/day), one perinatal-only exposure group (0.5:0 mg/kg/day), and four combined perinatal and adult exposure groups (0.05:0.15, 0.15:0.5, 0.5:0.5, and 0.5:1.5 mg/kg/day) (Chhabra et al. 1993; NTP 1992). Incidences of hepatocellular tumors were increased in adult-only exposed rats of both sexes. In males ingesting 0:0, 0:0.5, and 0:1.5 mg/kg/day, incidences of adenoma were 1 of 50, 10 of 49, and 38 of 50; of carcinoma, 0 of 50, 2 of 49, and 19 of 50; and of adenoma or carcinoma (combined), 1 of 50, 12 of 49, and 41 of 50, respectively. In females, incidences of adenoma were 0 of 50, 10 of 50, and 38 of 50; of carcinoma, 0 of 50, 2 of 50, and 4 of 50; and of adenoma or carcinoma (combined), 0 of 50, 12 of 50, and 39 of 50, respectively. These increases in liver tumor incidences were statistically significant ($p\leq0.002$) except for carcinoma in 0:0.5 mg/kg/day males and females and 0:1.5 mg/kg/day females ($p>0.05$). Combined perinatal and adult exposure significantly enhanced the development of liver tumors in female rats, as shown by comparisons with females receiving adult exposure only. Compared to the 0:0.5 mg/kg/day female adult-only exposed group, incidences of hepatocellular adenoma were 22 of 50 ($p=0.01$) and 35 of 50 ($p<0.001$); of carcinoma, 1 of 50 ($p=0.05$) and 8 of 50 ($p=0.048$); and of hepatocellular adenoma or carcinoma

(combined), 22 of 50 ($p=0.03$) and 39 of 50 ($p<0.001$) in the 0.15:0.5 and 0.5:0.5 mg/kg/day groups, respectively. Compared to the 0:1.5 mg/kg/day female adult-only exposed group, incidences of hepatocellular adenoma, carcinoma, and hepatocellular adenoma or carcinoma (combined) were 45 of 50 ($p=0.049$), 22 of 50 ($p<0.001$), and 47 of 50 ($p=0.016$), respectively, in the 0.5:1.5 mg/kg/day group. This enhancing influence of perinatal exposure did not occur in the males. Perinatal-only exposure did not cause significantly increased incidences of liver or other tumors in rats of either sex.

Increased incidences of mononuclear cell leukemia occurred in adult-only exposed rats but were not clearly related to treatment (Chhabra et al. 1993; NTP 1992). The incidences of this leukemia in the 0:0, 0:0.5, and 0:1.5 mg/kg/day groups were 25 of 50, 33 of 50, and 31 of 50, respectively, in males and 14 of 50, 22 of 50, and 23 of 50, respectively, in females; the incidences in the 0:0.5 mg/kg/day males and 0:1.5 mg/kg/day females were significantly ($p\leq0.05$) increased. Comparison of the combined perinatal and adult exposure groups with the adult-only exposed groups showed no significant enhancement; however, comparison with the unexposed control (0:0 mg/kg/day) incidences showed a consistent increase in the incidence of this neoplasm at higher doses. In the 0.15:0.5 and 0.5:0.5 mg/kg/day groups, the incidences of leukemia were 41 of 50 ($p\leq0.01$) and 37 of 50 ($p\leq0.01$) in males and 17 of 50 ($p>0.05$) and 27 of 50 ($p\leq0.01$) in females. In the 0.5:1.5 mg/kg/day groups, the incidences were 37 of 50 ($p\leq0.01$) in males and 25 of 50 ($p\leq0.05$) in females. The incidences in some of these groups fall outside the NTP historical control range. The incidences in males were as high as 82% and exceeded the upper historical control range of 62%. In females, the incidences were as high as 54% and exceeded the overall upper historical control range of 52% and the laboratory upper historical control range of 28%. A combined (life table) analysis of data from all eight experimental groups indicates that significant increases in the incidence of the leukemia are associated with increasing F_1 concentrations ($p\leq0.05$ in males; $p\leq0.01$ in females). For males, there was also a marginally significant ($p\leq0.05$) increase associated with F_0 exposure.

Eight F_0:F_1 doses were also tested in the mice among one unexposed control group (0:0 mg/kg/day), two adult-only exposure groups (0:1.3 and 0:3.9 mg/kg/day), one perinatal-only exposure group (3.9:0 mg/kg/day), and four combined perinatal and adult exposure groups (0.39:0.39, 1.3:1.3, 3.9:1.3, and 3.9:3.9 mg/kg/day) (NTP 1992). As in the rats, hepatocellular tumors were significantly ($p<0.001$) increased in adult-only exposed mice of both sexes. In males ingesting 0:0, 0:1.3, and 0:3.9 mg/kg/day, incidences of adenoma were 9 of 50, 48 of 49, and 42 of 50; of carcinoma, 8 of 50, 30 of 49, and 36 of 50; and of adenoma or carcinoma (combined), 16 of 50, 48 of 49, and 48 of 50, respectively. In adult-only exposed females, incidences of adenoma were 4 of 50, 39 of 50, and 46 of 48, carcinoma were 1 of 50,

28 of 50, and 41 of 48, and adenoma or carcinoma (combined) were 5 of 50, 42 of 50, and 47 of 48, respectively. Combined perinatal and adult exposure resulted in increased incidences of liver neoplasms in some treated groups. However, because adult-only exposure to 1.3 or 3.9 mg/kg/day resulted in such high incidences of liver neoplasms (84–98%), the possible enhancing effect of combined perinatal and adult exposure could not be adequately assessed in either sex. Compared to 0:1.3 mg/kg/day adult-only exposure, combined perinatal and adult exposure to 3.9:1.3 mg/kg/day caused significantly increased incidences of carcinoma in males (40 of 50, $p=0.01$) and females (44 of 50; $p<0.001$), adenoma in females (47 of 50, $p=0.005$) and adenoma or carcinoma (combined) in females (50 of 50, $p<0.001$). Compared to 0:3.9 mg/kg/day adult-only exposure, combined perinatal and adult exposure to 3.9:3.9 mg/kg/day caused significantly increased adenoma incidence in males (48 of 50; $p=0.007$) and decreased adenoma incidence in females (41 of 47; $p=0.022$). Perinatal-only exposure also caused significantly increased incidences of liver neoplasms in mice of both sexes. Comparison of the 0:0 and 3.0:0 mg/kg/day groups showed hepatocellular adenoma, carcinoma, and adenoma or carcinoma (combined) incidences of 31 of 50 ($p<0.001$), 17 of 50 ($p=0.033$), and 40 of 50 ($p<0.001$) in males and 19 of 50 ($p<0.001$), 7 of 50 ($p=0.213$), and 21 of 50 ($p<0.001$) in females. Combined perinatal and adult exposure to 3.9:1.3 mg/kg/day also caused a significant ($p=0.029$) increase in the incidence of thyroid follicular cell adenoma in male mice (5 of 48) compared to adult-only exposure to 0:1.3 mg/kg/day (0 of 49). This incidence of thyroid adenoma exceeds the historical control range of 0–4% in untreated males in NTP studies, but the effect was not seen in the higher dose groups (0:3.9 or 3.9:3.9 mg/kg/day). Perinatal-only exposure did not induce thyroid or other nonhepatic tumors in mice of either sex.

The existing evidence conclusively demonstrates that the liver is the main target of PBB carcinogenicity in animals. Results of a chronic study (Chhabra et al. 1993; NTP 1992) suggest that male rats are more sensitive than female rats (based on a higher carcinoma/adenoma ratio), and that mice are more sensitive than rats (based on earlier occurrence of hepatocellular adenomas, higher combined incidence of all liver neoplasms, and higher liver concentrations of PBBs). Based on findings in male rats and mice of both sexes in this study, there is some evidence that combined perinatal and adult dietary exposure to FireMaster FF-1 enhanced the susceptibility of hepatocellular neoplasms in animals receiving adult exposure.

The Cancer Effect Levels (CELs) for FireMaster FF-1 reported in Kimbrough et al. (1981), Groce and Kimbrough (1984), and NTP (1983, 1992) are recorded in Table 5-2 and plotted in Figure 5-2.

There are data indicating that FireMaster BP-6 has tumor promoting activity in rats and hamsters. In standardized liver tumor promotion assays, development of enzyme-altered hepatic foci (putative preneoplastic lesions) was assessed in rats that were 70% hepatectomized, initiated with a subcarcinogenic intraperitoneal dose of diethylnitrosamine 24 hours after the partial hepatectomy, and promoted with orally administered FireMaster BP-6 beginning 30 days later. Various promotion protocols caused significantly increased numbers of enzyme-altered hepatic foci with gamma-glutamyl transpeptidase (GGT) activity, including two gavage doses of 65 mg/kg on adjacent days (6.5 mg/kg was not effective) (Rezabek et al. 1987), estimated dietary dosages of 0.5 or 5 mg/kg for 180 days (Jensen et al. 1982), and estimated dietary dosages of 0.5 mg/kg/day for 140 days or 5 mg/kg for 15 days (Jensen et al. 1984). In a similar assay with rats that were not hepatectomized, a single gavage dose of 100 mg/kg FireMaster BP-6 administered 7–10 days after initiation with dimethylnitrosamine (NDMA) or N-nitroso-pyrrolidine (NPYR) promoted development of hepatic enzyme-altered foci (Rangga-Tabbu and Sleight 1992). A statistically significant increased number of tracheal papillomas (but not number of animals with papillomas) developed in a group of hamsters given a single subcutaneous initiating dose of diethyl-nitrosamine and fed an estimated dietary dosage of 8.3 mg/kg/day FireMaster BP-6 for 140 days (Wasito and Sleight 1989).

Individual PBB congeners have been examined for tumor promoting activity in rats that were partially hepatectomized and initiated with diethylnitrosamine (DENA). Numbers of hepatic GGT-altered foci and/or neoplastic nodules were increased following promotion with 3,3',4,4'-tetrabromobiphenyl (BB 77) (≈0.25 mg/kg/day in the diet for 180 days or 8 weekly intraperitoneal injections of ≈7 mg/kg), 2,2',4,4',5,5'-hexabromobiphenyl (BB 153) (≈0.5 mg/kg/day in the diet for 180 days) or 3,3',4,4',5,5'-hexa-bromobiphenyl (BB 169) (≈0.05 mg/kg/day in the diet for 140 days) (Buchmann et al. 1991; Dixon et al. 1988; Jensen et al. 1982, 1983). Dietary exposure to ≈5 mg/kg/day of BB 153 for 480 days similarly promoted hepatic development of altered foci and neoplastic nodules in rats, whereas ≈0.05 mg/kg/day of BB 169 did not, although an apparent synergistic effect was observed when these two congeners were fed together (Jensen and Sleight 1986). Additional information on structure-promotion relationships for PBBs is discussed in Section 5.5.2.

The tumor initiating potential of PBBs is not well characterized. Numbers of GGT-altered foci were significantly increased in partially hepatectomized rats that were administered a single 1–10 mg/kg oral dose of 3,3',4,4'-tetrabromobiphenyl (BB 77) followed by phenobarbital in the diet for 180 days (Dixon et al. 1988), indicating that PBBs may have initiating activity in hepatocarcinogenesis. The potential for

liver tumor initiation by PBBs appears to be weak compared to their potent promoting activity (Buchmann et al. 1991; Dixon et al. 1988; Jensen et al. 1984).

Polybrominated Diphenyl Ethers. 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 levels 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.

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. 1975b; 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.

The National Toxicology Program 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≤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 5.2.2.2. 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. 1975b). 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.

The Cancer Effect Levels (CELs) for decaBDE in the NTP (1986) study are recorded in Table 5-3 and plotted in Figure 5-2.

5.2.3 Dermal Exposure

A few studies have examined groups of chemical workers involved in the manufacture and distribution of PBBs and/or 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. 1978d). Results from these studies are discussed in this section, as well as in Section 5.2.1. 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.

5.2.3.1 Death

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

No deaths were observed over a 14-day period among a group of four rabbits exposed to up to 10,000 mg/kg of body weight of a commercial octabromobiphenyl mixture by application to abraded and occluded dorsal trunk skin (Waritz et al. 1977). The bromobiphenyl was applied as a 35% (w/v) paste in corn oil. The same group of investigators reported that four of four rabbits died over a 14-day period after application of 5,000 mg/kg of a commercial hexabromobiphenyl mixture in the same vehicle as the octabromobiphenyl mixture. A dose of 10,000 mg/kg applied for 24 hours killed two of four rabbits. The cause of death was not reported. A commercial mixture of decabromobiphenyl in corn oil was not lethal in rats that were observed for 14 days following application of a single dose as high as 5,000 mg/kg to covered intact skin (Millischer et al. 1980). The octabromobiphenyl LOAEL of 5,000 mg/kg is reported in Table 5-5. It is unclear whether the different lethality rates observed among the hexa-, octa-, and decabromobiphenyl mixtures reflect differences in lethal potency or in absorption rates or both.

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

Table 5-5 Levels of Significant Exposure to Polybrominated Biphenyls - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL Less Serious		LOAEL Serious		Reference Chemical Form
ACUTE EXPOSURE								
Death								
Rabbit (NZW)	24 hr		10000 mg/kg/day					Waritz et al. 1977 (OBB)
Rabbit (NZW)	24 hr					5000 mg/kg/day	(4/4 died)	Waritz et al. 1977 (HBB)
Systemic								
Rabbit (Albino)	once	Dermal	658 mg/kg/day					Millischer et al. 1980 (DBB)
Rabbit (NZW)	5 d 1x/d	Dermal		0.19 mg/kg/day	(hyperkeratosis)			Needham et al. 1982 (FF-1)
Rabbit (NZW)	24 hr	Hepatic	100 M mg/kg/day	1000 mg/kg/day	(increased liver weight; necrotic foci)			Waritz et al. 1977 (HBB)
		Bd Wt	1000 M mg/kg/day	5000 mg/kg/day	(11% weight loss)			
Rabbit (NS)	2 wk 5 d/wk 1x/d	Hepatic		1 mg/kg/day	(increased liver weight)			Waritz et al. 1977 (OBB)
		Dermal	1 mg/kg/day					
		Bd Wt	1 mg/kg/day					

Table 5-5 Levels of Significant Exposure to Polybrominated Biphenyls - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL		Reference Chemical Form
				Less Serious	Serious	
INTERMEDIATE EXPOSURE						
Systemic						
Gn Pig (Albino Hartley)	3 wk 3x/wk	Dermal	62 M mg/kg/day			Waritz et al. 1977 (OBB)

Bd Wt = body weight; d = days; DBB = deca-brominated biphenyl; Derm = dermal; FF-1 = FireMaster FF-1; HBB = hexa-brominated biphenyl; hr = hour(s); M = male; OBB = octa-brominated biphenyl; wk = week(s); x = time(s)

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.

5.2.3.2 Systemic Effects

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

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 PBBs and 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 5-5 and 5-6.

Hepatic Effects.

Polybrominated Biphenyls. No studies were located regarding hepatic effects in humans after dermal exposure to PBBs.

No significant changes in relative or absolute liver weight or gross pathological effects were reported in groups of four rabbits after application of a single dose of up to 10,000 mg/kg of octabromobiphenyl mixture in corn oil to abraded and occluded dorsal skin over a 24-hour period (Waritz et al. 1977). It was unclear if histopathological examinations were performed. Using the same protocol in rabbits, these investigators reported a significant increase ($p<0.01$) in relative and absolute liver weight, distinct lobular markings, and necrotic foci with doses ≥1,000 mg/kg of a commercial hexachlorobiphenyl mixture. A dose of 100 mg/kg was without effect. A significant increase ($p<0.01$) in relative liver weight was reported in rabbits after application of 1 mg/kg/day of a commercial mixture of octabromobiphenyl in corn oil to the intact and occluded shaved dorsal skin on 5 days/week for 2 weeks (Waritz et al. 1977). Histopathological examinations were not performed. A relatively low dose (0.0013 mg/kg) of FireMaster BP-6 dissolved in benzene/decame (1:9) applied once a day for 5 days to the ear of three rabbits caused no histopathological effects in the liver (Hass et al. 1978).

Table 5-6 Levels of Significant Exposure to Polybrominated Diphenyl Ethers - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL		Reference Chemical Form
				Less Serious	Serious	
ACUTE EXPOSURE						
Systemic						
Rabbit (New Zealand)	24 hr	Bd Wt	2000 mg/kg			IRDC 1974 DecaBDE
Rabbit (New Zealand)	24 hr	Bd Wt	2000 mg/kg			IRDC 1975a OctaBDE
Rabbit (New Zealand)	24 hr	Bd Wt	2000 mg/kg			IRDC 1975b PentaBDE

Bd Wt = body weight; d = days; Derm = dermal; hr = hour(s); M = male; wk = week(s); x = time(s).

No studies were located regarding hepatic effects following intermediate or chronic dermal exposure to PBBs.

Endocrine Effects.

Polybrominated Biphenyls. Hypothyroidism was diagnosed in 4 of 35 men who were occupationally exposed to unspecified PBBs and/or decaBDE (Bahn et al. 1980). The cohort consisted of workers (mean age 35.9 years) who had been employed at a production plant for at least 6 weeks during a 52-month period during which PBBs and decaBDE were the only chemicals manufactured and who had volunteered for a comprehensive medical evaluation performed 3 months after the end of the 52-month period. There was no further description of exposure, and it was assumed to have occurred by both inhalation and dermal routes. As detailed in Section 5.2.1.2, the results of this study suggest that occupational exposure to PBBs, decaBDE, and/or bromine affected the thyroid, but the mixed chemical exposure and a lack of data on serum or tissue levels of the chemicals preclude attributing effects solely to any particular congener or mixture of congeners.

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

Polybrominated Diphenyl Ethers. There is suggestive evidence of hypothyroidism in a small group of workers who were occupationally exposed to decaBDE as well as PBBs (Bahn et al. 1980) as summarized above and detailed in Section 5.2.1.2.

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

Dermal Effects.

Polybrominated Biphenyls. As discussed in Section 5.2.1.2, results from a medical history survey study of workers in a PBB manufacturing plant and a nonexposed group of Wisconsin farm residents indicated an association between occupational exposure to PBBs and the occurrence of acne (Chanda et al. 1982). The survey covered a period of 3 years of potential exposure, but exposure levels were not reported. No adverse dermal effects were observed on the arms or legs of subjects after a 6-day application of polymer fibers containing commercial octabromobiphenyl mixture under an occlusive covering; no additional information was reported (Waritz et al. 1977).

Several studies examined the acute dermal effects of commercial PBB mixtures in rabbits. Application of 0.19 mg/kg FireMaster FF-1 for 5 days, in toluene vehicle, to the inner surface of the left ear of two rabbits (right ear served as control) induced moderate hyperkeratosis, which included marked dilation of the hair follicles, with moderate proliferation of the epithelium and partial atrophy of the sebaceous glands (Needham et al. 1982). There was also evidence of excess keratin and debris in the subjacent hair follicles. Application of either a dry or water-moistened formulation of octabromobiphenyl mixture (amount not reported) for 24 hours did not adversely affect intact skin in rabbits, but slight erythema and edema were observed in abraded skin (Norris et al. 1975a). Repeated applications over a 2-week period of the dry octabromobiphenyl mixture formulation (amount not reported) to occluded intact or abraded skin caused no skin response, but the water-moistened formulation caused slight and transient erythema (Norris et al. 1975a). None of these studies reported the number of animals used. Rough skin with mild erythema was observed in occluded intact shaved dorsal skin of a group of rabbits after repeated applications of a dose of 1 mg/kg/day octabromobiphenyl mixture in corn oil over a 2-week period (Waritz et al. 1977). Application of a commercial decabromobiphenyl mixture in olive oil to covered intact or abraded skin for 4 hours, in an amount equivalent to 658 mg/kg, caused very slight erythema with or without edema in rabbits (Millischer et al. 1980).

Limited information is available regarding intermediate-duration dermal effects of PBBs in animals. A 10% chloroform solution of an unspecified commercial formulation of octabromobiphenyl did not induce bromacne when applied to the ear of rabbits for 30 days (Norris et al. 1975a). Only slight erythema and exfoliation was observed. Doses of 62 mg/kg of octabromobiphenyl mixture were not sensitizing when applied to the intact or abraded skin of guinea pigs over a 3-week period (Waritz et al. 1977).

No studies were located regarding dermal effects in animals after chronic application of PBBs.

Polybrominated Diphenyl Ethers. A human sensitization study was conducted in which 77.4% decaBDE (containing 21.8% nonaBDE and 0.8% octaBDE) as a 5% suspension in petrolatum was applied via patch, 3 times a week for 3 weeks, to 50 subjects (Norris et al. 1975a). No skin sensitization responses occurred during the sensitizing period or on challenge 2 weeks following the last application. No additional information was reported regarding the design and results of this study.

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) (IRCD 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 (IRCD 1975a, 1975b; Norris et al. 1975a). A similar single application of solid 77.4% decaBDE (21.8% nonaBDE and 0.8% octaBDE) to abraded skin caused a slight erythematous and edematous response in rabbits, although repeated applications of the same decaBDE mixture to intact skin (5 days/week for 2 weeks) or abraded skin (3 days) was non-irritating to rabbits (Norris et al. 1975a). All skin sites returned to normal appearance following cessation of exposure. (Norris et al. 1975a).

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. 1975b). 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.

Polybrominated Biphenyls. No studies were located regarding ocular effects in animals after dermal exposure to PBBs.

Transient irritation of the conjunctival membranes was observed after a single instillation of an unreported amount of dry solid octabromobiphenyl mixture to the eye in rabbits, but the cornea, iris, and lens were unaffected (Norris et al. 1975a). Commercial grade decabromobiphenyl did not cause eye irritation in rabbits when 0.05 mg in olive oil was instilled for 30 seconds followed by rinsing, but application of an unspecified amount of dry powder without rinsing was slightly irritating (Millischer et al. 1980). Mild conjunctival redness and swelling and a copious discharge was reported after application

of 100 mg of an unspecified commercial PBB powder mixture (either hexa- or octabromobiphenyl) for 20 seconds into the conjunctival sac of two rabbits (Waritz et al. 1977). These effects disappeared within 4 hours in both washed (with tap water) and unwashed eyes. The iris and cornea were unaffected.

Polybrominated Diphenyl Ethers. 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 (ICRD 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.

Polybrominated Biphenyls. No studies were located regarding body weight effects in humans after dermal exposure to PBBs.

No treatment-related effects on body weight were reported in rabbits given a dose of 1 mg/kg/day of a commercial mixture of octabromobiphenyl in corn oil via application to the intact shaved dorsal skin for 2 weeks (Waritz et al. 1977). No significant effect on final body weight was reported in groups of four rabbits after application of a single dose of up to 10,000 mg/kg of octabromobiphenyl mixture in corn oil to the abraded and occluded dorsal skin over a 24-hour period (Waritz et al. 1977). The observation period was 14 days. In a similar study with a commercial mixture of hexabromobiphenyl, rabbits treated with 1,000 mg/kg showed no weight gain over 14 days. Doses of 5,000 and 10,000 mg/kg induced an 11% and 20% weight loss, respectively, whereas, a dose of 100 mg/kg was without effect (Waritz et al. 1977).

Polybrominated Diphenyl Ethers. 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.

5.2.3.3 Immunological and Lymphoreticular Effects

Polybrominated Biphenyls. Several immunological parameters were evaluated in a group of 28 workers from the Michigan Chemical Company who were involved in the manufacturing and distribution of PBBs including FireMaster FF-1 (Stross et al. 1981). It is assumed that the main route of exposure was dermal, but inhalation and/or oral exposure cannot be ruled out. The subjects had worked directly with PBBs during the previous 5 years. Immunological studies included determination of immunoglobulin levels, skin testing, and lymphocyte transformation studies. No abnormalities in lymphocyte number or function could be determined when compared to an unexposed group. One of three blastogenic responses (PWM) was significantly reduced ($p<0.01$) relative to controls, but was within the normal range for the laboratory. No specific information was provided regarding the skin testing and immunoglobulin levels.

No studies were located regarding immunological effects in animals after dermal exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding immunological effects in humans or animals after dermal exposure to PBDEs.

5.2.3.4 Neurological Effects

Polybrominated Biphenyls. Twenty-five workers at a PBB manufacturing plant displayed mean scores on tests of memory and learning that were typical for people of their age, educational, occupational, and cultural backgrounds, even though they displayed an elevated mean PBB concentration in adipose tissue (9.33 ppm compared with 3.94 ppm for farm residents) (Brown et al. 1981). Workers with the highest concentrations of PBB in adipose tissue showed no evidence of memory dysfunction in these tests. Because 15/25 "directly handled PBBs or performed maintenance work in the area where PBBs were manufactured," it is likely that at least part of the exposure was by the dermal route.

No studies were located regarding neurological effects in animals after dermal exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding neurological effects in humans or animals after dermal exposure to PBDEs.

5.2.3.5 Reproductive Effects

Polybrominated Biphenyls. Eleven workers in a PBB manufacturing company in Michigan displayed no differences in the distribution of sperm counts, motility, or morphology compared with a control group of 52 unexposed men (Rosenman et al. 1979). PBBs were detected in the serum of all exposed subjects and in only one unexposed subject, but mean or individual serum PBB values were not reported.

No studies were located regarding reproductive effects in animals after dermal exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding reproductive effects in humans or animals after dermal exposure to PBDEs.

5.2.3.6 Developmental Effects

Polybrominated Biphenyls. No studies were located regarding developmental effects in humans or animals after dermal exposure to PBBs.

Polybrominated Diphenyl Ethers. No studies were located regarding developmental effects in humans or animals after dermal exposure to PBDEs.

5.2.3.7 Cancer

Polybrominated Biphenyls. No studies were located regarding cancer in humans after dermal exposure to PBBs.

An unspecified PBB mixture (purity not reported) was not tumorigenic when applied to the shaved dorsal skin of female CD-1 mice at a dose of 3.3 mg/kg twice weekly for 30 weeks; no tissues other than skin were examined (Berry et al. 1978, 1979). This same treatment did not promote the development of skin tumors in mice pretreated with a single application of a tumor initiator, dimethylbenzanthracene (DMBA), 1 week prior to PBB exposure (Berry et al. 1978, 1979). The results of these studies must be interpreted with caution, since a dose-response study was not done (i.e., only one dose level was tested, and the doses may have been too low). Toxic doses of FireMaster FF-1 promoted development of skin tumors in female HRS/J hairless mice (Poland et al. 1982). A single dermal application of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) initiator, followed by twice weekly applications of FireMaster FF-1 at

66.7 mg/kg for 5 weeks and then 33.3 mg/kg for 15 weeks, resulted in a 60% (9/15) incidence of papillomas compared to 0% (0/23) in MNNG-only controls. Toxic effects included mortality, which caused the dose reduction after 5 weeks, and severe hepatomegaly and hepatic porphyria.

Polybrominated Diphenyl Ethers. No studies were located regarding cancer in humans or animals after dermal exposure to PBDEs.

5.3 GENOTOXICITY

Polybrominated Biphenyls. No studies were located regarding genotoxic effects in humans following inhalation, oral, or dermal exposure to PBBs.

In vivo genotoxicity studies of PBBs in animals are summarized in Table 5-7. Administration of single oral doses between 50 and 1,000 mg of FireMaster FF-1/kg (purity not reported) by gavage in corn oil to male and female B6C3F1 mice and male Fischer-344 rats did not induce unscheduled deoxyribonucleic acid (DNA) synthesis in hepatocytes (Mirsalis et al. 1985, 1989). However, doses ≥200 mg/kg significantly increased hepatic cell proliferation in mice, but not in rats. The increase in cell proliferation without a change in unscheduled DNA synthesis suggests that PBBs acted as a promoter rather than directly causing DNA damage (initiator). A commercial mixture of decabromobiphenyl did not induce gene mutation in *Salmonella typhimurium* bacteria that were intraperitoneally injected into male CFLP mice in a host-mediated assay (Millischer et al. 1980). This decabromobiphenyl mixture also did not induce micronuclei in bone marrow erythrocytes of mice (Millischer et al. 1980). The mice in the host-mediated assay and micronucleus test were orally treated (method not specified) with total doses of 5,000, 10,000, or 20,000 mg/kg, administered in two equal doses 24 hours apart.

In vitro studies indicate that PBBs are not directly genotoxic. As summarized in Table 5-8, PBBs did not exhibit mutagenic activity when tested in the prokaryotic organisms *S. typhimurium* (Haworth et al. 1983; Millischer et al. 1980; NTP 1983) and *E. coli* (Rossman et al. 1991) with or without activation systems in the limited number of studies available. *In vitro* testing in eukaryotic cells resulted in negative genotoxic responses in hamster cells (Galloway et al. 1987; Kavanagh et al. 1985; Williams et al. 1984), rat liver cells (Kavanagh et al. 1985; Williams et al. 1984), mouse liver and lymphoma cells (Myhr and Caspary 1991; Williams et al. 1984), and human fibroblasts (Williams et al. 1984).

Table 5-7. Genotoxicity of PBBs and PBDEs *In Vivo*

Species (test system)	End point	Results	Reference
PBBs			
Mammalian cells			
Rat hepatocytes	Unscheduled DNA synthesis	–	Mirsalis et al. 1989 (FF-1)
Mouse hepatocytes	Unscheduled DNA synthesis	–	Mirsalis et al. 1985, 1989 (FF-1)
Host-mediated assays:			
Salmonella typhimurium (mouse hosted-mediated)	Gene mutation	–	Millischer et al. 1980 (DBB)
Micronucleus test:			
Mouse bone marrow erythrocytes	Chromosome aberration (micronuclei)	–	Millischer et al. 1980 (DBB)
PBDEs			
Cytogenicity			
Rat bone marrow cells (one-generation reproduction study)	Chromosome aberration	–	Norris et al. 1973, 1975 (77.4% decaBDE, 21.8% nonaBDE)

– = negative result; DBB = decabromobiphenyl commercial mixture; decaBDE = decabromodiphenyl ether; DNA = deoxyribonucleic acid; FF-1 = FireMaster FF-1; nonaBDE = nonabromodiphenyl ether; PBBs = polybrominated biphenyls; PBDEs = polybrominated diphenyl ethers

Table 5-8. Genotoxicity of PBBs and PBDEs *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
PBBs				
Prokaryotic organisms:				
Salmonella typhimurium (plate incorporation)	Gene mutation	–	–	NTP 1983 (FF-1)
Salmonella typhimurium (plate incorporation)	Gene mutation	–	–	Haworth et al. 1983 (HBB)
Salmonella typhimurium (plate incorporation)	Gene mutation	–	–	Millischer et al. 1980 (DBB)
Escherichia coli (culture)	Gene mutation	–	–	Rossman et al. 1991 (PBB)
Eukaryotic organisms				
Mammalian cells				
Chinese hamster CHO cells (cell culture)	Chromosomal aberration	–	–	Galloway et al. 1987 (HBB)
Chinese hamster CHO cells (cell culture)	Sister chromatid exchange	–	–	Galloway et al. 1987 (HBB)
Chinese hamster V79 cells (cell culture)	Gene mutation	–	–	Kavanagh et al. 1985 (BP-6)
Chinese hamster V79 cells (cell culture)	Gene mutation	No data	–	Kavanagh et al. 1985 (2,4,5-HBB)
Chinese hamster V79 cells (cell culture)	Gene mutation	No data	–	Kavanagh et al. 1985 (3,4,5-HBB)
Chinese hamster V79 cells (cell culture)	Gene mutation	–	–	Kavanagh et al. 1985 (3,4-TBB)
Rat liver cells WB (cell culture)	Gene mutation	No data	–	Kavanagh et al. 1985 (2,4,5-HBB)
Rat liver cells WB (cell culture)	Gene mutation	No data	–	Kavanagh et al. 1985 (3,4,5-HBB)
Mouse lymphoma cells L5178Y (cell culture)	Gene mutation	–	–	Myhr and Caspary 1991 (FF-1)
Rat liver cells (cell culture)	DNA repair	No data	–	Williams et al. 1984 (FF-1)
Mouse liver cells (cell culture)	DNA repair	No data	–	Williams et al. 1984 (FF-1)
Hamster liver cells (cell culture)	DNA repair	No data	–	Williams et al. 1984 (FF-1)
Rat liver cells (cell culture)	Gene mutation	No data	–	Williams et al. 1984 (FF-1)
Human fibroblast D-550 (cell culture)	Gene mutation	–	No data	Williams et al. 1984 (FF-1)

Table 5-8. Genotoxicity of PBBs and PBDEs *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
PBDEs				
Prokaryotic organisms:				
Salmonella typhimurium (plate incorporation)	Gene mutation	–	–	NTP 1986 (decaBDE)
Mammalian cells:				
Mouse lymphoma L5178Y cells (cell culture)	Gene mutation	–	–	NTP 1986 (decaBDE)
Chinese hamster Sp5/V79cells (cell culture)	Gene recombination	No data	–	Helleday et al. 1999 (2,2',4,4'-tetraBDE)
Chinese hamster SPD8/V79cells (cell culture)	Gene recombination	No data	+	Helleday et al. 1999 (2,2',4,4'-tetraBDE)
Chinese hamster Sp5/V79 cells (cell culture)	Gene recombination	No data	+	Helleday et al. 1999 (3,4-diBDE)
Chinese hamster SPD8/V79 cells (cell culture)	Gene recombination	No data	+	Helleday et al. 1999 (3,4-diBDE)
Chinese hamster Sp5/V79 cells (cell culture)	Gene recombination	No data	+	Helleday et al. 1999 (2-monoBDE)
Chinese hamster SPD8/V79 cells (cell culture)	Gene recombination	No data	+	Helleday et al. 1999 (2-monoBDE)
Chinese hamster ovary cells (cell culture)	Sister chromatid exchange	–	–	NTP 1986 (decaBDE)
Chinese hamster ovary cells (cell culture)	Chromosomal aberrations	–	–	NTP 1986 (decaBDE)

– = negative result; 2,2',4,4'-tetraBDE = 2,2',4,4'-tetrabromodiphenyl ether; 2,4,5-HBB = 2,2',4,4',5,5'-hexabromobiphenyl; 2-monoBDE = 2-bromodiphenyl ether; 3,4,5-HBB = 3,3',4,4',5,5'-hexabromobiphenyl; 3,4-diBDE = 3,4-dibromodiphenyl ether; 3,4-TBB = 3,3',4,4'-tetrabromobiphenyl; BDE = brominated diphenyl ethers; BP-6 = FireMaster BP-6; DBB = decabromobiphenyl commercial mixture; decaBDE = decabromobiphenyl ether; DNA = deoxyribonucleic acid; FF-1 = FireMaster FF-1; HBB = hexabromobiphenyl (unspecified); PBB = unspecified mixture; PBBs = polybrominated biphenyls; PBDEs = polybrominatedd diphenyl ethers

An *in vitro* study with a ^{14}C-PBB mixture containing 12 major components found only traces of radioactivity bound to rat liver microsomal macromolecules (Dannan et al. 1978a). Binding, however, was dependent upon the type of microsomes used to activate the PBB mixture. Microsomes isolated from animals pretreated with methylcholanthrene (MC) bound twice the amount of radioactivity compared with controls, whereas activation with phenobarbital (PB) or PBB bound 5 times more radioactivity than control microsomes. Also, the authors showed that no radioactivity was covalently bound to DNA following incubation with ^{14}C-PBB. The type of microsomes used or the presence or bsence of nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) in the incubation mixture made no difference.

Although it appears that PBBs are not mutagenic, due to their enzyme induction properties, they may potentiate the genotoxic activity of other compounds by activation to reactive intermediates.

Polybrominated Diphenyl Ethers. No studies were located regarding genotoxic effects in humans following inhalation, oral, or dermal exposure to PBDEs.

A limited amount of information has been published on the genotoxicity of PBDEs in animals *in vivo* or in prokaryotic and eukaryotic cells *in vitro* as summarized in Tables 5-7 and 5-8, respectively. Cytogenetic examination of bone marrow cells showed no increase in aberrations in maternal and neonatal rats following maternal oral exposure to ≤100 mg/kg/day of a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaBDE) for 90 days prior to mating and during mating, gestation, and lactation (Norris et al. 1973, 1975a). *In vitro* assays found that decaBDE did not induce gene mutations in bacterial cells (*S. typhimurium* TA98, TA100, TA1535, or TA1537) or mammalian cells (mouse lymphoma L5178Y cells), and did not induce sister chromatid exchange or chromosomal aberrations in Chinese hamster ovary cells (NTP 1986). *In vitro* exposure to the congeners 2,2',4,4'-tetraBDE (BDE 47), 3,4-diBDE (BDE 12), and 2-monoBDE (BDE 1) caused increased recombinogenic activity at the HGPRT locus in Chinese hamster SPD8 and Sp5 V79 cells (Helleday et al. 1999).

5.4 TOXICOKINETICS

Data regarding the toxicokinetics of PBBs in humans are limited to information derived from cases of accidental ingestion of food contaminated with PBBs and cases of occupational exposure by the

inhalation and dermal routes. These data provide qualitative evidence that PBBs are absorbed in humans by the inhalation, oral, and dermal routes. Limited quantitative data in animals indicate that some PBB congeners are well absorbed after oral exposure. Dermal absorption data for animals are insufficient for estimating absorption rates, and no inhalation absorption data were located. In blood, 80% of PBBs, are bound to protein and 20% are associated with lipids. The distribution pattern of PBBs did not differ significantly between humans and animals and among animal species. Due to their lipophilic nature, PBBs, especially the highly brominated congeners, tend to accumulate in lipid-rich tissues. Greater relative amounts of PBBs are usually found in the liver, adipose, skin, and breast milk. Certain components of PBB mixtures are metabolized by the microsomal monooxygenase system catalyzed by cytochrome P-450 of the type induced by phenobarbital. The rate of metabolism of some PBB congeners depends on the bromine substitution pattern. PBB congeners of low bromine content are transformed into hydroxylated derivatives that are predominately eliminated in the urine. Highly brominated congeners are either retained or excreted unchanged in the feces. As discussed in Section 5.3.2, the exact mechanism of PBB toxicity is not known. It has been suggested, however, that the mechanism for some congeners is related to the enhancement of gene expression triggered by initial binding to the same cytosolic receptor (Ah) involved in some effects of PCBs and PCDDs.

5.4.1 Absorption

5.4.1.1 Inhalation Exposure

Polybrominated Biphenyls. No studies were located regarding absorption of PBBs in humans after inhalation exposure. However, absorption of PBBs by inhalation (and by dermal contact) in humans can be inferred by the relatively high levels of PBB residues detected in adipose tissue and serum of workers involved in PBB manufacturing (Brown et al. 1981; Landrigan et al. 1979; Stross et al. 1981).

No studies were located regarding quantitative absorption of PBBs in animals after inhalation exposure to PBBs. However, increased bromine concentrations were found in the liver and adipose tissue of rats exposed continuously to a commercial mixture of octabromobiphenyl for 15 weeks, suggesting that absorption had occurred (Waritz et al. 1977).

Polybrominated Diphenyl Ethers. 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 2001a, 2001b). 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.

5.4.1.2 Oral Exposure

Polybrominated Biphenyls. Quantitative oral absorption data in humans were not located, but reports of increased levels of PBB residues in tissues and serum of individuals accidentally exposed to contaminated food indicate that gastrointestinal absorption of PBBs had occurred (Eyster et al. 1983; Humphrey and Hayner 1975; Landrigan et al. 1979; Miceli et al. 1985; Wolff et al. 1982).

Absorption of PBBs from the gastrointestinal tract in animals can be inferred from the numerous reports of adverse effects (Section 5.2.2) and increased residue levels in tissues following oral administration of these compounds (Section 5.4.2.2); however, few quantitative data exist. By comparing the amount of radioactivity in the feces of rats administered a single 1 mg/kg oral dose of ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl (BB-155) with that monitored after a single intravenous injection of the compound, it was estimated that ≈93% of the oral dose was absorbed over a 24-hour period (Matthews et al. 1977). Data obtained from similar experiments with PBB-155 later confirmed these results (Tuey and Matthews 1980). It was also demonstrated that absorption of this hexabromobiphenyl congener was independent of the dose, since ≥90% was absorbed over a dose range of 1–30 mg/kg (Matthews et al. 1977). In contrast with the high absorption rate for the hexabromobiphenyl congener, a commercial octabromobiphenyl mixture (45.2% octa, 47% nona, 5.7% deca, 1.8% hepta) appeared to be less well absorbed by rats after administration of a single dose of 1 mg/kg (Norris et al. 1975a). Within the first 24 hours after dosing, 61.9% of the dose was found in the feces. This indicates that at least 38.1% of the dose was absorbed, but absorption may have been higher, since biliary excretion may have occurred.

Polybrominated Diphenyl Ethers. No information was located regarding absorption of PBDEs in humans following oral exposure. Information regarding oral absorption in animals is available from studies of commerical PBDE mixtures and individual PBDE congeners. As summarized below, absorption of decaBDE is poor, whereas the lower brominated PBDEs are readily absorbed .

Studies with ^{14}C-labeled decaBDE (BDE 209) in rats consistently indicate that gastrointestinal absorption of this congener is low at 10% or less in rats (El Dareer et al. 1987; Klasson Wehler et al. 2001; Morck and Klasson Wehler 2001; Morck et al. 2003; Norris et al. 1973, 1975c). Following treatment with a

single 1 mg/kg dose of ^{14}C-decaBDE, administered as a former 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, 1975b). Two feeding studies were conducted in which rats were exposed to a higher purity 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 amount ingested in the 72 hours following ^{14}C-decaBDE intake 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 contents (<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).

A study of laboratory synthesized ^{14}C-decaBDE (>98% pure) was conducted in which a single 3 µmol/kg (≈3 mg/kg) dose was administered to normal or bile duct-cannulated male Sprague-Dawley rats by gavage (Klasson Wehler et al. 2001; Morck and Klasson Wehler 2001; Morck et al. 2003). The compound was suspended in a novel vehicle, Lutrol F127/soya phospholipone (34:16, w/w)/water, that was formulated to enhance solubility and improve absorption in comparison with previous studies of decaBDE, including those summarized above. In the normal rats, approximately 90% (86–93%, n=8) of the dose was found in the feces after 3 days; cumulative recovery after 7 days was 91% (87–95%, n=4). For the two bile duct-cannulated rats, an average of 88 and 9.5% of the dose was recovered in the feces and bile, respectively, within 3 days. There was a large variation in the fecal excretion of the two cannulated rats, which might have been due to the fact that no bile salts were added to compensate for the collected bile; the investigators concluded that this likely affected absorption. Because the biliary excretion of decaBDE was approximately 10% of the dose, it appears that at least this much of the decaBDE was absorbed. The investigators could not exclude that more than 10% of the dose had been absorbed because 65% of the radioactivity excreted in the feces was metabolites.

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 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). The doses were designed to mimic environmental exposure levels. 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 assessed the following six congeners: 2,2',4,4'-tetraBDE (BDE 47), 2,2',3,4,4'-pentaBDE (BDE 85), 2,2',4,4',5-pentaBDE (BDE 99), 2,2',4,4',6-pentaBDE (BDE 100), 2,2',4,4',5,5'-hexaBDE (BDE 153), and 2,2',4,4',5,6'-hexaBDE (BDE 154) (Hakk et al. 2001). Based on liver, carcass, and unrecovered levels 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 assessed the following eight congeners: 2,2',4,4',5,5'-hexaBDE (BDE 153), 2,2',4,4',5,6'-hexaBDE (BDE 154), 2,2',3,4,4',5',6-heptaBDE (BDE 183), 2,3,3',4,4',5,6-hepta-BDE (BDE 190), an unknown heptaBDE, and three unknown octaBDEs (Huwe et al. 2002b). Based on liver, carcass, and unrecovered levels 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.

A single 14.5 mg/kg (30 µmol/kg) gavage dose of ^{14}C-2,2',4,4'-tetraBDE (BDE 47) in corn oil was well absorbed by rats and 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 was 93–95%.

5.4.1.3 Dermal Exposure

Polybrominated Biphenyls. No studies were located regarding absorption of PBBs in humans or animals after dermal exposure to PBBs. However, absorption of PBBs through the skin in humans can be inferred by the relatively high levels of PBB residues detected in the adipose tissue and serum of workers involved in the manufacturing of these chemicals (Brown et al. 1981; Landrigan et al. 1979; Stross et al. 1981). It is assumed that dermal route predominates, but inhalation and/or oral exposure cannot be ruled out.

Similarly, dermal absorption in rabbits can be inferred from reports of lethality and liver effects observed after application of a commercial mixture of hexabromobiphenyl to abraded and occluded dorsal skin (Waritz et al. 1977).

Polybrominated Diphenyl Ethers. No information was located regarding dermal absorption of PBDEs in humans. The only information regarding dermal absorption in animals is that from a study of absorption in an *in vitro* preparation (Hughes et al. 2001). In that study, ^{14}C-decaBDE dissolved in tetrahydrofuran was applied to dorsal skin (three dose levels) excised from adult hairless female mice and fractions of receptor fluid were collected over a 24-hour period. Transfer of radioactivity to the receptor fluid was minimal, only 0.07 to 0.34% of the applied radioactivity. Two to 20% of the radioactivity was found in the skin, and the lowest dose applied had the highest percentage of the dose in the skin. Washing the skin with solvent 24 hours after application removed 77–92% of the applied dose.

5.4.2 Distribution

5.4.2.1 Inhalation Exposure

Polybrominated Biphenyls. No studies were located regarding distribution of PBBs in humans after inhalation exposure.

Limited information was located regarding distribution of PBBs in animals after inhalation exposure. Increased bromide concentrations were observed in the liver and adipose tissue of rats exposed continuously to vapors of a commercial octabromobiphenyl mixture (33% octa, 60% nona, 6% deca, 1% hepta) (3.5 pg octabromobiphenyl/L air at equilibrium) for 15 weeks (Waritz et al. 1977). Relative to controls, the concentration of bromide in liver and fat was increased by 39 and 100%, respectively; bromide concentration in skeletal muscle was not affected by treatment. No further details were provided.

Polybrominated Diphenyl Ethers. No information was located regarding distribution of PBDEs in humans following inhalation exposure.

The distribution of bromine was examined in tissues of rats after inhalation exposure 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 increases in the liver were not as marked as in the lungs or in adipose tissue.

5.4.2.2 Oral Exposure

Polybrominated Biphenyls. Numerous reports have been published regarding levels of PBBs in serum, adipose tissue, breast milk, placenta, and cord serum of humans exposed to PBBs via the diet (Anderson et al. 1978d; Eyster et al. 1983; Landrigan et al. 1979; Stross et al. 1979, 1981; Wolff et al. 1979a, 1982). By using paired sampling, several significant correlations were determined (Eyster et al. 1983). For example, in parturient women from Michigan, statistically significant correlations were found between PBB levels in maternal serum and placenta, cord serum, breast milk, and adipose; and between PBB levels in adipose tissue and breast milk. In addition, there was a significant correlation between PBB levels in serum and feces and between serum and biliary fluid samples in farmers and chemical workers in Michigan. In groups of pregnant, nonpregnant, and male chemical workers the serum to adipose tissue PBB concentration ratios ranged from 1:140 to 1:160, but in male farmers, this ratio was 1:325–329 (Eyster et al. 1983). The latter value is consistent with other reports regarding Michigan populations (Landrigan et al. 1979; Wolff et al. 1982). It is unclear why the partitioning ratios between male chemical workers and farmers should differ. The investigators noted that the group of farmers was much larger and might represent a better sample, as well as the possibilities that the farmers may have been more physically active or, for a variety of reasons, may have had lower total serum lipids (the amount of serum lipid might have affected the serum concentration of PBBs). PBB levels in body tissues and fluids are further discussed in Section 8.4.

Analysis of postmortem tissue samples from 15 subjects in the Grand Rapids, Michigan area indicated that renal fat had the highest single PBB concentration (1.65 µg/g wet weight) and the highest mean concentration (0.475 µg/g) (Miceli et al. 1985). In regards to adipose, PBB concentrations in different tissues, could be divided into three range groups: high (ratios of 0.45–0.56, adrenal, atheromatous aorta, and thymus), medium (ratios of 0.1–0.28, pancreas, liver, and left ventricle), and low (ratios of 0.02–0.09, kidney, lung, brain, skeletal muscle, thyroid, and nonatheromatous aorta).

As with the structurally related PCBs (Agency for Toxic Substances and Disease Registry 2000), PBBs are rapidly (minutes to hours) cleared from the blood and initially accumulate mainly in the liver, lungs, and muscle (Domino et al. 1982; Matthews et al. 1977). Due to their high affinity for lipid-rich tissues, PBBs are subsequently redistributed to adipose and skin for storage or metabolism in the liver, and a dynamic equilibrium of PBB concentrations is established among all tissues for each PBB homolog (Tuey and Matthews 1980).

In rats treated by gavage with one or four daily doses of ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl (BB 153), initial concentrations of radioactivity were highest in muscle, liver, and adipose tissue, but later redistribution to adipose tissue (4–7 days after the last dosing) resulted in lower concentrations in liver and muscle (Matthews et al. 1977). In rats dosed daily with ^{14}C-BB 153 over a 30-day period, tissue concentrations on day 31 were (in increasing order): blood, muscle, liver, skin, and adipose and were in general agreement with those predicted by a physiological compartment model (Tuey and Matthews 1980). When the model was scaled to nonlactating humans as discussed in Section 5.4.5 (Physiologically Based Pharmacokinetic/Pharmacodynamic Models), human intake of 9.8 g of the congener over a 230-day period would result in peak concentrations of 720 and 2.1 ppm in fat and blood, respectively, 5 years after the onset of exposure. The model also predicted that the body burden after 5 years would be 5.2 g and the half-life 6.5 years. This half-life is shorter than the 12 years (median, range 4–97 years) calculated for hexabromobiphenyl in a Michigan cohort (Lambert et al. 1990) (see Section 5.8.1).

In rats fed diets containing octabromobiphenyl mixture for several weeks, adipose tissue and liver accumulated much more bromine than did skeletal muscle (Lee et al. 1975b). For example, after 2 weeks of treatment, adipose of rats dosed with 50 mg/kg/day had 200 times more bromine than did adipose of control rats; the liver of these rats had 100 times more bromine than the livers of controls. Feeding a PBB-free diet for 2 weeks decreased PBB levels in liver and muscle, but not in fat. Eighteen weeks after exposure, the concentration of bromine in the adipose tissue of rats dosed with 50 mg/kg/day continued to increase to ≈840 times that of controls. Similar results were reported by Norris et al. (1975a, 1975b). These investigators also reported that 16 days after a single dose of octabromobiphenyl mixture in rats, PBB residues were present in the adrenals, adipose tissue, heart, and skin at levels ranging from 0.14 to 0.25% of the administered dose; the liver, pancreas, and spleen contained lesser amounts.

The distribution and elimination of PBBs from tissues were examined in rats over a period of 112 days after a single oral dose of FireMaster FF-1 (Domino et al. 1982). Elimination from blood was best described by a three-compartment model, and an elimination half-life from whole blood of 145 days was estimated. Relative to the three compartments (C1, C2, and C3): C1 consisted of heart, kidney, spleen, and whole blood; C2 included liver, lung, cerebrum, cerebellum, and testes; and C3 included subcutaneous fat. PBB residues in C1 rose quickly, peaked within 5 hours of dosing and then fell rapidly; a half-life of 3.62 hours was estimated. PBB peaked in C2 at 12 hours and then decreased; the half-life was 17.6 hours. In C3, levels of PBB peaked only after 1 week and remained elevated for several weeks; the estimated half-life was 31.1 days. Tissues with PBBs in order of increasing concentration were:

blood, spleen, kidney, and heart in C1, and testes, cerebrum, cerebellum, lung, and liver in C2. Simulations of different body fat proportions showed that reduction in body fat decreased the half-life of the chemical considerably. According to the investigators (Domino et al. 1982), tissues within C1 and C2 may be at greater risk of toxicity during the subacute phase of PBB ingestion. In their view, this could explain the fact that blood PBB levels in Michigan families were not positively correlated with toxic symptoms of exposure to PBBs (see Section 5.2).

In mink treated with FireMaster FF-1 in the diet for up to 11 months, the concentration of PBB residues in adipose tissue were higher than in brain and skeletal muscle at all times (Aulerich and Ringer 1979; Ringer et al. 1981). The source of the PBBs (FireMaster FF-1 versus food contaminated with PBBs) did not seem to have a significant influence on the qualitative or quantitative distribution of residues in tissues. Sows fed FireMaster BP-6 in the diet for 12 weeks also accumulated PBBs in adipose tissue; on a fat basis, the highest concentration of PBBs was found in the liver, followed by adipose, kidney, and brain (Werner and Sleight 1981). Distribution studies in guinea pigs after a single dose of FireMaster FF-1 showed preferential accumulation of residues in liver, kidneys, and lungs 2 days after dosing (Ecobichon et al. 1983). This was followed by a slow decrease in these organs, but levels in adipose tissue reached a maximum between 7 and 14 days after dosing and then decreased.

Several studies have examined the distribution of PBB residues in offspring after maternal exposure to PBBs during gestation and/or lactation. In 4-week-old pigs exposed *in utero* and via lactation to FireMaster BP-6, PBBs accumulated preferentially in adipose tissue and liver on a wet tissue basis. Over a wide range of doses, however, adipose had at least two times the PBB concentration compared to the liver (Werner and Sleight 1981). PBB levels in tissues of sows were comparable to those measured in tissues of 4-week-old pigs. On a fat basis, the liver had the highest concentration of PBBs in both sows and the young. In pigs exposed only *in utero*, PBB levels in liver and adipose were similar and considerably lower than in tissues of sows or 4-week-old pigs, suggesting that far more PBBs are transferred through lactation than through the placenta. A similar conclusion was reached in rat studies (McCormack and Hook 1982; Rickert et al. 1978). In contrast, PBB levels in liver and body fat of guinea pigs exposed briefly through lactation were considerably lower than the tissue levels acquired transplacentally in a 2-day period (Ecobichon et al. 1983). A biological half-life of 22 days in tissues of dams and pups was estimated in that study (Ecobichon et al. 1983).

Polybrominated Diphenyl Ethers. Results of tissue distribution studies of decaBDE are consistent with the poor absorption and rapid fecal elimination of this congener. In male rats administered a single

gavage dose of 1 mg/kg of a ^{14}C-labeled former commercial decaBDE mixture (77.4% pure containing 21.8% nonaBDE and 0.8% octaDBE), radioactivity could be detected on day 1 in all sampled tissues (adipose, skin, liver, heart, adrenals, spleen, pancreas) (Norris et al. 1975b). 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). Norris et al. (1975b) also examined the bromine content of tissues from rats administered 1 mg/kg/day doses of the 77.4% decaBDE mixture in the diet for 90 days, followed by an additional 90-day period on a control diet. During the recovery period, interim sacrifices were conducted on days 0, 10, 30, and 60. On recovery day 0, serum and kidneys had the same amounts of bromine as the controls, although adipose levels were increased approximately 4-fold and remained essentially unchanged over the 90-day recovery period. In the liver, the bromine content was elevated on day 0 of the recovery period, but decreased and stabilized near control values after recovery day 10. Tissue analyses in rats that were similarly exposed to 0.01-1 mg/kg/day of 77% decaBDE for 12 months showed adipose bromine levels that were slightly increased during the first 180 days and not significantly different than controls at the end of the year; no significant bromine accumulation occurred in serum, liver, kidneys, skeletal muscle, or testes (Norris et al. 1975b). Pregnant rats that were administered the 77% commercial decaBDE mixture by gavage on GDs 6–15 showed no significant increase in bromine content in the liver of fetuses on gestation day 21 compared to controls (Norris et al. 1975b). Placental transfer cannot be totally ruled out since no other fetal tissues were examined.

Two feeding studies were conducted in which rats were exposed to a higher purity 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, or 9–10, or 9–11 (El Dareer et al. 1987; NTP 1986). The compound was poorly absorbed in both studies. In the first study, dietary concentrations ranged from 238 to 51,100 ppm (≈20–4,500 mg/kg/day), and ≈91–100% of the ^{14}C was recovered in the feces dose in 72 hours. In the second study, rats were exposed to dietary concentrations of 277 or 48,000 ppm (≈20 or 4,300 mg/kg/day), and fecal recovery of ^{14}C ranged from ≈83 to 86% of the dose. Both studies found only trace levels of radioactivity in any organ or tissue at any time point, and the maximum total ^{14}C activity detected in the body at any time was only ≈1% of the dose. In general, the highest amounts of ^{14}C were found in the liver, and there was a tendency for rats fed the lower amounts of decaBDE to retain more radiolabel in tissues than rats fed higher amounts. Analysis of all major organs and tissues in the second study found the highest levels of ^{14}C in the gastrointestinal tract, followed by liver, kidney, lung, skin, and adipose.

A study of laboratory synthesized ^{14}C-decaBDE (>98% pure) was conducted in which a single 3 µmol/kg (≈3 mg/kg) dose was administered to male Sprague-Dawley rats by gavage (Klasson Wehler et al. 2001; Morck and Klasson Wehler 2001; Morck et al. 2003). The compound was suspended in a novel vehicle, Lutrol F127/soya phospholipone (34:16, w/w)/water, that was formulated to enhance solubility and optimize absorption. The amount of the ^{14}C dose remaining in the body after 3 and 7 days was approximately 9% (calculated by subtracting total urine and feces output from 100%). The highest concentrations of radioactivity on a fresh weight basis were in adrenals, kidneys, heart, and liver after both 3 and 7 days. Based on lipid weight, plasma and liver had the highest concentrations and adipose tissue had the lowest concentrations at both time points. The data indicate that the highest concentrations were in plasma and blood-rich tissues and that decaBDE is not readily distributed to adipose. The investigators speculated that decaDBD does not partition into lipids and is transported through aqueous compartments (e.g., serum and bile) due to binding to transport proteins. As discussed below, PBDEs with fewer numbers of bromine preferentially accumulate in adipose. The accumulation of lower brominated congeners appears to be due to their partitioning and retention in lipid-rich tissues, as well as rates of metabolism and elimination that are likely lower than for decaBDE.

The tissue half-life of a commercial pentaBDE product (Bromkal 70) was investigated in male and female Wistar rats following gavage administration of a single high dose (300 mg/kg) in corn oil (von Meyerinck et al. 1990). Groups of four rats/sex were sacrificed at weekly intervals until 10 weeks post-dosing for congeneric analysis of unspecified organs and perirenal fat. Distribution from extra-adipose tissues into the fat was essentially complete after 4 days. Five congeners were detected in the fat and incompletely characterized as a tetraBDE, two pentaBDEs, and two hexaBDEs. The half-life of the congeners generally increased with increasing bromination. The mean half-life of the tetraBDE congener was 19.1 days in males and 29.9 days in females and significantly ($p=0.01$) different between the sexes. The mean half-lives of the penta- and hexaBDE congeners did not significantly differ between the sexes, ranging from 36.8–47.4 days for pentaBDE congener 1, 24.9–25.2 days for pentaBDE congener 2, 44.6–55.1 days for hexaBDE congener 1, and 90.9–119.1 days for hexaBDE congener 2. Although the congeners were not fully identified (likely due to a lack of analytical standards), it is relevant to note that the predominant congeners in Bromkal 70 are 2,2',4,4'-tetra BDE (BDE 47) and 2,2',4,4',5-penta BDE (BDE 99).

Information on the tissue distribution 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 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). The doses were designed to mimic environmental exposure levels. Liver, carcass, and feces were analyzed for major congeners in the penta- and octaBDE formulations 24 hours after the final feeding. The study of the pentaBDE mixture assessed the following six congeners: 2,2',4,4'-tetraBDE (BDE 47), 2,2',3,4,4'-pentaBDE (BDE 85), 2,2',4,4',5-pentaBDE (BDE 99), 2,2',4,4',6-pentaBDE (BDE 100), .2,2',4,4',5,5'-hexaBDE (BDE 153), and 2,2',4,4',5,6'-hexaBDE (BDE 154) (Hakk et al. 2001). An average of 0.4–1.2 and 27.4–45.2% of the dosed congeners occurred in the liver and carcass, respectively, and 11.7–59.1% of the doses were not recovered. The congener distribution patterns in the liver and carcass resembled that of the commercial pentaBDE mixture, suggesting that there was no preferential tissue accumulation of the tested congeners. The study of the octaBDE mixture assessed the following eight congeners: 2,2',4,4',5,5'-hexaBDE (BDE 153), 2,2',4,4',5,6'-hexaBDE (BDE 154), 2,2',3,4,4',5',6-heptaBDE (BDE 183), 2,3,3',4,4',5,6-heptaBDE (BDE 190), an unknown heptaBDE, and three unknown octaBDEs (Huwe et al. 2002b). Four of congeners (BDE 90, the unknown heptaBDE, and two of the unknown octaBDEs) were not detected in the liver, and one congener (an unknown octaBDE) was not detected in the carcass. Of the detected congeners, an average of 1.1–1.7% and 16.2–62.9% of the dose occurred in the liver and carcass, respectively. Unlike the lower brominated congeners (tetra- to hexaBDEs) in the study of the pentaBDE mixture, tissue accumulation generally decreased with increasing bromination; total retentions in the liver and carcass were 64.4% for one of the hexaBDEs (BDE 153), 27.0–37.1% for the three heptaBDEs, and 16.2–22.8% for two of the octaBDEs. An average of 19.6–83.3% of the congener doses were not recovered. The brain uptake and retention of PBDE congeners was studied in neonatal NMRI male mice that were given a single 1.5 mg/kg dose of ^{14}C-2,2',3,3',4,4',5,5',6,6'-decaDBE (BDE 209), or 0.8 mg/kg of ^{14}C-2,2',4,4',5-pentaDBE (BDE 99), by gavage in a 20% fat emulsion vehicle, on PNDs 3, 10, or 19 and killed 24 hours or 7 days after exposure (Eriksson et al. 2002b, Viberg et al. 2001b, 2003a). In the study with BDE 209, after 24 hours, the mice exposed on PND 3 or 10 had 4.8 or 4.0% of the total administered radioactivity in the brain, respectively, whereas the brain of those exposed on PND 19 had only 0.6% of administered amount (Viberg et al. 2001b, 2003a). Seven days after exposure, the amount of radioactivity in the brain had increased approximately 2-fold in the mice exposed on PND 3 or 10 (to 7.4 or 10.5% of the administered amount), whereas those exposed on PND 19 showed the same amount as at 24 hours post-exposure. These findings suggest that brain uptake of ^{14}C-BDE 209 was more efficient in the younger animals and that the amount of radioactivity reaching the brain increased with time. The pattern of brain retention for BDE 209 was different than that for BDE 99, which showed a decrease over the 7-day period (Eriksson et al. 2002a). In particular, 24 hours after exposure to ^{14}C-BDE 209, the amount of radioactivity in the brain was between 3.7 and 5.1% of the total administered

amount in the three different age categories, whereas the amount declined to 1.3–2.8% of the administered dose after 7 days.

Rats that were administered a single 2.2 mg (≈10 mg/kg) gavage dose of ^{14}C-2,2',4,4'-5-pentaBDE (BDE 99) in corn oil and examined after 72 hours had the largest amounts of radioactivityin the carcass, adipose (epididymal), and gastrointestinal tract (38.8, 3.8, and 6.1% of the dose, respectively (Hakk et al. 1999, 2002). No other tissues contained contained >1% of the radioactivity. Fractionation of the carcass into skin, bone, brain, eyes, and muscle showed that the majority of the ^{14}C was in the skin. When deposition was expressed on a concentration basis, the highest levels of radioactivity.occurred in the most lipid-rich tissues, i.e., adipose, adrenals, gastrointestinal tract, and skin.

A single 14.5 mg/kg dose of ^{14}C-2,2',4,4'-tetraBDE (BDE 47) in corn oil was administered by gavage to rats and mice (Örn and Klasson-Wehler 1998). Approximately 86% of the absorbed dose in rats and 80% of the dose in mice remained in tissues (liver, lung, kidney, brain) at 5 days, mainly in the adipose. In rats, adipose tissue had an approximately 70 times higher concentration of label than the other tissues on a fresh weight basis and 3.5-fold higher on a lipid weight basis. The lungs had the second highest concentration of radiolabel with approximately twice that in the liver and kidneys. In mice, adipose tissues had an approximately 10-fold higher level of label than other tissues on a fresh weight basis. On a lipid weight basis, adipose tissue had a concentration of ^{14}C similar to liver and twice that in lung and kidney.

5.4.2.3 Dermal Exposure

Polybrominated Biphenyls. No studies were located regarding distribution of PBBs in humans after dermal exposure.

Increased liver weight and necrosis were observed in rabbits after application of an unspecified hexabromobiphenyl mixture to the skin, suggesting that PBBs or metabolites reached that organ (Waritz et al. 1977). No further information was available.

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

5.4.2.4 Other Routes of Exposure

Polybrominated Biphenyls. In general, the distribution pattern of PBBs after parenteral administration is similar to that obtained after oral exposure. In rats, immediately after intravenous injection of ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl (BB-153) adipose, skin, muscle, liver, and blood contained ≈29, 20, 40, 10, and 1.5% of the dose, respectively (Matthews et al. 1977). Seven to 42 days postdosing, most of the residue in liver and muscle was redistributed to adipose tissue. The percent of the dose remaining in liver and muscle on day 42 was 0.8 and 3.5%, respectively. The concentration of radioactivity in skin remained relatively constant over a 42-day period. In a similar study in rats, the adipose/blood equilibrium distribution rate was found to be much higher than for any other tissue examined, and 4 days after dosing, adipose tissue contained ≥60% of the body burden (Tuey and Matthews 1980).

The elimination half-times from blood and several tissues were determined in rats administered a single intraperitoneal dose of 10 mg/kg FireMaster BP-6 (Miceli and Marks 1981). Elimination from serum followed first-order kinetics, and a half-time of 23.1 weeks was calculated over a 36-week period after dosing. Adrenal and adipose tissue had the highest PBB concentrations at week 6, and these levels were maintained throughout the 36-week observation period. Concentrations of PBBs were also elevated in the liver, lungs, and pituitary at week 6, whereas PBB levels in brain, kidney, and spleen were several-fold lower. Elimination half-times from adrenal, brain, fat, liver, lung, and spleen were 43.3, 63.0, 69.3, 11.5, 11.2, and 9.0 weeks, respectively. Elimination from heart, kidney, and pituitary did not appear to follow first-order kinetics; thus, elimination half-times from these tissues were not calculated. The concentration of PBB in adipose tissue was at least 4 times higher than in any other tissue, and unlike other tissues, continued to increase, reaching a maximum at week 12 postdosing. The adipose/serum ratio of PBB concentration increased from 222 at 6 weeks to 722 at 36 weeks, reflecting the much more rapid elimination of PBB from serum than from adipose tissue. The investigators estimated that, given the elimination half-time from fat of 69 weeks, >1 µg/g of PBB would remain in fat by the time the rats reached 2 years of age, the end of their lifespans.

The distribution of PBB residues was also examined in pregnant mink and ferrets after injection of a mixture of 2,2',4,4',5,5'-hexabromobiphenyl (BB 153) and 2,2',3,4,4',5,5'-heptabromobiphenyl (BB 180) (Bleavins et al. 1980). Two hours after a single injection in the jugular vein on gestation day 37, the liver, kidney, and adipose tissue of ferret dams had 1.625, 0.108, and 0.124% of the dose/g tissue, respectively. PBB levels in fetal tissues did not exceed 0.013% of the dose (liver). In mink, PBB levels in maternal liver, kidney, and adipose tissue were 1.622, 0.087, and 0.031% of the dose/g tissue, respectively. Fetal liver had the highest amount of PBBs, 0.005% of the dose. In a different experimental series, the

investigators (Bleavins et al. 1980) also showed that the dam's milk was the major route of offspring exposure since PBB levels, on a per gram or per kit basis, were significantly higher in 2-week-old kits than in newborn kits. The ratio of the 2-week PBB concentration to the birth concentration was 3.94/g and 36.66/kit. On a per kit basis, treated newborn kits accumulated 0.80% of the maternal dose through *in utero* exposure.

Polybrominated Diphenyl Ethers. No relevant information was located regarding distribution of PBDEs following exposure by non-natural routes of administration.

5.4.3 Metabolism

Polybrominated Biphenyls. Information regarding the metabolism of PBBs in humans is limited. Chromatographic analysis of serum samples from Michigan dairy farmers and from Michigan Chemical Corporation employees revealed some differences in peak profile between these two groups and between these two groups and the peak profile of FireMaster BP-6 (Wolff and Aubrey 1978; Wolff et al. 1979a). The concentration of two pentabromobiphenyls was lower in the farmers than in the chemical workers. Both farmers and workers had a significantly lower amount of 2,2',3,4,4',5,5'-heptabromobiphenyl than FireMaster BP-6. Other minor differences between the groups were also apparent. The differences in peak profiles between farmers and chemical workers were attributed to different routes of exposure. Farmers had predominantly dietary exposure to PBBs which, according to the authors (Wolff and Aubrey 1978), could have undergone partial metabolism in the animal food source (see below). It should be noted that chemical transformation of the PBBs due to cooking of meat or pasteurization of milk whould not be expected since the temperatures reached during these processes is probably not high enough. As discussed in Chapter 6, temperatures must exceed ≈500 °C for structural alterations of PBBs to occur. Nevertheless, a significant reduction (36–52%) in the concentration of PBBs in pressure-cooked meat relative to raw meat (due to loss of fat) has been reported (Zabik et al. 1978). The decreased heptabromobiphenyl peak in farmers and workers relative to FireMaster BP-6 may reflect poor absorption of this congener since it is not expected to be metabolized readily (Wolff and Aubrey 1978; Wolff et al. 1979a).

Human exposure to PBBs in the Michigan contamination episode occurred primarily through consumption of contaminated meat and dairy products. The limited information available regarding the metabolism of PBBs in dairy cattle is insufficient to ascertain whether humans ingested PBBs or metabolic products of PBBs. In a controlled study, cows fed single or repeated doses of FireMaster BP-6 excreted 50% of the dose in the feces as parent compound (Willet and Durst 1978). Tissues, feces, or

urine were not analyzed for metabolites. Results of studies in rats, and also *in vitro* studies (see below), have shown that highly brominated PBB congeners, such as the major components of FireMaster BP-6, undergo little or no metabolic transformation. Based on the existing information, it seems reasonable to assume that in the Michigan contamination episode, humans consumed mainly unchanged penta-, hexa-, and heptabromobiphenyls.

The *in vivo* metabolism of some PBB congeners and of commercial PBB mixtures has been investigated in a limited number of animal studies. For example, in pigs, intraperitoneal injection of 4-bromobiphenyl yielded three urinary metabolites: 4'-bromo-4-biphenylol (3% of the dose), bromobiphenylol (traces), and 4'-bromobiphenylol (0.5% of the dose) (Kohli and Safe 1976). 4,4'-Dibromobiphenyl (BB 15) yielded four urinary metabolites: 4,4'-dibromo-3-biphenylol (5% of the dose), 3,4'-dibromo-4-biphenylol (1% of the dose), 4'-bromo-3-methoxy-4-biphenylol (1% of the dose), and traces of a dibromomethoxybiphenyl. The authors suggested these results indicate that metabolism of BB 15 occurs through the formation of an arene oxide. The major urinary metabolite of FireMaster BP-6 was a pentabromobiphenylol (1% of the dose), which could have resulted from direct hydroxylation of the minor pentabromobiphenylol isomers in FireMaster BP-6 or by debromination/hydroxylation of the major congener, 2,2',4,4',5,5'-hexabromobiphenyl (BB 153).

In rabbits, metabolism of 2-bromobiphenyl yielded two polar metabolites, one metabolite was identified as 2'-bromo-4-biphenylol (1% of the dose), and the other metabolite (traces) was also a monohydroxylated derivative, but the position of the hydroxyl group was not determined (Kohli et al. 1978). 3-Bromobiphenyl produced a major metabolite (4% of the dose) identified as either 3-bromo-4-biphenylol or 5-bromo-2-biphenylol; a minor dihydroxylated metabolite was also detected. 4-Bromobiphenyl yielded two metabolites: 4'-bromo-4-biphenylol (2% of the dose) and 4'-bromo-3,4-biphenyldiol (1.5% of the dose). Experiments with tritiated 4-bromobiphenyl suggest that the metabolism of this congener involves the formation of an arene oxide.

Similar results have been reported in rats (Sparling et al. 1980). 4'-Bromo-4-biphenylol was the major metabolite of 4-bromobiphenyl (BB 3). 2-Bromobiphenyl (BB 1) was metabolized to 2-bromo-4,4'-biphenyldiol and 2-bromo-4',5-biphenyldiol; 2-bromo-5-biphenylol was a minor metabolite. 3-Bromobiphenyl (BB 2) also yielded diols as major metabolites: 3-bromo-4,4'-biphenyldiol and an unknown diol. The main conclusions of this experiment were: the major site of hydroxylation is at the *para* position of the unsubstituted phenyl ring, and also at the *para* position of the ring for BB 1 and BB 2; substitutions in positions 2 and 3 tend to direct hydroxylation to position *para* and *ortho* (minor) to

the substituents; and the 2- and 3-hydroxylated products are subsequently dehydroxylated, whereas the 4'-hydroxy congener is not.

In contrast to the lower brominated congeners, no major metabolites were identified in the urine or feces of rats treated with a single intraperitoneal dose of 2,2'4,4'5,5'-hexabromobiphenyl, suggesting that this congener is stable and persistent (Safe et al. 1978). Analyses of the feces of dogs administered FireMaster BP-6 orally revealed the presence of a metabolite identified as 6-hydroxy-2,2',4,4',5,5'-hexa-bromobiphenyl (Gardner et al. 1979). This metabolite was not found in the liver, but the parent compound was identified. Since hydroxylation in position 6 of highly substituted congeners is unlikely, it was postulated that the metabolite found in the feces was formed by microbial metabolism of the PBB in the intestinal tract. The *in vitro* metabolism of 2,2',4,4',5,5'-hexabromobiphenyl (BB 153) with liver microsomes of rats induced with either BB 153 or FireMaster BP-6 produced three major metabolic fractions: lipophilic ether soluble polar metabolites, trichloroacetic acid (TCA) soluble conjugates, and macromolecular adducts (Purdy and Safe 1980).

The NADPH-dependent metabolism of a PBB mixture was studied *in vitro* with liver microsomes of rats induced with PB, PBB, or 3-MC (Dannan et al. 1978a). Of the 12 major components of the mixture, only 2,2',4,5,5'-pentabromobiphenyl (BB 101) and a hexabromobiphenyl were metabolized by microsomes from PB- or PBB-treated rats. Of seven structurally identified PBB components, only BB 101 had a bromine-free *para* position. Although BB 101, 2,3',4,4',5-pentabromobiphenyl (BB 118), and 2,2',3,4,4',5'-hexabromobiphenyl (BB 138) have two adjacent unsubstituted carbons, only BB 101 was metabolized. No significant metabolism occurred when the PBB mixture was incubated with microsomes of control rats or MC-induced rats. When 2,2'-dibromobiphenyl (BB 4) and 4,4'-dibromobiphenyl (BB 15) were incubated with liver microsomes of PB-treated rats, only BB 4 was metabolized. These results suggest that the presence of a free *para* position is required for the metabolism of brominated biphenyls and that the bromine content of the molecule is less important in determining metabolism than the position of bromines on the biphenyl nucleus.

A more recent study with hepatic microsomes of induced rats showed that MC pretreatment increased the NADPH-dependent metabolism of PBB congeners (di-, tri-, and tetrabrominated), which had adjacent unsubstituted *ortho* and *meta* positions on at least one ring (Mills et al. 1985). Some penta- and hexabromobiphenyls that have adjacent unsubstituted *ortho* and *meta* positions were not metabolized, suggesting that further bromination prevents metabolism. Pretreatment with PB increased the microsomal metabolism of congeners that have adjacent unsubstituted *meta* and *para* positions on at least one ring. It

was concluded that the rates of metabolism of PBB congeners depends on the position of the bromines and the form of the cytochrome P-450 induced. The ability to metabolize PBBs also depends on the species. For example, hepatic microsomes isolated from rats have a greater potential to metabolize PBBs than hepatic microsomes isolated from pigeons (Borlakoglu and Wilkins 1993).

Polybrominated Diphenyl Ethers. DecaBDE (BDE 209) is predominantly excreted in the feces as unabsorbed parent molecule. Data are available indicating that the small amount of decaBDE that is absorbed can be metabolized (El Dareer et al. 1987; Morck et al. 2003; NTP 1986). A feeding study was conducted in which rats were exposed to a high purity 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, or 9–10, or 9–11, at dietary concentrations of 277 or 48,000 ppm (El Dareer et al. 1987; NTP 1986). 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 decaBDE dose level or time of sacrifice (24, 48, or 72 hours after consumption of ^{14}C-decaBDE). For both dose levels, the percent of ^{14}C dose remaining in the gut contents (<4%) and gut contents (<0.04%) decreased with time. Of the radioactivity recovered, >99% was in the feces and gut contents. HPLC and UV spectral analyses of extracts of feces collected on days 9–11 indicated the presence of decaBDE (81% of recovered radioactivity) and three main unidentified metabolites. The total radioactivity present as the metabolites tended to increase with increasing dietary level of decaBDE (1.5 and 27.9% of total recovery at the low and high dose, respectively). Since absorption of decaBDE was minimal, El Dareer et al. (1987) speculated that the greater extent of metabolism in the high dose rats may be due to induction of hepatic metabolizing enzymes, or gastrointestinal tract bacteria, during the period of feeding unlabeled decaBDE. Analysis of feces from rats following a single 1.07 mg/kg intravenous dose of ^{14}C-decaBDE similarly indicated that the excreted material was predominantly unchanged compound and three main unidentified metabolites (El Dareer et al. 1987; NTP 1986). Unchanged decaBDE comprised 36.5 and 40.4% of the totals for the 0–48-hour and 48–72-hour collection periods, respectively. The HPLC retention times of the metabolites were similar in the oral and intravenous studies. A single metabolite was detected in the bile of rats following a single 0.9 mg/kg intravenous dose of ^{14}C-decaBDE (El Dareer et al. 1987). Approximately 7% of the administered dose appeared in the bile in 4 hours, and <1% of this amount was unchanged decaBDE.

A study of laboratory synthesized ^{14}C-decaBDE (>98% pure) was conducted in which a single 3 µmol/kg (≈3 mg/kg) dose was administered to normal or bile duct-cannulated male Sprague-Dawley rats by gavage (Morck and Klasson Wehler 2001; Morck et al. 2003). The compound was emulsified in a novel vehicle, Lutrol F127/soya phospholipone (34:16, w/w) in water, that was formulated to enhance solubility

and optimize absorption. Feces, bile, and several tissues (liver, kidney, lung, small intestine wall, and adipose) were qualitatively analyzed for metabolites using gel permeation chromatography (GPC) and gas chromatography-mass spectrometry (GC-MS). In the normal rats, approximately 90% of the dose was excreted in the feces in 3 days, mainly as metabolites (65% of dose). In the the two bile duct-cannulated rats, an average of 9.5% of the dose was excreted via the bile in 3 days, almost all of which represented metabolites. Metabolites were characterized as nonextractable, water-soluble, lipid-bound, phenolic metabolites, and parent compound/neutral metabolites. Feces from normal rats showed formation of phenolic and neutral metabolites. Metabolites in the nonconjugated phenolic fraction included six penta- to heptaBDEs containing a guaiacol structure (a hydroxy and a methoxy group) on one of the rings (proposed to be on vicinal carbons), as well as traces of monohydroxylated diphenyl ethers with at least six bromine atoms and uncharacterized metabolites having eight bromine atoms. DecaBDE was the main component in the neutral fraction of the feces, but trace amounts (<0.5% of the decaBDE) of three nonaBDEs also formed. The mechanism by which decaBDE was debrominated to the methoxy-hydroxylated penta- to hepatBDEs was not clear, but was inferred to include a catechol (dihydroxy compound) and/or other reactive intermediates. Analysis of bile showed metabolites that were mainly lipid-bound on day 1 and subsequently water soluble. Neutral compounds in the bile consisted mainly of parent decaBDE and traces of the three nonaBDEs that were found in the feces. Phenolic metabolites in the bile included eight compounds that were the same as those found in the feces. The tissue evaluations showed a high concentration of radioactivity in the liver and small intestine wall (27 and 61%, respectively) that was largely nonextractable, and therefore was assumed to be bound covalently to macromolecules and indicative of adducts formed by metabolism via reactive metabolites. The metabolites identified in the feces of the rats were probably due to debromination of decaBDE, followed by metabolic substitution of vicinal carbons by methoxy or hydroxy groups. The authors did not rule out that the intestinal endoderm or gut microflora could play a role in some of this metabolism. PBDEs with fewer bromines probably have lower rates of metabolism (and elimination) than decaBDE because lower brominated PBDEs partition into, and are retained in, lipid-rich tissues, whereas decaBDE does not partition into lipids.

Information on the metabolism 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 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). The doses were designed to mimic environmental exposure levels. The predominant congeners in the pentaBDE formulation were 2,2’,4,4’-tetraBDE (BDE 47) and 2,2’,4,4’,5-pentaBDE (BDE 99). The octaBDE formulation was mainly comprised of hexaBDE through

nonaBDE congeners and only contained tetra- and pentaBDEs as minor components. Liver, carcass, and feces were analyzed for major congeners in the penta- and octaBDE formulations 24 hours after the final feeding. Urine was not analyzed because the lipophilic nature of the compounds and previous studies indicated that PBDEs are not excreted in the urine.

The study of the pentaBDE mixture assessed the following six congeners: 2,2',4,4'-tetraBDE (BDE 47), 2,2',3,4,4'-pentaBDE (BDE 85), 2,2',4,4',5-pentaBDE (BDE 99), 2,2',4,4',6-pentaBDE (BDE 100), 2,2',4,4',5,5'-hexaBDE (BDE 153), and 2,2',4,4',5,6'-hexaBDE (BDE 154) (Hakk et al. 2001). An average of 0.4–1.2 and 27.4–45.2% of the dosed congeners occurred in the liver and carcass, respectively. The congener distribution patterns in the liver and carcass resembled that of the commercial pentaBDE mixture, suggesting that there was no preferential tissue accumulation of the tested congeners. An average of 11.7–59.1% of the congener doses was not recovered, suggesting that significant metabolism of some of the congeners occurred. The study of the octaBDE mixture assessed the following eight congeners: 2,2',4,4',5,5'-hexaBDE (BDE 153), 2,2',4,4',5,6'-hexaBDE (BDE 154), 2,2',3,4,4',5',6-heptaBDE (BDE 183), 2,3,3',4,4',5,6-heptaBDE (BDE 190), an unknown heptaBDE, and three unknown octaBDEs (Huwe et al. 2002b). Four of congeners (BDE 90, the unknown heptaBDE, and two of the unknown octaBDEs) were not detected in the liver, and one congener (an unknown octaBDE) was not detected in the carcass. Of the detected congeners, an average of 1.1–1.7% and 16.2–62.9% of the dose occurred in the liver and carcass, respectively. Unlike the lower brominated congeners (tetra- to hexaBDEs) in the study of the pentaBDE mixture, tissue accumulation generally decreased with increasing bromination; total retentions in the liver and carcass were 64.4% for one of the hexaBDEs (BDE 153), 27.0–37.1% for the three heptaBDEs, and 16.2–22.8% for two of the octaBDEs. An average of 19.6–83.3% of the congener doses were not recovered, indicating that most of the congeners were metabolized to some extent

The metabolism of ^{14}C-2,2',4,4'-5-pentaBDE (BDE 99) was investigated in normal and bile duct-cannulated rats that were administered a single 2.2 mg (≈10 mg/kg) dose in corn oil by gavage (Hakk et al. 1999, 2002). The feces was the major route of elimination as shown by fecal recovery of 43 and 86% of the administered ^{14}C in 72 hours in the normal and bile-duct cannulated rats, respectively. The feces from the normal rats contained predominantly parent compound and minor amounts of metabolites (>90 and <10% of the extracted radioactivity, respectively). The fecal metabolites were incompletely identified as two mono-OH-pentaBDEs and two mono-OH-tetraBDEs, indicating that some debromination occurred. Metabolites found in the bile included two mono-OH-pentaBDEs, three di-OH-pentaBDEs, and two possible thiol-substituted pentaBDEs. The possible thiol-substituted pentaBDEs

could have been formed through the mercapturic acid pathway, but no glucuronide or sulfate conjugates were found in the bile. Evidence for reactive intermediates in the feces of normal rats was indicated by high nonextractable fractions ranging from 18–52%. Cumulative excretion of radiolabel in the urine at 72 hours, consisting exclusively of uncharacterized metabolites, was 1 and 0.3% of the dose in the normal and bile duct-cannulated rats, respectively. Absorbed BDE 99 was preferentially distributed to lipophilic tissues, in which it accumulated as parent compound; the only tissue in which metabolites were detected was the liver, which contained only trace amounts of OH-metabolites.

Rats given a single gavage dose of 14.5 mg/kg dose of ^{14}C-2,2',4,4'-tetraBDE (BDE 47) in corn oil excreted 14% of the dose in the feces and <0.5% in the urine over a 5-day period (Örn and Klasson-Wehler 1998). The majority of the fecal ^{14}C (≈85% of the parent compound/metabolite fraction) was parent compound. Six metabolites were detected in the feces, but were not precisely identified; the metabolites were tentatively characterized as hydroxlated derivatives (two *ortho*-, one *meta*-, and two *para*-OH-tetraBDEs) and a trace amount of a thiol-tetraBDE. Mice treated in the same manner excreted 20% of the dose in the feces and 33% in the urine over 5 days, indicating that the mouse is more capable of metabolizing BDE 47 than rats. The majority of the fecal ^{14}C in mice (≈70% of the parent compound/metabolite fraction) was the metabolite fraction, which contained the same six metabolites characterized in the rat feces. Approximately 20% of the mouse urinary ^{14}C was characterized as parent compound; the remainder could not be identified, but was speculated to arise from decomposition of a labile metabolite(s). Unchanged tetraBDE was the major compound in all tissues analyzed in both species; minor amounts of hydroxylated tetraBDE metabolites were also detected. A water-soluble metabolite was detected in urine from mice, but could not be isolated.

Following oral exposure of male Sprague-Dawley rats to decaBDE (BDE-209), 13 phenolic metabolites were determined in the plasma (Sandholm et al. 2003). The major metabolites were characterized as a hydroxyl-octaBDE, a hydroxyl-nonaBDE, and a hydroxyl-methoxy-hexaBDE. In addition to the debromination reactions, the presence of a methoxy group is suggestive of methylation, and possibly other metabolic processes, by bacteria of the gut.

5.4.4 Elimination and Excretion

5.4.4.1 Inhalation Exposure

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

5.4.4.2 Oral Exposure

Polybrominated Biphenyls. No studies were located that provide information on percentage of ingested PBBs excreted by humans. However, PBBs in biliary fluid of a group of farmers and chemical workers ranged from undetected to 70 µg/L, and the correlation between serum PBB levels and levels in bile was statistically significant (Eyster et al. 1983). Similarly, PBB levels in feces ranged from undetected to 862 µg/kg, and the correlation between serum PBB levels and fecal levels was also statistically significant (Eyster et al. 1983).

Serum half-life values have been estimated using human data from the Michigan PBB cohort (Blanck et al. 2000b; Lambert et al. 1990; Rosen et al. 1995). A median half-life of 12.0 years (95% CI 4–97 years) was estimated based on two serum measurements from 15 women (≥20 years of age) with an initial serum PBB level of ≥5 ppb (Lambert et al. 1990). An analysis of 51 women (≥18.8 years of age) and 112 men (≥18.1 years of age) with at least two measurements 1 year apart and an initial PBB level of ≥20 ppb found a median half-life of 13.0 years (95% CI 6.3–infinite years) and 10.0 years (95% CI 6.7–20.0 years), respectively (Rosen et al. 1995). Based on a median half-life of 10.8 years (95% CI 9.2–14.7 years) for the entire group (163 persons, median PBB level 45.5 ppb), it was estimated that it will take more than 60 years for their PBB levels to fall below a detection limit of 1 ppb.

Determinants of PBB serum decay were investigated in 380 Michigan women (≥16 years of age) who had an initial PBB level of at least 2 ppb and at least two measurements taken when they were not pregnant (Blanck et al. 2000b). The mean initial PBB level was 20.9 ppb (standard deviation 78.7), and the mean time between the first and last measurement was 4.2 years (range 16.0–75.2 years). A total of 109 women (29%) did not have a reduction in serum PBBs over time. Assuming that PBBs reached equilibrium in the body before substantial amounts were eliminated and before the first serum measurements were taken, the entire body was modeled as a single compartment for PBBs with exponential decay. The median PBB half-life in the entire group was 13.5 years (95% CI 10.5–23.2 years). Subject-specific decay rate estimates were regressed on predictor variables including initial age, body mass index (BMI), smoking history, breast-feeding duration, and parity. The serum PBB decay rate was slower, resulting in a longer half-life, with higher initial PBB levels; women with initial PBB levels of <10 and >10 ppb had median half-lives of 12.9 and 28.7 years, respectively. The PBB decay rate was also slower ($p=0.03$) in women with an initial BMI above the median (BMI≥23). Increasing number of pregnancies between the first and last measurement was also associated with slower decay, but the effect was of borderline statistical

significance (p=0.06). Breast feeding as either a continuous variable or as categorized by tertiles of duration (<3, 3–9, or >9 months), age, and smoking were not associated with serum PBB decay.

Lactation constitutes the most important route of excretion of PBB in lactating women. Numerous studies reported PBB levels in breast milk from Michigan women (Brilliant et al. 1978; Eyster et al. 1983; Humphrey and Hayner 1975; Jacobson et al. 1984; Landrigan et al. 1979). PBB levels in breast milk on a lipid basis ranged from undetected to 92,667 µg/kg, with a median of 250 µg/kg, in a group of parturient women from Michigan (Eyster et al. 1983). Regression analysis of the data revealed that on a lipid basis, PBBs are 107–119 times more concentrated in milk than in serum. Also, adipose PBB levels are 1.1–1.5 times higher than the breast milk levels when milk levels were ≥100 µg/kg.

The importance of PBB transfer through lactation in experimental animals is discussed in Section 5.4.2.2.

There is limited information regarding excretion of PBBs in experimental animals. Rats dosed once with ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl (BB 153) excreted 7.9% of the dose in the feces within the first 24 hours; urinary excretion data were not provided (Matthews et al. 1977). It was estimated that <10% of the administered dose would ever be excreted. These results are consistent with those of other investigators who report that this congener is stable and persistent (Safe et al. 1978). In rats gavaged with ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl for 22 days, between 10 and 20% of the daily dose was excreted daily in the feces; this value was predominantly due to elimination of unabsorbed PBB (Tuey and Matthews 1980). In monkeys, the main route of excretion of hexabromobiphenyl residues was also in the feces (Rozman et al. 1982). Between 60 and 70% of the administered dose was excreted in the feces in the first 11 days after dosing; urinary excretion was minimal. The difference between the absorption rate reported by Matthews et al. (1977) and that reported by Rozman et al. (1982) can probably be accounted for by differences in the experimental designs.

Rats treated with a single gavage dose of ^{14}C-octabromobiphenyl excreted <1% of the administered dose in urine and expired air over a 16-day period (Norris et al. 1975a). Within the first 24 hours after dosing, 61.9% of the dose was present in the feces. The proportion that represents unabsorbed PBB is not known. By day 16, 74% of the administered dose had been recovered in the feces.

Polybrominated Diphenyl Ethers. Oral studies with ^{14}C-labeled decaBDE consistently show that this PBDE is rapidly and predominantly eliminated in the feces (El Dareer et al. 1987; Klasson Wehler et al. 2001, Morck and Klasson Wehler 2001; Morck et al. 2003; Norris et al. 1973, 1975c). In rats treated by

gavage with a single 1 mg/kg dose of a low purity former commercial mixture (77.4% ^{14}C-decaBDE, 21.8% nonaBDE, 0.8% octaBDE) in corn oil, most of the excreted radioactivity measured over a 16-day period was in the feces and <1% of the label was detected in the urine and expired air (Norris et al. 1973, 1975c). No significant differences in excretion patterns were observed between males and females. Approximately 91% of the decaBDE-derived radioactivity was found in the feces in the first 24 hours and almost all radioactivity (>99%) was accounted for by day 2 after dosing. Two feeding studies were conducted in which rats were exposed to a higher purity 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, or 9–10, or 9–11 (El Dareer et al. 1987; NTP 1986). In the first study, dietary concentrations ranged from 238–51,100 ppm (six levels) (≈20–4600 mg/kg/day). Recovery of radioactivity in the feces ranged from 91.3±4.0 to 101±4% of the amount ingested in the 72 hours following ^{14}C-decaBDE intake and was not related to dose level. In the second study, rats were exposed to dietary concentrations of 277 or 48,000 ppm (≈25 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 contents (<0.04%) decreased with time. Of the radioactivity recovered, >99% was in the feces and gut contents. Urinary excretion of ^{14}C accounted for ≤0.01% of the dose.

In a study of laboratory synthesized ^{14}C-decaBDE (>98% pure), a single 3 µmol/kg (≈3 mg/kg) dose was administered to normal or bile duct-cannulated male Sprague-Dawley rats by gavage (Klasson Wehler et al. 2001; Morck and Klasson Wehler 2001; Morck et al. 2003). The compound was suspended in a novel vehicle, Lutrol F127/soya phospholipone (34:16, w/w)/water, that was formulated to enhance solubility and optimize absorption. In the normal rats, approximately 90% (86–93%, n=8) of the dose was found in the feces after 3 days; cumulative recovery after 7 days was 91% (87-95%, n=4). For the two bile duct-cannulated rats, an average of 88 and 9.5% of the dose was recovered in the feces and bile, respectively, within 3 days. There was a large variation in the fecal excretion of the two cannulated rats that might have been due to the fact that no bile salts were added to compensate for the collected bile. Urinary excretion of ^{14}C was negligible for all groups at <0.1% of the dose. DecaBDE was speculated to have higher rates of metabolism and elimination than PBDEs with fewer numbers of bromines because it is unlikely to partition into lipids and is transported through aqueous compartments (e.g., serum and bile), whereas lower brominated congeners preferentially accumulate in the body due to their partitioning and retention in lipid-rich tissues.

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 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). The doses were designed to mimic environmental exposure levels. 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 assessed the following six congeners: 2,2',4,4'-tetraBDE (BDE 47), 2,2',3,4,4'-pentaBDE (BDE 85), 2,2',4,4',5-pentaBDE (BDE 99), 2,2',4,4',6-pentaBDE (BDE 100), 2,2',4,4',5,5'-hexaBDE (BDE 153), and 2,2',4,4',5,6'-hexaBDE (BDE 154) (Hakk et al. 2001). One of the penta congeners, BDE 85, occurred at a relatively high level in the feces (55.8% of the adminstered amount). Fecal excretion of the five other tetra- to hexaBDE congeners ranged from 7.6 to 15.8% of the dose. The study of the octaBDE mixture assessed the following eight congeners: 2,2',4,4',5,5'-hexaBDE (BDE 153), 2,2',4,4',5,6'-hexaBDE (BDE 154), 2,2',3,4,4',5',6-heptaBDE (BDE 183), 2,3,3',4,4',5,6-heptaBDE (BDE 190), an unknown heptaBDE, and three unknown octaBDEs (Huwe et al. 2002b). Fecal excretion ranged from 4.9 to 15.9% of the dose for the hexaBDE congeners, 20.9–31.5% of the dose for the heptaBDE congeners, and 16.7–44.3% of the dose for the octaBDE congeners.

The elimination of ^{14}C-2,2',4,4'-5-pentaBDE (BDE 99) was investigated in normal and bile duct-cannulated rats that were administered a single 2.2 mg (≈10 mg/kg) dose in corn oil by gavage (Hakk et al. 1999, 2002). The main route of elimination was via the feces as shown by cumulative fecal recovery of 43 and 86% of the administered radiolabel in 72 hours in the normal and bile-duct cannulated rats, respectively. The difference in the excreted amounts of radioactivity suggests that bile salts are needed for the intestinal uptake of BDE 99. Cumulative urinary excretion of radiolabel over 72 hours was ≈1% and ≈0.3% of the dose in the normal and bile duct-cannulated rats, respectively. The cumulative biliary excretion of radiolabel in cannulated rats was ≈4%.

Rats given a single gavage dose of 14.5 mg/kg dose of ^{14}C-2,2',4,4'-tetraBDE (BDE 47) in corn oil excreted 14% of the dose in the feces and <0.5% in the urine over a five day period (Örn and Klasson-Wehler 1998). Mice treated in the same manner excreted 20% of the dose in the feces and 33% in the urine over the same time period. The amount of non-absorbed material in the feces on day 1 was approximately 5 and 7% of the dose in rats and mice, respectively, suggesting efficient absorption in both species (Örn and Klasson-Wehler 1998).

5.4.4.3 Dermal Exposure

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

5.4.4.4 Other Routes of Exposure

Polybrominated Biphenyls. Rats given a single intravenous dose of ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl (BDE 153) excreted a cumulative 0.96, 3.3, and 6.6% of the dose in the feces 1, 7, and 42 days after dosing, respectively (Matthews et al. 1977). Only traces (0.1% of the dose) were excreted in the urine. Two decay components were calculated from excretion data; an initial decay rate of 1.05% of the dose/day and a later rate of 0.15% of the dose/day. Biliary excretion accounted for 0.68% of the dose between 0 and 4 hours after dosing. Analysis of bile and feces showed that at least 95% of the radioactivity corresponded to the parent compound. Moreover, in rats, ≈35% of the radioactivity excreted in the bile during the first week after a single dosing was reabsorbed (Tuey and Matthews 1980).

Parenteral administration of mono- and dibromobiphenyls to rats, rabbits, and pigs suggests that the urine is an important route of excretion for polar metabolites (Kohli and Safe 1976; Kohli et al. 1978; Sparling et al. 1980). However, cumulative urinary excretion did not account for more than 5% of the administered doses. Data regarding fecal excretion were not provided.

Polybrominated Diphenyl Ethers. No relevant information was located regarding elimination of PBDEs following exposure by non-natural routes of administration.

5.4.5 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.

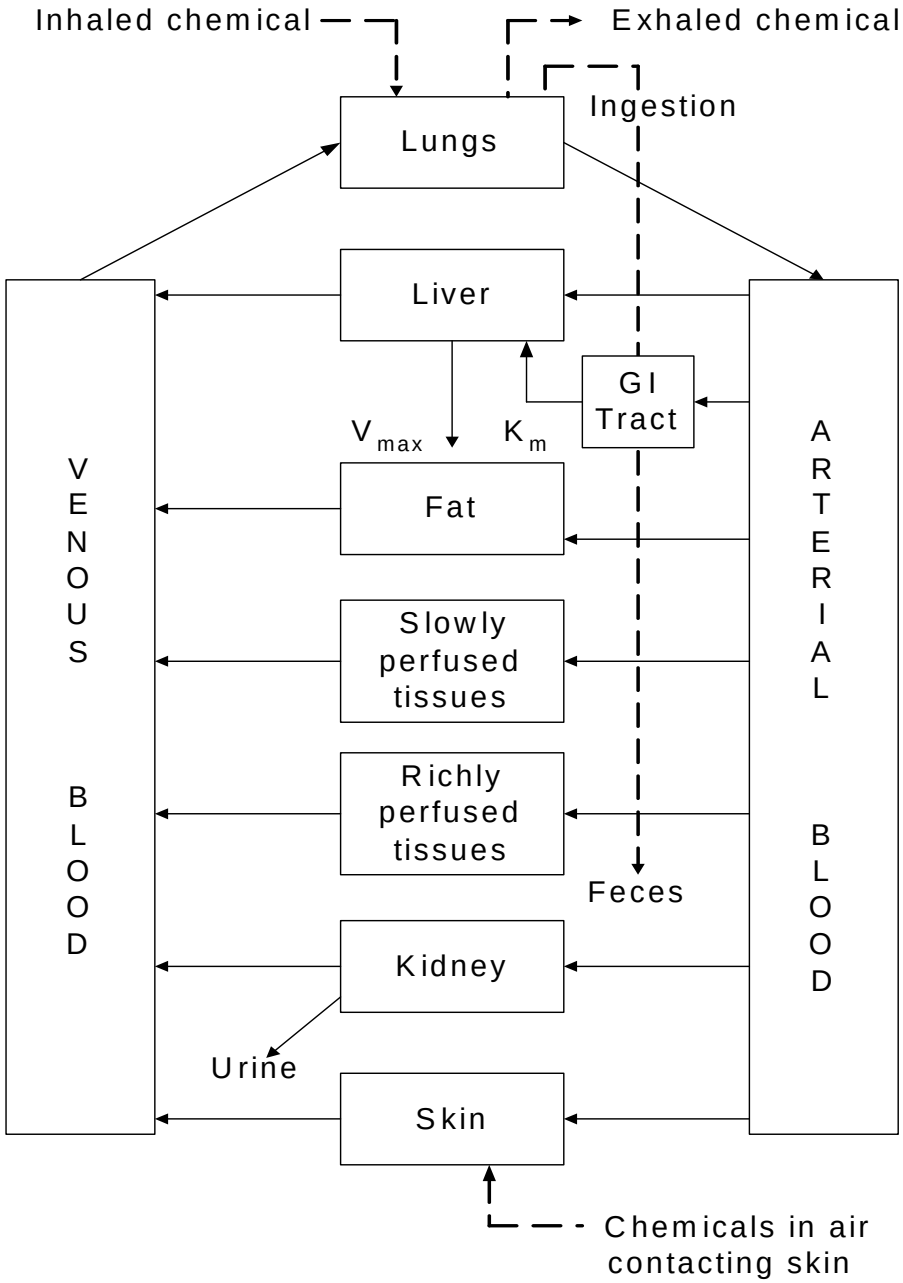
PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). 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 parametrization, (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 5-5 shows a conceptualized representation of a PBPK model.

Figure 5-5. Conceptual Representation of a Physiologically Based Pharma cokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

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.

Polybrominated Biphenyls. A PBPK model that incorporates tissue volume, affinity for PBBs, and rate of perfusion was developed to describe the distribution and body burden of the major component of FireMaster PBB mixtures, 2,2',4,4',5,5'-hexabromobiphenyl (BB 153), in the rat (Tuey and Matthews 1980). The modeling methods are an extension of those used to predict the disposition of PCBs (Matthews et al. 1977). The model predicts that at equilibrium, changes in the PBB concentration or changes in tissue volume of any tissue will lead to a corresponding change in all tissues. For example, if the concentration of a PBB congener in the liver is reduced by metabolism or excretion, then the concentration of that PBB congener in all tissues will be reduced proportionally. Congeners that cannot be readily metabolized (as is the case for BB 153) or excreted will concentrate in adipose tissue, but will still circulate to other tissues. Exposure to other tissues will be proportional to the respective tissue/blood ratios and the concentration in main storage tissues. This dynamic distribution results in accumulation of persistent congeners in all tissues and depletion from all tissues of those congeners that can be cleared. In rats orally dosed daily with ^{14}C-2,2',4,4',5,5'-hexabromobiphenyl over a 30-day period, tissue concentrations on day 31 were (in increasing order): blood, muscle, liver, skin, and adipose, and were in general agreement with those predicted by the PBPK model. When the model was scaled to nonlactating humans by adjusting for tissue volume, blood flow, and clearance and rate constant parameters, human intake of 9.8 g of the congener from milk consumption over a 230-day period would result in peak concentrations of 720 and 2.1 ppm in fat and blood, respectively. The model also predicted that the body burden after 5 years would be 5.2 g and the half-life 6.5 would be years. The half-life of 6.5 years predicted using the rat-based PBPK model is shorter than mean half-life values of ≈10-15 years estimated using human sera data from the Michigan PBB cohort (Blanck et al. 2000b; Lambert et al. 1990; Rosen et al. 1995), as discussed in Section 5.4.4 (Elimination and Excretion). The shorter predicted half-life from the rat model compared to estimated values based on human sera might be due to differences in adipose content between rats and man; fat acts as a depot for these chemicals, and most rat studies use young animals with a fat content less than in many people.

Polybrominated Diphenyl Ethers. No PBPK/PD models were located for PBDEs.

5.5 MECHANISMS OF ACTION

5.5.1 Pharmacokinetic Mechanisms

Polybrominated Biphenyls. The mechanism by which PBBs enter the blood stream from the lungs, skin, or gastrointestinal tract is not known and little information is available on how PBBs are distributed in the

body. The available data indicate that the absorption mechanism is likely passive diffusion. Results from studies of Michigan subjects showed that in the blood stream, 80% of the PBBs were bound to protein and 20% was associated with lipids (Greaves et al. 1984). Of the fraction bound to protein, 73% was bound to apolipoprotein B and the remaining percent was bound to apolipoprotein A. In an *in vitro* model, shown to be representative of environmentally contaminated blood, the distribution of PBBs among plasma, erythrocytes, mononucleocytes, and polymorphonucleocytes was 89:9:<1:<1, respectively (Roboz et al. 1985).

In an *in vitro* study in an adipocyte cell line (3T3L1 cells), >75% of the 2,2',4,4',5,5'-hexabromobiphenyl (BB 153) taken up by the cells was associated with subcellular fractions that contained 85% of the cellular triglyceride, with only 20% of the compound found in the microsomal plasma-membrane fraction (Kraus and Bernstein 1986). This study also found that inhibition of respiration by cyanide at a concentration that completely inhibited oxygen consumption did not affect uptake of BB 153, supporting the assumption that because of their lipophilic nature, PBBs penetrate membranes by passive diffusion.

Polybrominated Diphenyl Ethers. No studies were located regarding pharmacokinetic mechanisms for PBDEs.

5.5.2 Mechanisms of Toxicity

PBBs and PBDEs share some toxicological properties with other structurally similar polyhalogenated aromatic compounds, particularly PCBs, chlorinated dibenzo-*p*-dioxins (CDDs), and chlorinated dibenzofurans (CDFs) (Agency for Toxic Substances and Disease Registry 1994, 1998, 2000). However, although these chemicals are structurally similar in two dimensions, PBDEs (and PCDEs) differ from the other classes on a three dimensional basis. In particular, the oxygen bridge of the ether linkage in the diphenyl oxide molecule increases the distance between the biphenyl rings. This apparently reduces steric interactions between *ortho* substituents on the adjacent rings, such that the presence of *ortho* bromines is unlikely to present a barrier to rotation that would prevent the two aromatic rings from assuming a fully coplanar configuration (Chen et al. 2001; Hardy 2002; Howie et al. 1990). In other words, the ether bridge makes PBDEs more non-coplanar in nature, and introducing *ortho* substitutions into PBDEs does not create a spatial impediment for the two phenyl rings to assume a semi-flat position with respect to each other, as it does for PBBs or PCBs. Therefore, for PBDEs, the influences of the ether bridge and bromine position preclude clearly classifying the congeners as either dioxin-like (coplanar) or non-dioxin-like (non-coplanar). This has implications not only for dioxin-type toxicities, which are

mediated by the Ah (aryl hydrocarbon) receptor (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 planar conformation usually considered characteristic of AhR ligands. Structure-activity relationships have been incompletely examined 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 2002a; 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 2000, 2002). 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 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 geometrical differences in bromine and chlorine also have implications for understanding the mechanism(s) of effects for nondioxin-like PBB congeners, which are not as well characterized as for PCBs. In particular, it cannot necessarily be assumed, on the basis of two-dimensional structure, that the mechanisms and effects for nondioxin-like PBBs and PCBs are similar.

Polybrominated Biphenyls. The mechanism of toxicity for PBBs has been extensively studied, but is not completely understood (Akoso et al. 1982a, 1982b; Andres et al. 1983; Dannan et al. 1982a, 1982b; Goldstein et al. 1979; Parkinson et al. 1983; Render et al. 1982; Robertson et al. 1982; Safe 1984). Many PBBs, PCBs, chlorinated dibenzo-*p*-dioxins (CDDs), chlorinated dibenzofurans (CDFs), and other structurally related halogenated aromatic hydrocarbons are believed to share a common mechanism of action strongly related to similarities in their structural configuration. Most of what is known regarding the mechanism of action of these compounds is based on structure-receptor binding relationships, structure-induction relationships, and structure-toxicity relationships (Goldstein and Safe 1989; Safe 1990). Most of the studies providing this information used parenteral routes of exposure and/or *in vitro* test systems, as explained below. It is beyond the scope of this profile to discuss these studies in detail.

A limited number of studies have shown that some PBB congeners bind to the cellular AhR, which regulates the synthesis of a variety of proteins. This receptor was identified in the cytosol of mouse liver cells (Poland et al. 1976) and, subsequently, in extrahepatic tissues of laboratory animals, mammalian cell cultures, and human organs and cell cultures (Goldstein and Safe 1989). The structure-binding relationships for the coplanar 3,3',4,4',5-pentabromobiphenyl (BB 126), the monoortho substituted congener 2,3,3',4,4',5-hexabromobiphenyl (BB 156), and the diortho substituted analog 2,2',5,5'-tetrabromobiphenyl (BB 52) were examined in rat liver cytosol (Safe et al. 1985). At PBB concentrations 1,000-fold (10 µM) greater than tetrachlorordibenzo-*p*-dioxin (TCDD) concentrations (10 nM), the coplanar congener completely displaced radiolabeled 2,3,7,8-TCDD from the cytosolic AhR protein, the monoortho analog partially displaced the radiolabel, and 2,2',5,5'-tetrabromobiphenyl (BB 52) was the least active competitor. The latter congener is relatively nontoxic and does not induce AHH. The Ah-binding characteristics of 3,3',4,4'-tetrabromobiphenyl (BB 77) and 3,3'4,4'5,5'-hexabromobiphenyl, both coplanar, were also examined in rat and mice liver cytosol (Millis et al. 1985). The results showed than the tetrabromobiphenyl was 10 times more effective than the hexabromobiphenyl in displacing radiolabeled 2,3,7,8-TCDD from the receptor. The stereospecific nature of the binding (high affinity seen with congeners substituted in both *para* and two or more *meta* positions) strongly suggests that a biological receptor mediates the responses caused by some PBBs.

The ability of PBBs to induce hepatic Phase I xenobiotic metabolizing enzymes (cytochrome P-450-dependent monooxygenases) is well documented (Dannan et al. 1978b, 1982a, 1982b, 1983; Ecobichon et al. 1979; Moore et al. 1978, 1979; Parkinson et al. 1983; Robertson et al. 1982; Schramm et al. 1985). PBB mixtures were classified as "mixed-type" inducers of hepatic microsomal monooxygenases and resembled a mixture of phenobarbital (PB)-like plus 3-methylcholanthrene (MC) as inducers of P-450 isozymes from CYP1A and CYP2B families. The CYP1A1 and CYP1A2 genes are induced by AhR agonists, such as 2,3,7,8-TCDD and MC, and the structure-induction relationships for PBBs as inducers of these P-450 isozymes and their related activities have also been determined (Dannan et al. 1983; Parkinson et al. 1983; Robertson et al. 1982). For example, when injected intraperitoneally to immature male Wistar rats, the coplanar derivatives, 3,4,4'-tribromobiphenyl (BB 37), 3,4,4',5-tetrabromobiphenyl (BB 81), 3,3',4,4'-tetrabromobiphenyl (BB 77), 3,3',4,4'5-pentabromobiphenyl (BB 126), and 3,3',4,4',5,5'-hexabromobiphenyl (BB 169) had a pattern of induction resembling that of MC (Robertson et al. 1982). Similar type experiments have shown that monoortho-bromo-substituted analogs of the coplanar PBBs, such as 2,3',4,4'-tetrabromobiphenyl (BB 66), 2,3',4,4',5-pentabromobiphenyl (BB 118), and 2,3',4,4',5,5'-hexabromobiphenyl (BB 167), exhibit a mixed-type induction activity and resemble FireMaster BP-6 in their mode of induction (Dannan et al. 1978b; Parkinson et al. 1983). Yet a third

group of PBB congeners, the diortho-bromo analogs of the coplanar PBBs, resemble PB in their mode of induction (PB induces the CYP2B1 and CYP2B2 genes). Among the diortho-bromo-substituted PBBs studied are 2,2',5,5'-tetrabromobiphenyl (BB 52), 2,2',4,5,5'-pentabromobiphenyl (BB 101), 2,2',4,4',5,5'-hexabromobiphenyl (BB 153), and 2,2',3,4,4',5,5'-heptabromobiphenyl (Moore et al. 1979; Parkinson et al. 1983). Results from studies with some dibromobiphenyls revealed that 4,4'-dibromobiphenyl (BB 15) resembled PB in its mode of induction (Robertson et al. 1982), whereas 2,2'-dibromobiphenyl (BB 4) had no significant effect on hepatic microsomal drug-metabolizing enzymes (Moore et al. 1979). The results of these experiments indicated that coplanar PBB congeners substituted in both *para* and one or more *meta* positions are MC-type inducers, diortho substituted congeners are PB-type inducers, and monoortho analogs of the coplanar PBBs are mixed-type inducers. These results were qualitatively similar to those obtained with PCBs and support the idea of a common receptor-mediated mechanism of action for PBBs. PBBs are also efficacious inducers of hepatic phase II metabolizing enzymes such as glutathione transferases, UDP glucuronyl transferases, and epoxide hydrolase (Parkinson et al. 1983; Schramm et al. 1985). For example, when intraperitoneally injected in rats, FireMaster BP-6 efficaciously induced hepatic glutathione transferases while concomitantly depressing selenium-dependent glutathione perioxidase activity, an important antioxidant enzyme in the liver (Schramm et al. 1985).

Many studies that examined structure-induction relationships for several PBB congeners also studied structure-toxicity relationships. Thymus and spleen weight were significantly reduced in rats by a series of MC-type inducers (Robertson et al. 1982). Further experiments in rats revealed that of a series of PBB congeners, only MC-type inducers significantly decreased thymus weight and body weight; PB-type, mixed-type, and MC-type inducers increased relative liver weight (Parkinson et al. 1983). Results from feeding studies in rats indicate that 3,3',4,4',5,5'-hexabromobiphenyl (BB 169) (MC-type) and 2,2',4,4',5,5'-hexabromobiphenyl (BB 153) (PB-type) increased liver weight; however, only the MC-type inducer decreased body weight and thymic and splenic weight, and caused lymphocytic depletion in the thymus (Render et al. 1982). Similar results were obtained when the toxicities of 3,3',4,4'-tetrabromobiphenyl (BB 77) (MC-type) and 2,2'5,5'-tetrabromobiphenyl (weak PB-type) were compared in rats (Robertson et al. 1983a). Only BB 77 caused significant reductions in growth rate and thymus size and marked depletion of lymphocytes from the thymic cortex. Results from studies with FireMaster BP-6 revealed that the pattern of toxic responses and the magnitude of the responses attributed to this mixture are consistent with it being composed of both MC-type and PB-type congeners; the most toxic responses being attributed to the MC-type congeners (Akoso et al. 1982a, 1982b; Dannan et al. 1982a, 1982b; Ecobichon et al. 1979; Parkinson et al. 1983; Render et al. 1982). These results suggest a correlation

between immunological and hepatic effects and the ability to induce AHH activity and that the most toxic congeners are those that resemble the structural configuration of 2,3,7,8-TCDD. This relationship further supports the idea of a common receptor-mediated mechanism of action. Other PBB congeners (*ortho*-substituted) induce other types of effects, such as neurotoxicity, by yet unknown but AhR independent mechanisms.

Some information on structure-promotion relationships for PBBs is available from studies that used two-stage liver and skin carcinogenesis models. In the liver promotion studies, development of enzyme-altered hepatic foci (putative preneoplastic lesions) was assessed in rats that were partially hepatectomized, initiated with diethylnitrosamine and promoted with PBBs (Buchmann et al. 1991; Dixon et al. 1988; Evans and Sleight 1989; Jensen and Sleight 1986; Jensen et al. 1982, 1983; Sleight 1985). Both MC-type (3,3',4,4',5,5'-hexabromobiphenyl [BB 169] and 3,3',4,4'-tetrabromobiphenyl [BB 77]) and PB-type (2,2',4,4',5,5'-hexabromobiphenyl [BB 153]) congeners showed hepatic promoting activity with varying potencies. FireMaster BP-6 was a more effective promoter than its major constituent congener BB 153 (Jensen et al. 1982), which also indicates that other congeners are very effective as promoters, or possibly that the combination of congeners with mixed- or PB-type activity have a synergistic or additive effect. Although both MC- and PB-type congeners promote two-stage hepatic tumor activity, it appears that the MC-type congeners may exert their effects indirectly by causing hepatotoxic (cytotoxic effects and necrosis), whereas the PB-type congeners may act as mitogens (stimulate cellular growth and division). In a skin tumor assay, HRS/J hairless mice were initiated with MNNG and promoted with PBBs (Poland et al. 1982). FireMaster FF-1 and BB 169 were effective skin tumor promoters, but BB 153 showed no activity, suggesting that, unlike rat liver tumor promotion, promoter activity in the mouse skin tumor model is AhR-dependent. Another indication that promotion of tumors by PBBs is not solely an Ah-receptor mediated process is provided by the results of an *in vitro* gap junctional intercellular intercommunication assay (Kang et al. 1996). Gap junctional intercellular intercommunication in normal human breast epithelial cells was inhibited by 2,2',4,4',5,5'-hexaCB (CB 153) in a dose-dependent manner, but not by the coplanar congener 3,3',4,4',5,5'-hexaCB (CB 169). Inhibition of gap junctional intercellular communication is generally regarded as a mechanistic marker for tumor promotion (as well as several other toxicological end points).

Expression of the dioxin-type toxic response, which is species and strain dependent, is initiated by the binding of individual congeners with the AhR. The responsiveness of a particular organ or cell depends on the affinity of the receptor for the ligand molecule (Goldstein and Safe 1989). For example, certain inbred strains of mice, typified by C57BL/6J, have a cytosolic AhR protein with a relatively high binding

affinity for inducers of benzo[a]pyrene hydroxylase such as 3-MC, ß-naphthoflavone, 2,3,7,8-TCDD, and other isostereomers of 2,3,7,8-TCDD. In contrast, other inbred mouse strains, such as DBA/2J, have reduced AhR binding affinity. Responsiveness to aromatic hydrocarbons is inherited in a simple autosomal dominant mode. Nonresponsiveness has been attributed to a mutation resulting in a receptor with a diminished affinity (Okey et al. 1989). This defective receptor is almost completely unresponsive to weak inducers such as 3-MC and has reduced sensitivity to more potent inducers such as 2,3,7,8-TCDD. Studies with PBBs have shown that treatment of C57BL/6J and DBA/2J with FireMaster BP-6 resulted in the induction of hepatic microsomal benzo[a]pyrene hydroxylase only in the C57BL/6J strain and aminopyrine N-demethylase (PB inducible) in both strains of mice (Robertson et al. 1984a). However, 3,3',4,4'-tetrabromobiphenyl (BB 77), a more potent MC-type inducer than the BP-mixture, induced benzo[a]pyrene hydroxylase in both strains of mice but did not induce aminopyrine N-demethylase in either strain of mice. Also, after treatment with the dioxin-like congener BB 77, thymic atrophy was only observed in the responsive strain (Robertson et al. 1984a). In general, studies summarized in Section 5.2 in which more than one strain was tested (mice or other species) do not address the possible strain-dependency of the toxic responses observed. It must be mentioned, however, that differences in the response between tissues, strains, or species, do not exclusively indicate differences in receptor affinities, but most likely reflect the fact that the battery of enzyme activities (see below) controlled by the Ah locus varies within the tissue, strain, and animal species.

Initial binding of a PBB congener to the AhR is followed by an activation or transcription step and subsequent accumulation of occupied nuclear receptor complexes. These complexes interact with a specific DNA sequence in the CYP1A1 gene (which regulates the expression of cytochrome P-450IA1 isozymes), changing its secondary or supersecondary structure (Elferinck and Whitlock 1990), which leads to enhancement of the CYP1A1 gene expression. Newly synthesized enzymes and macromolecules resulting from the pleiotropic response to the ligand-receptor complex are believed to be responsible for many of the effects caused by PBBs and other halogenated aromatic hydrocarbons. In other words, the binding of a congener to the AhR initiates a transcriptional upregulation of a battery of genes that modulates biochemical and endocrine pathways, cell cycle regulation (e.g., apoptosis, proliferation, and differentiation), morphogenesis, oxidative stress response, and other processes, and is ultimately expressed as a diverse spectrum of well characterized toxic responses.

No studies were located regarding the mechanism of endocrine effects (thyroid toxicity, estrogenicity) of PBBs.

Polybrominated Diphenyl Ethers. As discussed in the introduction to this section, bromination at the *ortho* position does not appear to significantly change the planarity of PBDE molecules or their biological effects. 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-substituted to heptabromo-substituted, and with the exception of 4,4'-diBDE (BDE 15)and 3,3',4,4'-tetraBDE (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, 3,3’,4,4’,5-pentaBDE (BDE 126), induced a significant AhR-mediated gene expression response in the H4IIE-luc cells, but the magnitude of induction was only 13% of that caused by TCDD. With the exception of 2,3,3’,4,4’-pentaBDE (BDE 105), which induced a response of 1.7% of the TCDD maximum, no other congener, including the environmentally prominent congeners 2,2',4,4'-tetraBDE (BDE 47), 2,2',4,4',5-pentaBDE (BDE 99), and 2,2',4,4',5,5’-hexaBDE (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.

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 10^{-2}–10^{-5} times that of 2,3,7,8-TCDD. The congener with the highest affinity among the tested congeners was 2,2',3,4,4'-pentaBDE (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 in rat hepatocytes (Chen and Bunce 2001).

Congeners 3,3’,4,4’-tetraBDE (BDE 77), 2,3’,4,4’,6-pentaBDE (BDE 119), and 3,3’,4,4’,5-pentaBDE (BDE 126) were moderately active towards DRE (dioxin response element) binding and induced responses of both CYP1A1 mRNA and CYP1A1 protein equivalent 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. Congeners 2,2’,4,4’,6-pentaBDE (BDE 100), 2,2’,4,4’,5,5’-hexaBDE (BDE 153), and 2,2’,3,4,4’,5’,6-heptaBDE (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 2,2’,4,4’-tetraBDE (BDE 47) and 2,2’,4,4’,5-pentaBDE (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 end points evaluated in the Chen et al. (2001) and Chen and Bunce (2003) studies, it was concluded that the relative induction potencies (REPs) of the most active PBDEs toward CYP1A1 are $\approx 10^{-4}$ that of TCDD (similar to some mono-*ortho*-PCBs and two orders of magnitude less than those of coplanar PCBs), and the REPs for the environmentally prominent congeners are essentially zero.

The enzyme induction properties of PBDEs have been less studied than for 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 PBDDs/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, 3,3',4,4'-tetraBDE (BDE 77), 2,2',4,4',6-pentaBDE, 2,3',4,4'-tetraBDE (BDE 66), and 3,3',4,4',5-penta-BDE (BDE 126) were the strongest inducers. Congeners 2,2',4,4',5,5'-hexaBDE (BDE 153) and 2,2',3,4,4',5',6-heptaBDE were weak inducers in all cell types, whereas BDE 66 and 2,2',3,4,4'-pentaBDE (BDE 85) were very weak inducers in rat hepatocytes and inactive in the other cells. Congeners 2,2',4,4'-tetraBDE (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 2,4,4'-triBDE (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 2,2',3,4,4'-pentaBDE (BDE 85), which despite its relatively strong AhR binding affinity (see above), showed no evidence of activating the AhR to its dioxin-response element (DRE) binding form and was only a weak EROD inducer.

Relevant information on structure-toxicity relationships is available for PCDEs. Howie et al. (1990) examined the immunotoxic potencies of various PCDE congeners on the inhibition of the splenic PFC response to SRBC antigen and found the following 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. In general, this potency order paralleled their potencies as inducers of hepatic microsomal AHH and EROD. Worth noticing 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 PFC response to challenge with SRBC 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 polyCDE 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.

Evidence for thyroid hormone involvement in PBDE toxicity includes observations in rats and mice that were orally exposed to commercial mixtures of deca-, octa-, or pentaBDE (see Section 5.2.2.2, Endocrine Effects). DecaBDE appears to be much less potent than the lower brominated mixtures and the main effects include (1) histological changes in the thyroid indicative of glandular stimulation (e.g., follicular cell hyperplasia similar to that induced by a hypothyroid state) (IRDC 1976; Norris et al. 1973, 1975b; NTP 1986; WIL Research Laboratories 1984), and (2) decreased serum thyroxine (T_4) levels with no accompanying 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). Considering these data, the structural resemblance of PBDEs to T_4, and information from studies of individual congeners as summarized below, it is hypothesized that, depending on dose, duration, and mixture/congener, PBDEs can disrupt the production, transport, and disposition of thyroid hormones.

The mechanism(s) by which PBDEs decrease serum T_4 levels is is unclear. 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. 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 et al. 2001; 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). As discussed below, increased microsomal enzyme activity 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.

The possible interaction of PBDEs with T_4 binding to human transthyretin (TTR) was investigated in an *in vitro* competitive binding assay (Meerts et al. 1998, 2000). Testing of 17 congeners, ranging from di- to heptaBDEs, showed that none of the parent compounds competed with T_4 for binding to human TTR. Incubation of the congeners with rat liver microsomes induced by PB (CYP2B enriched), β-naptho-flavone (CYPIA enriched), or clofibrate (CYP4A3 enriched) indicated that metabolism is necessary to compete with T_4-TTR binding and that potency is likely to be both congener and metabolic enzyme-specific. The CYP2B-enriched liver microsomes were the most potent, causing 9 of the 17 congeners to generate metabolites (not identified) that were effective in displacing T_4 from TTR (60% inhibition): 4,4'-diBDE (BDE 15), 2,4,4'-triBDE (BDE 28), 2,4,6-triBDE (BDE 30), 2,2',4,4'-tetraBDE (BDE 47), 2,2',4,6'-tetraBDE (BDE 51), 2,4,4',6-tetraBDE (BDE 75), 3,3,4,4'-tetraBDE, 2,2',4,4',6-pentaBDE, and 2,3',4,4',6-pentaBDE (BDE 119). No T_4-TTR inhibition occurred with the higher brominated diphenyl ethers (i.e., 2,2',3,4,4',5'-hexaBDE (BDE 138), 2,2',4,4',5,5'-hexaBDE (BDE 153), 2,3,4,4',5,6-hexaBDE (BDE 166), and 2,3,3',4,4',5,6-heptaBDE), although it was not verified that these PBDEs were metabolized during the *in vitro* microsomal incubations. Three pure hydroxylated PBDEs, synthesized for their structural resemblance with the thyroid hormones 3,5-diiodothyronine (3,5-T_2), 3,3',5-triiodo-thyronine (T_3), and 3,3',5,5'-tetraiodothyronine (T_4), were also tested in the T_4-TTR competition binding assay. The relative potencies 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-tribromophenoxy]phenol) hydroxylated PBDEs were 1.42- and 1.22-fold more potent, respectively, than T_4, and the percentage competition at 500 nM exceeded that of the natural ligand; 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). Because the PBDEs were able to compete with T_4-TTR binding only after metabolic conversion by induced rat liver microsomes, and considering that thyroid hormones are also hydroxy-halogenated diphenyl ethers. Studies with PCBs and hydroxylated derivatives similarly showed that the congener patterns most closely resembling the diiodophenolic ring of thyroxine had the highest TTR binding activity (Chauhan et al. 2000; McKinney et al. 1987; Rickenbacher et al. 1986).

Although the findings discussed above suggest that hydroxylation of PBDEs could be involved in the mechanism of thyroid toxicity, there are indications that hydroxylated metabolites might not play a large role for commercial penta- and octaBDE products. For example, of the *in vitro*-generated metabolites displacing T_4 from TTR (Meerts et al. 1998, 2000), only two (2,2',4,4'-tetraBDE [BDE 47] and 2,2',4,4',6-pentaBDE [BDE 100]) are known to be present in a commercial product (pentaBDE). An *in vivo* study in rats (Om and Klasson-Wehler 1998) showed that BDE 47 is well absorbed (~95%), but poorly metabolized, because (1) only 3% of the amount excreted in feces over a 5-day period was in the form of metabolites, (2) the parent molecule was the major compound detected in all tissues analyzed (and the only brominated compound detectable in kidney, brain, and adipose tissue), and (3) plasma levels were low and mainly due to the parent molecule. Additionally, the three pure hydroxylated PBDEs synthesized by Meerts et al. (1998, 2000) for their structural similarity to thyroid hormones are not based on congeners known to be present in commercial penta- or octaBDE mixture; this suggests that the congeners in these mixtures do not share close structural relationships with thyroid hormones. It is relevant to note that high concentrations of hydroxy and methoxy metabolites of PBDEs have been detected in fish from the Baltic Sea (Asplund et al. 1999), indicating that these compounds can potentially directly impact thyroid function in humans consuming fish.

Three hydroxylated PBDEs, the 4'-hydroxyl derivatives of 1,3,5-triBDE, 1,3,3',5'-tetraBDE, and 1,3,3',5,5'-pentaBDE, were tested for affinity to the human thyroid hormone receptor proteins THR-α and THR-β *in vitro* (Marsh et al. 1998). 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 4–>1,000 times less than for T_4 and T_3). Because the tested congeners were the most likely to have affinity for the thyroid hormone receptor, it was speculated that other hydroxylated PBDE congeners will have even lower potential for receptor binding. DecaBDE (not hydroxylated) had no effect on thyroid hormone receptor-mediated transcriptional activation by T_3 in HeLaTRDR4-luc human cells; no other congeners were tested in this assay (Sakai et al. 2003).

The extent that PBDEs affect circulating levels of T_4 or T_3 is likely to vary with species and rats are generally regarded as more sensitive than humans. As discussed in Section 5.5.3, 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; that latter being, in part, related to the absence of thyroid binding globulin (TBG) in rats (Capen 1997). Transthyretin (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 cerebrospinal fluid (CSF) in both 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). 2,2',4,4'-TetraBDE (BDE 47) competitively inhibited binding of T_4 to sites in rat choroid plexus following *in vivo* (but not *in vitro*) exposure (Sinjari et al. 1998). Choroid plexus homogenate from rats that were orally treated with 6 or 18 mg/kg/day for 14 days showed T_4 binding that was 80 and 63%, respectively, of that in controls. At least one group of mammals is known to exist without TTR (Palha et al. 1997; Schussler 2000). The TTR-nul mouse has decreased protein-bound and total T_4, normal free T_4, and has apparent good health (Palha et al. 1997, 2000). Interference with the blood-choroid-plexus-CSF-TTR-mediated route of T_4 to the brain caused by the absence of TTR did not produce measurable features of hypothyroidism (Palha et al. 2000).

Neurobehavioral developmental alterations have been induced in mice that were neonatally or perinatally exposed to individual PBDE congeners, including 2,2',4,4'-tetraBDE (BDE 47), 2,2',4,4',5-pentaBDE (BDE 99), 2,2',4,4',5,5'-hexaBDE (BDE 153), and 2,2',3,3',4,4',5,5',6,6'-decaBDE (BDE 209) (Branchi et al. 2001, 2002; Eriksson et al. 1999, 2001a, 2002a; Viberg et al. 2001a, 2001b, 2002a, 2002b, 2003). As detailed in Section 5.2.2.4 Neurological Effects, the main effects were observed at adulthood and included reduced spontaneous motor activity, impaired habituation capability, and learning impairment in a maze task. The mechanisms for these behavioral and cognitive effects have not been elucidated. One possibility involves the well-documented key role of thyroid hormones in brain development. As discussed above, *in vivo* and *in vitro* exposure to PBDEs has caused changes in hormone levels and other thyroid end points in animal models. Some studies suggest that the effects might be related to alterations in cholinergic functions. For example, neonatal exposure to a single 8 mg/kg oral dose of BDE 99 on PND 10 altered the behavioral response to nicotine, a cholinergic agent, in adult mice (Viberg et al. 2002b). 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; 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). Adult mice that were exposed to single 9 mg/kg oral dose of BDE 153 on PND 10 had a decrease in specific α-Bungarotoxin binding sites (cholinergic nicotinic receptors) in the brain hippocampus (Viberg et al. 2001b, 2002a).

Effects of PBDEs on intracellular signaling processes in rat neuronal cultures and cerebellar fractions have been reported. *In vitro* exposure to commercial pentaBDE mixture DE-71 or tetra congener BDE 47 stimulated arachidonic acid release in rat cerebellar granule neurons; this effect was not seen with the commercial octaBDE product 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 penta mixture DE-71and tetra congener BDE 47 also caused translocation of protein kinase C, as indicated by increased phorbol ester binding; octaBDE mixture 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. 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). The *in vitro* uptake of the neurotransmitter dopamine into rat brain synaptic vesicles was inhibited by penta mixture DE-71 mainly tetra and penta congeners), but not by commercial mixtures of octaBDE (DE-79) or decaBDE (DE-83R) (Mariussen and Fonnum 2002, 2003).

The ability of PBDE congeners to induce estrogen receptor (ER)-mediated gene expression in MVLN recombinant human breast carcinoma cells, or displace steroid hormones (^{3}H-testosterone or ^{3}H-estradiol) from carp serum proteins, was assessed by Villeneuve et al. (2002). Of 10 tested congeners, including the environmentally prominent congeners 2,2',4,4'-tetraBDE (BDE 47), 2,2',4,4',5-pentaBDE (BDE 99), and 2,2',4,4',5,5'-hexaBDE (BDE 153), induced a significant response in either *in vitro* assay. Another study found that a hydroxylated metabolite of BDE 47, 6-OH-2,2',4,4'-tetraBDE, did not inhibit aromatase (CYP 19) enzyme activity in human H295R adrenocortical cells (Canton et al. 2003). Aromatase is a steroidogenic enzyme that mediates the conversion of androgens to estrogens. No other PBDEs were tested in this *in vitro* study.

The estrogenic and antiestrogenic activities of several PBDE congeners and three hydroxylated PBDEs were also assessed *in vitro* using human breast cell line assays based on ER-dependent luciferase reporter gene expression (Meerts et al. 2001). The hydroxylated PBDEs, tribromophenoxy)phenol, 2-bromo-

4-(2,4,6-tribromophenoxy)phenol, and 2,6-dibromo-4-(2,4,6-tribromophenoxy)phenol, have bromine substitution patterns similar to those of the thyroid hormones T_2 (3,5-diiodothyronine), T_3 (3,3',5-triiodothyronine), and T_4 (3,3',5,5'-tetraiodothyronine), respectively. Eleven of 17 PBDE congeners showed estrogenic activity (dose-dependent luciferase induction) in the ER-CALUX assay with T47D.Luc cells, although the most potent PBDE congeners (2,2',4,4',6-pentaBDE [BDE 100] > 2,4,4',6-tetraBDE [BDE 75] > 2,2',4,6'-tetraBDE [BDE 51] > 2,4,6-triBDE [BDE 30] > 2,3',4,4',6-pentaBDE [BDE 119]) had EC_{50} values that were 250,000–390,000 times less potent than 17β-estradiol (E_2). In contrast, the T_3- and T_2-like hydroxylated PBDEs showed estrogenic potencies exceeding that of E_2 (no estrogenic activity was induced by the T_4-like hydroxylated PBDE). Antiestrogenic potencies were determined in the ER-CALUX assay by treating T47D.Luc cells with the PBDEs and hydroxylated PBDEs in the presence of E_2. Only 2,2',4,4',5,5'-hexaBDE (BDE 153), 2,3,4,4',5,6-hexaBDE (BDE 166), and 2,3,3',4,4',5,6-heptaBDE, which did not induce luciferase activity alone, caused reductions in E_2-induced luciferase activity. Three of the compounds with potent estrogenic activity in the ER-CALUX assay, 2,4,6-triBDE (BDE 30), 2,2',4,4',6-pentaBDE (BDE 100), and 4-(2,4,6-tribromophenoxy)phenol, were also tested for estrogenicity in ERα-specific and ERβ-specific human embryonic kidney cell lines (293-ERα-Luc and ERβs-Luc cells, respectively). The hydroxylated PBDE was potent in the ERα-specific cells (maximum luciferase induction similar to E_2) and also showed activity in the ERβ-specific cells (maximum 50% induction compared to E_2), whereas the ERα- and ERβ-specific cell lines were less responsive to 2,4,6-triBDE (BDE 30) (34.2 and 7.8% induction compared to E_2) and 2,2',4,4',6-pentaBDE (≈20 and <2% relative induction). These results indicate that pure and hydroxylated congeners of PBDEs can be agonists of both ERα and ERβ receptors and that metabolism of PBDEs may produce more potent pseudoestrogens. The common structural features among the estrogenic PBDEs in this study are two *ortho* (2,6)-bromine atoms on one phenyl ring, at least one *para*-bromine atom (preferably on the same phenyl ring as the *ortho* bromines), and nonbrominated *ortho-meta* or *meta* carbons on the other phenyl ring (Meerts et al. 2001).

The predominant congeners in biological samples, 2,2',4,4'-tetraBDE (BDE 47) and 2,2',4,4',5-pentaBDE (BDE 99), did not demonstrate estrogenic activity in the Meerts et al. (2001) study. The hydroxy-PBDE derivatives that showed minimal activity were *para*-substituted with a hydroxyl group, whereas the *para* position of the predominant isomers in biological samples is occupied by bromine atom, suggesting that the relevance of the results to commercial BDE mixtures is unclear. Additionally, all of the available estrogenicity studies were *in vitro*, and there is, as yet, no evidence either for estrogenicity or anti-estrogenicity of PBDEs and/or PBDE metabolites *in vivo*.

5.5.3 Animal-to-Human Extrapolations

Residue levels of PBBs and 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 PBB and PBDE congeners in human milk do not resemble the pattern of any of the commercial mixtures, 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 8, residue analyses indicate that tetra- to hexa-congeners predominate in humans, aquatic mammals, birds, fish, and other biota, indicating that the biological fate of PBB and 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 PBBs and 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).

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 thyroid-binding globulin (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. Transthyretin (TTR) is the major thyroid hormone binding protein in rats, but not in man. In most mammals, including humans, thyroxin binding globulin (TGB) is the principal thyroid hormone binding protein; 74% of the total bound-T_4 is bound to TGB, 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). The increased metabolic clearance of serum T_4 is thought to involve the induction of hepatic microsomal enzymes. Although it is well documented that PBDEs are microsomal enzyme inducers, there is little evidence that induction of hepatic enzymes leads to greater clearance of thyroid hormones, indicating that it is

misleading to assume that PBDEs are unlikely to affect thyroid function in humans, or that humans are less sensitive to these effects than rats.

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.

5.6 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 Colborn and Clement (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 1997a). 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 PBBs and PDBEs, 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.

Polybrominated Biphenyls. PBBs have the potential to interact with the endocrine system based on effects that mainly include changes in levels of thyroid and female reproductive hormones. No studies were located that investigated the estrogenic and antiestrogenic activity of PBBs *in vitro* or *in vivo* at the level of the estrogen receptor.

The thyroid gland is an unequivocal target of PBBs in animals, and evidence in humans is suggestive of a similar relationship. A spectrum of effects has been observed in rats exposed for acute and intermediate durations, ranging from decreases in serum levels of T_4 and T_3 to histological and ultrastructural changes in the follicles (Akoso et al. 1982b; Allen-Rowlands et al. 1981; Byrne et al. 1987; Gupta and Moore 1979; Kasza et al. 1978a; Norris et al. 1975a, 1975b; NTP 1983; Sepkovic and Byrne 1984; Sleight et al. 1978). The preponderance of these studies tested FireMaster FF-1 or FireMaster BP-6 in rats, although chronic exposure to FireMaster FF-1 induced thyroid follicular hyperplasia in mice (NTP 1992). Thyroid effects also occurred in offspring of treated rats and pigs (Meserve et al. 1992; Werner and Sleight 1981). Effects in workers exposed to unspecified PBBs and/or decabromobiphenyl included increased serum FSH, low or borderline low serum T_4, and increased thyroid antimicrosomal antibody titers (Bahn et al. 1980).

Serum levels of the adrenal hormones corticosterone B, dehydroepiandrosterone, and dehydro-epiandrosterone sulfate were decreased in rats fed ≥0.25 mg/kg/day FireMaster BP-6 for 5–7 months (Byrne et al. 1988). Serum corticosterone levels and adrenal weight did not change in rats exposed to ≤6 mg/kg/day of an unspecified PBB mixture for a shorter duration of 20 days (Castracane et al. 1982).

Increased menstrual cycle duration and prolonged implantation bleeding were observed in female monkeys fed FireMaster FF-1 in approximate daily dose levels of 0.012 mg/kg for 7 months before breeding and during pregnancy (Allen et al. 1979; Lambrecht et al. 1978). A corresponding decrease in serum levels of progesterone suggests that the reproductive effects in the monkeys are related to PBB-induced endocrine imbalance. Implantation was completely blocked in 40–67% of female rats treated with FireMaster BP-6 by gavage in dose levels ≥28.6 mg/kg on alternate days between gestation days 0 and 14 (Beaudoin 1979).

Delayed vaginal opening, an effect suggesting retarded sexual maturation, was observed in F_1 generation rats whose only PBB exposure was from the mothers fed a diet providing 5 mg/kg/day FireMaster FF-1 from day 8 of pregnancy until weaning at 28 days postpartum (McCormack et al. 1981).

Two studies of women exposed during the Michigan contamination episode found no associations between serum levels of PBBs and breast feeding (Blanck et al. 2000b; Thomas et al. 2001). Determinants of PBB serum decay were investigated in women who had a mean initial PBB level of 20.9 ppb, a mean time between the first and last measurement of 4.2 years, and at least two measurements taken when they were not pregnant (Blanck et al. 2000b). The median PBB half-life was estimated to be 13.5 years. Subject-specific decay rates were regressed on various predictor variables. Results included the finding that breast feeding, as either a continuous variable or as categorized by duration (<3, 3–9, or >9 months), was not associated with serum PBB decay, although increasing number of pregnancies was weakly associated with a slower rate of serum PBB decay (the effect had borderline statistical significance). Additional information on the design and results of this study is provided in Section 5.8.1. Thomas et al. (2001) found no relationship between serum levels of PBBs and the frequency and duration of lactation in Michigan women. Characteristics of the study cohort included a mean initial serum PBB level of 17.5 ppb, an estimated mean serum PBB level at delivery of 9.4 ppb, mean duration of breast-feeding as main source of nutrition of 2.6 months, and mean total duration of breast feeding of 4.1 months. Exposure was treated as a categorical variable by dividing the women into groups of low (reference) exposure (≤1 ppb), moderate exposure (>1–≤7 ppb), and high exposure (>7 ppb), and three outcomes of interest were analyzed: (1) the decision to breast feed (yes/no), (2) the duration (months) of breast-feeding as the main source of nutrition, and (3) the total duration (months) of breast-feeding. None of the three outcomes was significantly associated with serum PBB levels. Additional information on the design and results of this study is provided in Section 5.2.2.5.

The issue of breast cancer has received attention following reports of high levels of organochlorine compounds in breast cancer patients. A relationship between increasing serum levels of PBBs and increasing risk of breast cancer was indicated in case-control studies of women exposed during the Michigan contamination episode (Henderson et al. 1995; Hoque et al. 1998), but the results are only suggestive due to factors such as small number of cases, insufficient information on known breast cancer risk factors, and confounding exposures to other organochlorine chemicals. The evidence for an association between breast cancer and PCBs is also not conclusive (Agency for Toxic Substances and Disease Registry 2000), and the hypothesis that environmental exposure to PCBs can cause breast cancer in humans is controversial (Safe and Zacharewski 1997; Wolff and Toniolo 1995). Overall, the evidence for an association between breast cancer and PBBs is inconclusive and needs further study.

Polybrominated Diphenyl Ethers. Results of *in vitro* estrogen receptor and thyroid hormone transport protein binding assays, as well as *in vivo* thyroid hormone homeostasis studies in animals, suggest that there is a potential for some PBDEs to disrupt thyroid and other endocrine system functions in humans.

The estrogenic and antiestrogenic activities of 17 PBDE congeners and 3 hydroxylated PBDEs were tested *in vitro* using human breast cell line assays based on ER-dependent luciferase reporter gene expression (Meerts et al. 2001). Eleven of 17 PBDE congeners showed estrogenic activity (dose-dependent luciferase induction) in the ER-CALUX assay with T47D.Luc cells, although the most potent PBDE congeners (2,2',4,4',6-pentaBDE > 2,4,4',6-tetraBDE [BDE 75] > 2,2',4,6'-tetraBDE [BDE 51] > 2,4,6-triBDE [BDE 30] > 2,3',4,4',6-pentaBDE [BDE 119]) had EC_{50} values that were 250,000–390,000 times less potent than that of the natural ligand, 17β-estradiol (E_2). In contrast, two of the hydroxylated PBDEs, 4-(2,4,6-tribromophenoxy)phenol, 2-bromo-4-(2,4,6-tribromophenoxy)phenol (which have bromine substitution patterns similar to the thyroid hormones T_2 [3,5-diiodothyronine] and T_3 [3,3’,5-triiodothyronine], respectively) had estrogenic potencies exceeding that of E_2. Three of the compounds with potent estrogenic activity in the ER-CALUX assay, 2,4,6-triBDE (BDE 30), 2,2',4,4',6-pentaBDE, and 4-(2,4,6-tribromophenoxy)phenol, were also tested for estrogenicity in ERα-specific and ERβ-specific human embryonic kidney cell lines (293-ERα-Luc and ERβs-Luc cells, respectively). The hydroxylated PBDE was potent in the ERα-specific cells (maximum luciferase induction similar to E_2) and also showed activity in the ERβ-specific cells (maximum 50% induction compared to E_2), whereas the ERα- and ERβ-specific cell lines were less responsive to BDE 30 and 2,2',4,4',6-pentaBDE. These results indicate that pure congeners of PBDEs can be agonists of both ERα and ERβ receptors and that metabolism to hydroxylated PBDEs may increase estrogenic potency. However, no estrogenic activity was demonstrated by the predominant congeners in biological samples, 2,2’,4,4’-tetraBDE (BDE 47) and

2,2',4,4',5-pentaBDE (BDE 99). The hydroxy-PBDE derivatives that showed minimal activity were *para*-substituted with a hydroxyl group, whereas the *para* position of the predominant isomers in biological samples is occupied by bromine atom, suggesting that the relevance of the results to commercial PBDE mixtures is unclear. Additionally, because all of the available estrogenicity data are *in vitro*, there is, as yet, no evidence either for estrogenicity or anti-estrogenicity of PBDEs and/or PBDE metabolites *in vivo*.

The same 17 PBDE congeners and three hydroxylated PBDEs were also tested for possible interaction with T_4 binding to human TTR, a plasma transport protein of thyroid hormones, in an *in vitro* competitive binding assay (Meerts et al. 1998, 2000). None of the pure congeners competed with T_4 for binding to human TTR. 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: 4,4'-diBDE (BDE 15), 2,4,4'-triBDE (BDE 28), 2,4,6-triBDE (BDE 30), 2,2',4,4'-tetraBDE (BDE 47), 2,2',4,6'-tetraBDE (BDE 51), 2,4,4',6-tetraBDE (BDE 75), 3,3,4,4'-tetraBDE, 2,2',4,4',6-pentaBDE, and 2,3',4,4',6-pentaBDE (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-tribromophenoxy]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). Studies with hydroxylated derivatives of PBBs similarly showed that the congener patterns most closely resembling the diiodophenolic ring of thyroxine had the highest TTR binding activity (Chauhan et al. 2000; McKinney et al. 1987; Rickenbacher et al. 1986).

Because PBDEs were able to compete with T_4-TTR binding only after metabolic conversion, and considering that thyroid hormones are also hydroxy-halogenated diphenyl ethers, the results of the Meerts et al. (1998, 2000) study suggest that hydroxylation of PBDEs could be involved in the mechanism of thyroid toxicity. However, there are indications that hydroxylated metabolites might not play a large role for thyroid effects of commercial penta- and octaBDE mixtures. For example, of the *in vitro*-generated metabolites displacing T_4 from TTR (Meerts et al. 1998, 2000), only two (2,2',4,4'-tetraBDE (BDE 47) and 2,2',4,4',6-pentaBDE) are known to be present in a commercial mixture (pentaBDE). An *in vivo* study in rats (Om and Klasson-Wehler 1998) showed that BDE 47 is well absorbed (~95%), poorly metabolized (only 3% of the amount excreted in feces was in the form of metabolites), and occurs in

blood and other tissues mainly as the parent compound. Additionally, the three pure hydroxylated PBDEs synthesized by Meerts et al. (1998, 2000) for their structural similarity to thyroid hormones are not based on congeners known to be present in commercial penta- or octaBDE mixtures; this suggests that the congeners in these mixtures do not share close structural relationships with thyroid hormones. In another study, 4'-hydroxyl derivatives of 1,3,5-triBDE, 1,3,3',5'-tetraBDE, and 1,3,3',5,5'-pentaBDE were tested for affinity to the human thyroid hormone receptor proteins THR-α and THR-β *in vitro* (Marsh et al. 1998). 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 4–>1,000 times less than for T_4 and T_3). Because the tested congeners were the most likely to have affinity for the thyroid hormone receptor, it is likely that other hydroxylated PBDE congeners will have even lower potential for receptor binding.

Studies of serum hormone levels and organ histology indicate that the thyroid is a sensitive target for some PBDEs. Reduced serum T_4 levels and follicular cell hyperplasia have been consistently observed in rats and mice orally exposed to PBDEs. Acute-duration studies showed decreases in serum T_4 in rats exposed to ≥10 mg/kg/day octaBDE or ≥30 mg/kg/day pentaBDE for 4 days and in rats and mice exposed to ≥18 mg/kg/day pentaBDE for 14 days (Darnerud and Sinjari 1996; Hallgren et al. 2001; Zhou et al. 2001). Effects observed in intermediate-duration studies include thyroid hyperplasia in rats exposed to ≥8 mg/kg/day octaBDE for 30 days (Norris et al. 1973, 1975a, 1975b) and reduced serum T_4 in rats exposed to ≥10 mg/kg/day pentaBDE for 90 days (WIL Research Laboratories 1984). Exposure to pentaBDE on gestation day 6 through postnatal day 21 caused serum T_4 reductions at 30 mg/kg/day in maternal rats and at ≥10 mg/kg/day in their offspring (Zhou et al. 2002). Intermediate-duration exposure to a 77% decaBDE/22% nonaBDE commercial mixture caused thyroid hyperplasia in rats at doses of ≥80 mg/kg/day for 30 days (Norris et al. 1973, 1975a, 1975b). Chronic (103-week) exposure to high-purity commercial decaBDE (≥97%) did not induce thyroid histopathological changes in rats at ≤2,550 mg/kg/day, although follicular cell hyperplasia developed in mice exposed to 2,240 mg/kg/day (NTP 1986).

As summarized above, evidence for thyroid hormone involvement in PBDE toxicity includes observations in rats and mice that were orally exposed to commercial mixtures of deca-, octa- or pentaBDE. The main effects include (1) histological changes in the thyroid indicative of glandular stimulation (e.g., follicular cell hyperplasia similar to that induced by a hypothyroid state) (IRDC 1976; Norris et al. 1973, 1975b; NTP 1986; WIL Research Laboratories 1984), and (2) decreased serum thyroxine (T_4) levels with no accompanying 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), and decaBDE appears to be much less potent than the lower brominated mixtures. Considering these data, the structural resemblance of some PBDEs to T_4, and information from binding studies of individual congeners as summarized above, it is hypothesized that, depending on dose, duration, and mixture/congener, PBDEs can disrupt the production, transport, and disposition of thyroid hormones.

The extent that PBDEs affect circulating levels of T_4 or T_3 is likely to vary with species, and rats are generally regarded as more sensitive than humans. 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; that latter being, in part, related to the absence of thyroid binding globulin (TBG) in rats (Capen 1997). Transthyretin (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 cerebrospinal 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). 2,2',4,4'-TetraBDE (BDE 47) competitively inhibited binding of T_4 to sites in rat choroid plexus homogenates following *in vivo* exposure (Sinjari et al. 1998).

5.7 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 8.6 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 infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). 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).

Children are exposed to PBBs and PBDEs in the same manner as the general population, primarily via consumption of contaminated foods. Exposure also may occur by transfer of PBBs and PBDEs that have accumulated in women’s bodies to the fetus across the placenta. Because PBBs and PBDEs are lipophilic substances, they can additionally accumulate in breast milk and be transferred to nursing infants.

Placental transfer, although it may be limited in absolute amounts, is a concern because of possible effects of PBBs and PBDEs on sensitive immature tissues, organs, and systems, with potentially serious long-lasting consequences. Transfer of PBBs and PBDEs via breast milk could be relatively considerable and, like prenatal exposure, has the potential to contribute to altered development.

Although embryos, fetuses, and nursing infants may be exposed to relatively high amounts of PBBs and PBDEs during sensitive periods of development, it is not known if the susceptibility of children to the health effects of these chemicals differs from that of adults. Younger children may be particularly vulnerable to PBBs and 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. No specific information was located regarding the pharmacokinetics of PBBs in children or nutritional factors that may influence the absorption of PBBs.

No biomarkers of exposure or effects for PBBs or PBDEs have been validated in children or in adults exposed as children. There also are no biomarkers in adults that identify previous childhood exposure. No studies were located regarding interactions of PBBs or PBDEs with other chemicals in children or adults. No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to PBBs or PBDEs, reducing body burden, or interfering with the mechanism of action for toxic effects. In addition, no data were located regarding whether methods for reducing toxic effects in adults might be contraindicated in children.

Polybrominated Biphenyls. Information on health effects of PBBs in children is available from several studies of the Michigan contamination episode. A 1976 medical history questionnaire study of 342 Michigan children likely to have been exposed to PBBs found that the number of subjectively reported symptoms of ill health, including several symptoms of neurological effects, did not increase with increasing serum PBB levels (assayed in 1976), but rather decreased; general neurological examinations did not reveal a pattern of abnormality among the Michigan children (Barr 1980). Studies of fetal mortality rates in Michigan (Humble and Speizer 1984) and of physical and neuropsychological development in Michigan children exposed during the contamination episode (Schwartz and Rae 1983; Seagull 1983; Weil et al. 1981) did not conclusively correlate the ingestion of PBBs with effects, as summarized below.

Examination of approximately 100 children presumably exposed *in utero* or in early infancy during the peak of the Michigan contamination episode and whose families lived on farms known to be contaminated with PBBs has not revealed any consistent or marked abnormalities. No significant abnormalities were found by physical and neuropsychological examination of 33 of these exposed children when they had a mean age of 37.2 months, compared with a group of 20 age-matched, non-exposed control children (Weil et al. 1981). However, subjective interviews with parents suggested that more exposed children than control children had frequent upper respiratory illnesses such as colds, runny noses, and sore throats (Weil et al. 1981). Administration of 5 of 18 possible neuropsychological development tests from the McCarthy Scales of Children's Abilities to 19 of these exposed children at ≈2.5–4 years of age showed a statistically significant negative correlation between PBB levels in fat tissue and developmental abilities in four of the five tests (Seagull 1983). Subsequent administration of the full battery of 18 neuropsychological tests, as well as I.Q. tests, to the same group of children when ≈4–6 years old, found that the exposed children's performances were within the normal range in all areas assessed (Schwartz and Rae 1983). Due mainly to the small data set and the inconsistency of the results, the available data do not conclusively establish or eliminate the possibility that *in utero* and early infancy exposure to PBBs might adversely affect the development of human children.

Neurobehavioral alterations have been observed in animals following gestational and lactational exposure to PBBs. Performance deficits in tests of operant behavior were seen in 6-month-old offspring of rats that were exposed to ≥0.2 mg/kg/day of FireMaster BP-6 by gavage from day 6 of gestation until weaning (Henck and Rech 1986), but not in 75-day-old offspring of rats exposed to ≥0.5 mg/kg/day for 4 weeks prior to mating (Geller et al. 1985). Effects on acquisition of forward locomotion, cliff avoidance, cage emergence, and open-field activity were found in offspring of rats exposed to ≥0.2 mg/kg/day of FireMaster BP-6 in the diet from day 6 of gestation through PND 24 and tested through PND 60 (Henck et al. 1994). Testing of mouse offspring at 30–120 days of age following maternal exposure to FireMaster FF-1 by gavage on every other day during gestation and through weaning showed altered negative geotaxis and avoidance response latencies at ≥3 mg/kg/day and reduced acoustic startle responsiveness and motor activity at 10 mg/kg/day (Tilson 1992).

Animal studies of FireMaster FF-1 and FireMaster BP-6 have also shown that hexabromobiphenyl PBB mixtures can induce non-neurological developmental toxicity. Embryolethal effects or increased mortality among nursing young were observed in rats (Beaudoin 1977, 1979; Groce and Kimbrough 1984) and mice (Luster et al. 1980) after oral exposure during gestation and in monkeys after exposure before conception and during pregnancy (Allen et al. 1979; Lambrecht et al. 1978). Structural

malformations in fetuses, including cleft palate, were also observed in rats (Beaudoin 1977) and mice (Corbett et al. 1975) after exposure to these PBBs during gestation. Increased incidences of fetuses with extra ribs were found in a study of rats orally exposed to a commercial octabromobiphenyl mixture during gestation (Waritz et al. 1977), although decabromobiphenyl was not embryotoxic, fetotoxic, or teratogenic in rats (Millischer et al. 1980). Other studies with FireMaster FF-1 and FireMaster BP-6 found that body weight gain was reduced in the offspring of rats and mice after exposure during gestation and/or lactation (Corbett et al. 1975; Groce and Kimbrough 1984; McCormack et al. 1981, 1982c; Meserve et al. 1992). Liver effects, including increased liver weight and hepatic cytochrome P-450 enzymic activity, hepatocyte enlargement, vacuolization, and/or other degenerative changes, occurred in the offspring of rats, mice, and swine fed FireMaster FF-1 or FireMaster BP-6 during gestation and/or lactation (Chhabra et al. 1993; Moore et al. 1978; NTP 1992; Werner and Sleight 1981).

Other effects in offspring of animals exposed to PBBs during gestation and lactation include altered thyroid hormone levels. Serum T_4 levels were reduced in 15-day-old offspring of rats that were exposed to 2.5 mg/kg/day FireMaster BP-6 in the diet from GD 0 through PND 15 (Meserve et al. 1992). The pups had received pituitary stimulation by an injection of corticotropin-releasing factor or adrenal stimulation by an injection of adrenocorticotropic hormone. Serum concentrations of T_3 and T_4 were significantly reduced in 4-week-old nursing offspring of swine that were fed ≥1.25 mg/kg/day dietary doses of FireMaster BP-6 during the second half of gestation and throughout lactation (Werner and Sleight 1981). These effects in offspring are consistent with evidence that the thyroid gland is an unequivocal target of PBBs in adult animals. A spectrum of thyroid effects, ranging from decreases in serum T_4 and T_3 levels to histological and ultrastructural changes in the follicles, has been documented in adult rats orally exposed to PBBs (mainly FireMaster BP-6 and FF-1) for acute and intermediate durations (Akoso et al. 1982b; Allen-Rowlands et al. 1981; Byrne et al. 1987; Gupta and Moore 1979; Kasza et al. 1978a; Norris et al. 1975a, 1975b; NTP 1983; Sepkovic and Byrne 1984; Sleight et al. 1978). Additionally, there is suggestive limited evidence of thyroid effects in adult humans; effects in workers exposed to unspecified PBBs and/or decaBDE included increased serum FSH, low or borderline low serum T_4, and increased thyroid antimicrosomal antibody titers (Bahn et al. 1980).

No information was located on possible immunological effects of PBBs in children, and data in adult humans are limited and largely inconclusive. Altered lymphocyte transformation responses among populations exposed to PBBs during the Michigan contamination episode have been reported in some studies (Bekesi et al. 1978; Roboz et al. 1985), but other investigations were not able to confirm these findings (Landrigan et al. 1979; Silva et al. 1979; Stross et al. 1981). No correlation can be established

between altered immune parameters and serum PBB levels based on the available data. Exposure to PBBs caused altered immune responses in a variety of animal species, which suggests that humans may also be affected. Studies in animals, mostly intermediate-duration studies in rodents, showed that a variety of immunological parameters such as spleen and thymus weights (Gupta and Moore 1979; Gupta et al. 1981; NTP 1983), antibody production (Loose et al. 1981), and lymphoproliferative responses (Howard et al. 1980; Luster et al. 1978, 1980) can be affected by treatment with commercial PBB mixtures, although some of these effects were only seen at PBB levels that cause overt toxicity (Luster et al. 1978, 1980).

Levels of PBBs in breast milk have been measured in women exposed as a result of the Michigan contamination episode. The milk concentrations of PBBs in women from the lower peninsula of Michigan (exposed area) were generally higher than in breast milk of females from the upper peninsula (farthest from the sources) (Brilliant et al. 1978). PBB levels in breast milk of five women from exposed farms ranged from 0.21–92.7 ppm (Cordle et al. 1978; Humphrey and Hayner 1976). On a lipid basis, the ratio of PBBs in breast milk to maternal serum was 107–122 to 1 and in adipose tissue to breast milk was 1.1–1.5 to 1 in a cohort of Michigan residents (Eyster et al. 1983; Landrigan et al. 1979). No monitoring information was located on PBBs in breast milk for U.S. populations outside of Michigan.

Determinants of PBB serum decay were investigated in Michigan women who had a mean initial PBB level of 20.9 ppb, a mean time between the first and last measurement of 4.2 years, and at least two measurements taken when they were not pregnant (Blanck et al. 2000b). Assuming that PBBs reached equilibrium in the body before substantial amounts were eliminated and before the first serum measurements were taken, the authors estimated the median PBB half-life to be 13.5 years. Subject-specific decay rates were regressed on various predictor variables. Results of the analysis included the finding that an increasing number of pregnancies between the first and last measurement was associated with a slower rate of serum PBB decay (the effect had borderline statistical significance). Breast feeding as either a continuous variable or as categorized by duration (<3, 3–9, or >9 months) was not associated with serum PBB decay. Additional information on the design and results of this study is provided in Section 5.8.1. Another study of women exposed to PBBs during the Michigan contamination episode similarly found no relationship between serum levels of PBBs and the frequency and duration of lactation (Thomas et al. 2001). Characteristics of the study cohort included a mean initial serum PBB level of 17.5 ppb, an estimated mean serum PBB level at delivery of 9.4 ppb, a mean duration of breast-feeding as the main source of nutrition for 2.6 months, and a mean total duration of breast-feeding of 4.1 months. Exposure was treated as a categorical variable by dividing the women into groups of low (reference)

exposure (≤1 ppb), moderate exposure (>1–≤7 ppb), and high exposure (>7 ppb). Three outcomes of interest were analyzed: (1) the decision to breast feed (yes/no), (2) the duration (months) of breast-feeding as the main source of nutrition, and (3) the total duration (months) of breast-feeding. None of the three outcomes was significantly associated with serum PBB levels, even after controlling for various confounding determinants, including histories of previous breast-feeding and thyroid disorders. Additional information on the design and results of this study is provided in Section 5.2.2.5.

Polybrominated Diphenyl Ethers. No specific information was located on health effects of PBDEs in newborn or older children. Thyroid and neurobehavioral alterations have been observed in developing animals following pre- and/or postnatal exposure to lower brominated BDEs, indicating that infants and children might be particularly susceptible to these effects. The potential for thyroid and neurodevelopmental effects of decaBDE is expected to be relatively low due to differences in the toxicokinetics and toxicity of decaBDE compared to lower brominated BDEs.

Thyroid hormones regulate cell proliferation, migration, and differentiation during development, and maintenance of normal levels is essential to normal growth and development. Disruption of circulating hormone levels can have markedly different effects, depending on the stage of development, and even transient disruptions can produce permanent effects. Effects can include 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 lower brominated 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 T4 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).

Although there is no evidence that exposure to PBDEs causes thyroid effects in humans, the thyroid is a well-documented target of lower brominated commercial mixtures and single congeners in adult and neonatal rats and mice. The main effects in animals are reduced serum T_4 hormone levels and follicular cell hyperplasia, with no accompanying changes in serum T_3 or TSH levels. In adult animals, acute-duration oral exposure caused decreases in serum T_4 in rats exposed to ≥10 mg/kg/day octaBDE or ≥30 mg/kg/day pentaBDE for 4 days, and in rats and mice exposed to ≥18 mg/kg/day pentaBDE for 14 days (Darnerud and Sinjari 1996; Hallgren and Darnerud 1998; Hallgren et al. 2001; Zhou et al. 2001). Effects observed in intermediate-duration oral studies included thyroid hyperplasia in rats exposed to ≥8 mg/kg/day octaBDE for 30 days (Norris et al. 1973, 1975a, 1975b) and reduced serum T_4 in rats exposed to ≥10 mg/kg/day pentaBDE for 90 days (WIL Research Laboratories 1984).

Other animal studies have shown reduced serum levels of thyroid hormones in offspring of rats exposed to lower brominated PBDEs during gestation and lactation, as well as in rats orally exposed as weanlings. Exposure to pentaBDE from GD 6 through the end of lactation caused serum T_4 decreases in maternal rats (on GD 20 and PND 22) at 30 mg/kg/day and in their offspring (on PNDs 4 and 14) at ≥10 mg/kg/day (Zhou et al. 2002). Assessment of weanling (28-day-old) rats that were orally exposed to PBDEs for 4 days and evaluated for thyroid hormone changes on the day after the last exposure showed that octaBDE caused significantly reduced serum T_4 and T_3 levels at ≥10 and ≥60 mg/kg/day, respectively (Zhou et al. 2001). Similar exposure to pentaBDE caused serum T_4 and T_3 decreases at higher dose levels of ≥30 and ≥100 mg/kg/day, respectively. The results of these studies are consistent with findings of reduced serum T_4 hormone levels and follicular cell hyperplasia in adult rats and mice confirming that the thyroid is a sensitive target of octaBDE and pentaBDE at doses as low as 10 mg/kg/day (Darnerud and Sinjari 1996; Hallgren et al. 2001; WIL Research Laboratories 1984).

Although alterations in thyroid hormone homeostasis can cause neurodevelopmental effects, little specific information is currently available on the potential neurotoxic effects of PBDEs. Data are mainly limited to the results of three behavioral tests in animals showing some alterations in spontaneous locomotion behavior and learning and memory ability in mice that were tested at 2 months of age and as adults (4 months), following neonatal exposure (PNDs 3, 10, or 19) to single low oral doses of the congeners 2,2',4,4'-tetraBDE (BDE 47) or 2,2',4,4',5-pentaBDE (BDE 99) (Eriksson et al. 1998, 1999, 2001, 2002a). Effects on spontaneous activity were observed in adult mice treated at either 3 or 10 days of age, but not at 19 days of age, suggesting that there is a critical phase of neonatal brain development for the induction of behavioral disturbances (Eriksson et al. 1999, 2002a). No studies were located that sufficiently evaluated neurological effects of PBDEs in animals exposed as adults. None of the commercial decaBDE,

octaBDE, and pentaBDE products have been screened for neurotoxicity using comprehensive test batteries that typically include functional observational, motor activity, and neuropathology evaluations. There were no indications of neurotoxicity in rats and mice fed high dietary dose levels of a 94–97% pure commercial decaBDE mixture for 14 days (≤19,000 mg/kg/day), 13 weeks (≤9,500 mg/kg/day), or 103 weeks (≤7,780 mg/kg/day), as assessed by overt clinical signs (all exposures) and nervous system histopathology (chronic exposure only) (NTP 1986). Although the high doses and extended exposure durations in the NTP (1986) studies provided opportunities for the development of effects, neurotoxicity is only incompletely evaluated due to the lack of testing for subtle behavioral and other sensitive neurological end points.

The human relevance of the thyroid and neurodevelopmental 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 thyroid binding globulin (TBG) in rats (Capen 1997). Transthyretin (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 cerebrospinal 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, mechanism by which lower brominated BDEs cause decreased serum T4 might involve hepatic microsomal enzyme induction and consequent increased metabolic formation of hydroxy-metabolites, but humans are not particularly sensitive to this effect. Additionally, the animal studies in which neurobehavioral alterations were observed were performed using an atypical design that has not been validated, and used exposure levels much higher than those likely to be experienced by humans.

The potential for induction of thyroid and neurodevelopmental effects by decaBDE is low in comparison to commercial penta- and octaBDE mixtures due to toxicokinetic and toxicity differences. The lower brominated BDEs preferentially accumulate in the body due to their partitioning and retention in lipid-rich tissues and lower rates of metabolism and elimination relative to decaBDE (Hardy 2002b; Morck et al. 2001, 2003). These characteristics seem to be a function of the number and location of bromines on the diphenyl oxide molecule . The tetra- and pentaBDEs appear to be relatively well absorbed, whereas the fully brominated decaBDE, a large poorly soluble molecule, is very poorly absorbed (≈1% or less of

an oral dose) and rapidly eliminated (≈99% of the dose within 72 hours). Further, decaBDE is significantly less toxic than lower brominated BDE mixtures. For example, a study in weanling rats showed that acute oral exposure to commercial decaBDE had no effect on thyroid hormones, while similar exposures to commercial octaBDE or pentaBDE mixtures caused decreases in serum T_4 and T_3 levels (Zhou et al. 2001). Similarly, acute postnatal oral exposure to pure decaBDE congener (BDE 99) was less potent than BDE 47, BDE 99, and 2,2',4,4',5,5'-hexaBDE (BDE 153) in inducing behavioral effects in adult mice (Eriksson et al. 1999, 2001a, 2002a; Viberg et al. 2001a, 2001b, 2002a, 2002b, 2003). Chronic (103-week) exposure to a high-purity (≥97%) commercial decaBDE mixture induced thyroid follicular cell hyperplasia in mice, but the dose levelwas very high (2,240 mg/kg/day) and no thyroid histological changes occurred in similarly exposed rats (NTP 1986).

No information is available regarding the immunosuppressive potential of PBDEs in young animals, and data on immune function in adult animals are mainly limited to findings in acute-duration oral studies of relatively high doses of pentaBDE. The 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 mitogen-stimulated splenocytes was reduced in mice exposed to 36 mg/kg/day pentaBDE for 14 days (Darnerud and Thuvander 1998). Other 14-day studies in mice found no changes in natural killer cell activity at dosages ≤72 mg/kg/day (Fowles et al. 1994) or numbers of splenic and thymic lymphocyte subsets at dosages ≤36 mg/kg/day (Darnerud and Thuvander 1998), although 18 mg/kg/day of the single congener 2,2',4,4'-tetraBDE (BDE 47) caused significantly reduced numbers of total lymphocytes and CD4+, CD8+, and CD45R+ subtypes in spleen (Darnerud and Thuvander 1998). Histological examinations of spleen, thymus, lymph node, and/or bone marrow tissues in systemic toxicity studies showed no effects of repeated dietary administration in intermediate-duration studies in rats exposed to ≤8,000 mg/kg/day decaBDE for 13 weeks (NTP 1986), in mice exposed to ≤9,500 mg/kg/day decaBDE for 13 weeks, in rats exposed to ≤750 mg/kg/day octaBDE for 13 weeks (IRDC 1977), or in rats exposed to ≤100 mg/kg/day pentaBDE for 90 days (WIL Research Laboratories 1984). Chronic ingestion of decaBDE caused lesions in the spleen of rats exposed to ≥1,200 mg/kg/day (splenic hematopoiesis in males) or 2,240 mg/kg/day (splenic fibrosis and lymphoid hyperplasia in females) for 103 weeks (NTP 1986). None of these studies reported changes in clinical condition that could be indicative of reduced immunocompetence. Although the high doses and extended exposure durations provided opportunities for the development of histopathology in immune system tissues and relevant clinical signs, no comprehensive immunotoxicity evaluations of PBDEs have been performed.

Information on the reproductive toxicity of PBDEs mainly consists of a one-generation study of decaBDE in rats that found no exposure-related functional effects (Dow Chemical Co. 1975; Norris et al. 1975a, 1975b). No histopathological changes were observed in male or female reproductive tissues from rats that were exposed to PBDEs in dietary systemic toxicity studies, including decaBDE in rats at doses ≤8,000 mg/kg/day for 13 weeks or ≤2,550 mg/kg/day for 103 weeks (NTP 1986), decaBDE in mice at doses ≤9,500 mg/kg/day for 13 weeks or ≤7,780 mg/kg/day for 103 weeks (NTP 1986); octaBDE in rats at ≤750 mg/kg/day for 13 weeks (IRDC 1977); or pentaBDE in rats at doses ≤100 mg/kg/day for 90 days (WIL Research Laboratories 1984). Developmental toxicity studies showed no evidence of teratogenicity of deca-, octa-, and pentaBDE in rats and rabbits, although fetotoxic effects, including skeletal ossification variations at maternally toxic doses have been observed (Argus Research Laboratories 1985b; Breslin et al. 1989; Dow Chemical Co. 1975, 1985; Life Science Research Israel Ltd. 1987; Norris et al. 1975a, 1975b; WIL Research Laboratories 1986).

Results of *in vitro* estrogen receptor assays suggest that there is a potential for some PBDEs to disrupt endocrine system functions in humans. The estrogenic and antiestrogenic activities of 17 PBDE congeners and 3 hydroxylated PBDEs were tested *in vitro* using human breast cell line assays based on ER-dependent luciferase reporter gene expression (Meerts et al. 2001). Eleven of 17 PBDE congeners showed estrogenic activity (dose-dependent luciferase induction) in the ER-CALUX assay with T47D.Luc cells, although the most potent PBDE congeners (2,2',4,4',6-pentaBDE > 2,4,4',6-tetraBDE [BDE 75] > 2,2',4,6'-tetraBDE [BDE 51] > 2,4,6-triBDE [BDE 30] > 2,3',4,4',6-pentaBDE [BDE 119]) had EC_{50} values that were 250,000–390,000 times less potent than that of the natural ligand, 17β-estradiol (E_2). In contrast, two of the hydroxylated PBDEs, 4-(2,4,6-tribromophenoxy)phenol, 2-bromo-4-(2,4,6-tribromophenoxy)phenol (which have bromine substitution patterns similar to the thyroid hormones T_2 [3,5-diiodothyronine] and T_3 [3,3’,5-triiodothyronine], respectively) had estrogenic potencies exceeding that of E_2. Three of the compounds with potent estrogenic activity in the ER-CALUX assay, 2,4,6-triBDE (BDE 30), 2,2',4,4',6-pentaBDE, and 4-(2,4,6-tribromophenoxy)phenol, were also tested for estrogenicity in ERα-specific and ERβ-specific human embryonic kidney cell lines (293-ERα-Luc and ERβs-Luc cells, respectively). The hydroxylated PBDE was potent in the ERα-specific cells (maximum luciferase induction similar to E_2) and also showed activity in the ERβ-specific cells (maximum 50% induction compared to E_2), whereas the ERα- and ERβ-specific cell lines were less responsive to BDE 30 and 2,2',4,4',6-pentaBDE. These results indicate that pure congeners of PBDEs can be agonists of both ERα and ERβ receptors and that metabolism to hydroxylated PBDEs may increase estrogenic potency. However, no estrogenic activity was demonstrated by the predominant congeners in biological samples, 2,2’,4,4’-tetraBDE (BDE 47) and 2,2’,4,4’,5-pentaBDE (BDE 99). The hydroxy-

PBDE derivatives that showed minimal activity were *para*-substituted with a hydroxyl group, whereas the *para* position of the predominant isomers in biological samples is occupied by bromine atom, suggesting that the relevance of the results to commercial PBDE mixtures is unclear. Additionally, because all of the available estrogenicity data are *in vitro*, there is no evidence for estrogenicity or anti-estrogenicity of PBDEs or PBDE metabolites *in vivo,* including possible effects on reproductive development in children.

Lower brominated PBDEs are pervasive environmental contaminants that bioaccumulate in the mother's body and can be transferred to infants through the placenta and breast milk. Considering the continued widespread production and use of PBDEs, particularly commercial pentaBDE mixtures whose constituent tetra- to hexaBDE congeners are highly bioaccumulative, as well as the time lag from current-year usage to exposure via the food chain, it is probable that tissue concentrations among the general population will continue to rise. The accumulation of decaBDE in breast milk does not appear to be appreciable, in large part because the amount in the maternal bloodstream and available for transfer to breast milk is low due to poor gastrointestinal absorption and rapid elimination in the feces (Hardy 2002b). The increasing temporal trend for PBDEs in human tissues is illustrated by findings of an exponential increase of PBDEs in Swedish human breast milk from 1972 to 1997 with a doubling rate of 5 years (Norén and Meironyté 1998, 2000). The milk concentrations of PBDEs (sum of eight congeners) on a lipid basis were 0.07 ppb in 1972 and 4 ppb in 1997 (Meironyté et al. 1999). Analysis of samples from 11 Finnish women showed that PBDE concentrations (sum of four congeners, lipid basis) were similar in breast milk and placenta, with ranges of 0.99–5.89 and 1.00–4.40 ppb, respectively (Strandman et al. 2000). The four highest sum concentrations were from women following their first childbirth. No PBPK models have been developed for PBDEs that could be used to quantitatively predict transfer of PBDEs via breast milk or across the placenta.

Assessments of potential health risks to children were performed for commercial pentaBDE, octaBDE, and decaBDE products by industry as part of the EPA Voluntary Children's Chemical Evaluation Program (VCCEPP) (BFRIP 2002; ENVIRON 2003a, 2003b). Based on evaluations of toxicity and potential exposure data, the reports concluded that none of the three commercial mixtures were expected to be harmful to children. These assessment documents have been independently reviewed as part of the VCCEP Peer Consultation process, but final reports reflecting peer review findings and EPA conclusions are not yet available (EPA 2004a, 2004b, 2004c).

5.8 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).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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 or 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 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 PBBS and PBDEs are discussed in Section 5.8.1.

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 PBBS and PBDEs are discussed in Section 5.8.2.

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 5.10 “Populations That Are Unusually Susceptible”.

5.8.1 Biomarkers Used to Identify or Quantify Exposure to PBBs and PBDEs

Polybrominated Biphenyls. PBBs are environmental contaminants found mainly, but not exclusively, in body tissues and fluids of populations with known exposure to PBBs. Because they are lipophilic and have long half-lives, certain PBB congeners preferentially accumulate in lipid-rich tissues, especially adipose, and are present in serum and human milk. Both serum and adipose PBB levels are indicators of exposure, but monitoring PBBs simultaneously in samples of both types is more reliable than in serum only. The serum/adipose partition ratios for groups of pregnant and nonpregnant Michigan women and chemical workers ranged between 1:140 and 1:260; the value for Michigan male farmers was 1:325–329 (Eyster et al. 1983). These values agree with those reported by other investigators for similar populations (Landrigan et al. 1979; Wolff et al. 1982). The importance of a dual determination of PBBs in serum and adipose can be illustrated with the following example. In a Michigan cohort, 70% of 839 subjects were identified as having had exposure by their serum PBB levels. When adipose tissue results were added, an additional 24% indicated exposure (Wolff et al. 1982). The larger number of people with measured exposure when adipose tissue results were included reflects the higher fat content of adipose compared to serum and the lipophilicity of the chemicals. The partition ratio of ≈1:300 made the adipose limit of detection a more sensitive indicator of exposure, even though the limit of detection in adipose was one order of magnitude higher than in serum. Partition ratios below those reported from groups expected to be in equilibrium may indicate current or recent exposure (Anderson 1985).

Using an animal physiological compartment model scaled to humans by adjusting tissue volume, blood flow, and clearance and rate constant parameters, it was predicted that human intake of 9.8 g of 2,2',4,4',5,5'-hexabromobiphenyl (BB 153) from milk consumption over a 230-day period would result in peak tissue concentrations of 720 and 2.1 ppm in adipose and blood, respectively, at 8 months, and 443 and 1.3 ppm, respectively (Tuey and Matthews 1980). The elimination rate after 5 years would be 1.63 mg/day, the body burden would be 5.2 g, and the half-life would be 6.5 years. When a dose of 0.1 mg/day for 10 months was simulated, the excretion rate in a lean individual was estimated at 10.2 µg/day; overweight individuals had an excretion rate of 4.1 µg/day. PBB in adipose tissue from the lean and overweight subjects were predicted to be 2,769 and 1,103 ppb, respectively. PBB in serum would be 8.1 ppb in lean subjects and 3.2 ppb in overweight subjects, indicating that $t_{1/2}$ increases with increasing fat content. These predictions point to the importance of the percentage of body fat in the equilibrium dynamics of PBBs and indicate that because lean individuals have a smaller fat compartment, all of their body tissues will have higher concentrations of PBB than those in fatter individuals of the

same exposure (Tuey and Matthews 1980). The assumptions on which the predictions are based do not reflect possible differences in fat and lean subjects due to the way that PBBs are compartmentalized and/or excreted as a percent of the total body burden, or in decay rates due to differential partitioning.

As indicated above, PBBs are persistent chemicals due to their lipophilicity. Some studies have reported practically no change in serum PBB levels over a 12–18-month period (Wolff et al. 1979b) or over a 3-year period (Landrigan et al. 1979). The half-life of 6.5 years predicted by Tuey and Matthews (1980) is shorter than half-life values determined using sera data from the Michigan PBB cohort (Blanck et al. 2000b; Lambert et al. 1990; Rosen et al. 1995). A median half-life of 12.0 years (95% CI 4–97 years) was estimated based on two serum measurements from 15 women (≥20 years of age) with an initial serum PBB level of ≥5 ppb (Lambert et al. 1990). An analysis of 51 women (≥18.8 years of age) and 112 men (≥18.1 years of age) with at least two measurements 1 year apart and an initial PBB level of ≥20 ppb found a median half-life of 13.0 years (95% CI 6.3–infinite years) and 10.0 years (95% CI 6.7–20.0 years), respectively (Rosen et al. 1995). Based on a median half-life of 10.8 years (95% CI 9.2–14.7 years) for the entire group (163 persons, median PBB level 45.5 ppb), it was estimated that it will take more than 60 years for their PBB levels to fall below a detection limit of 1 ppb.

Determinants of PBB serum decay were investigated in a group of 380 Michigan women (≥16 years of age) who had an initial PBB level of at least 2 ppb and at least two measurements taken when they were not pregnant (Blanck et al. 2000b). The mean initial PBB level was 20.9 ppb (standard deviation 78.7), and the mean time between the first and last measurement was 4.2 years (range 16.0–75.2 years). A total of 109 women (29%) did not have a reduction in serum PBB over time. Assuming that PBBs reached equilibrium in the body before substantial amounts were eliminated and before the first serum measurements were taken, the entire body was modeled as a single compartment for PBBs with exponential decay. The median PBB half-life in the entire group was 13.5 years (95% CI 10.5–23.2 years). Subject-specific decay rate estimates were regressed on predictor variables including initial age, body mass index (BMI), smoking history, breast-feeding duration, and parity. The serum PBB decay rate was slower, resulting in a longer half-life, with higher initial PBB levels; women with initial PBB levels of <10 and >10 ppb had median half-lives of 12.9 and 28.7 years, respectively. The PBB decay rate was also slower ($p=0.03$) in women with an initial BMI above the median (BMI≥23). Increasing number of pregnancies between the first and last measurement was also associated with slower decay, but the effect was of borderline statistical significance ($p=0.06$). Breast feeding as either a continuous variable or as categorized by tertiles of duration (<3, 3–9, or >9 months), age, and smoking were not associated with serum PBB decay.

The average concentration of PBBs (on an adipose basis and as hexabromobiphenyl) in pooled extracts of several hundred individual tissue samples collected in a statistically valid manner from all nine regions of the continental United States was 1–2 ppb (Lewis and Sovocool 1982). Chemical workers involved in the PBB manufacturing process had a median adipose PBB concentration of 6,000 ppb (range 400–350, 500 ppb); Michigan male farmers and chemical workers not involved in the PBB manufacturing process had a median of 1,050 ppb (range 70,000–350,000 ppb) (Eyster et al. 1983).

Polybrominated Diphenyl Ethers. Lower brominated PBDEs are persistent environmental contaminants that accumulate in adipose tissue, serum, and breast milk serum of the general population, whereas decaBDE does not appear to be bioaccumulative. The accumulation of lower brominated congeners is likely due to ease of absorption, metabolism, and elimination compared to decaDBE (i.e., related to each molecule's specific structure and not solely due to lipophilicity). Serum, adipose, and breast milk levels are indicators of exposure for lower brominated PBDEs. Lower brominated congeners in breast milk are useful as markers of maternal body burdens as well as lactational and *in utero* exposures. The predominant congeners identified in milk and other human tissues are 2,2',4,4'-tetraBDE (BDE 47), 2,2'4,4',5-pentaBDE (BDE 99), and 2,2'4,4',5,5'-hexaBDE (BDE 153) (all *ortho-para* substituted congeners). Considering the continued widespread production and use of PBDEs, particularly commercial pentaBDE mixtures whose tetra- to hexaBDE congeners are highly bioaccumulative, as well as the time lag from current-year usage to exposure via the food chain, it is highly likely that tissue concentrations among the general population will continue to rise. The increasing temporal trend for PBDEs in human tissues is suggested by findings of an exponential increase of PBDEs in Swedish human breast milk from 1972 to 1997 with a doubling rate of 5 years (Norén and Meironyté 1998, 2000). In the 1997 sample, the concentration of PBDE (sum of eight congeners) was 4 ppb on a lipid basis, whereas the 1972 sample contained 0.07 ppb (Meironyté et al. 1999). PBDEs have been detected in human placenta at concentrations similar to those in breast milk from the same women; concentrations (sum of four congeners, lipid basis) ranged from 0.99 to 5.89 ppb in milk and from 1.00 to 4.40 ppb in placenta (Strandman et al. 2000).

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 median percentage decrease 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 2,2',4,4'-tetraBDE (BDE 47),

2,2',4,4',5,5'-hexaBDE (BDE 153), 2,2',4,4',5,6'-hexaBDE (BDE 154), 2,2',3,4,4',5',6-heptaBDE (BDE 183), and 2,2',3,3',4,4',5,5',6,6'-decaBDE (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.

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, whereas 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.

5.8.2 Biomarkers Used to Characterize Effects Caused by PBBs and PBDEs

Polybrominated Biphenyls. Biomarkers of effects for PBBs are likely to be common to the general class of halogenated aromatic hydrocarbons, rather than specific for PBBs, because PCBs, PBDEs, and other structurally similar chemicals cause generally similar effects. A potential biomarker for PBBs is related to their effect on the thyroid gland. As discussed in Sections 5.2.2.2, Endocrine Effects, the thyroid gland is a sensitive and unequivocal target of PBBs in animals, and evidence in humans is suggestive of a similar relationship. Effects in workers exposed to unspecified PBBs and/or decabromobiphenyl included increased serum thyrotropin, low or borderline low serum T_4, and increased thyroid antimicrosomal antibody titers (Bahn et al. 1980). A spectrum of thyroid effects has been observed in exposed rats, ranging from decreases in serum levels of serum T_4 and T_3 to histological and ultrastructural changes (Akoso et al. 1982b; Allen-Rowlands et al. 1981; Byrne et al. 1987; Gupta and Moore 1979; Kasza et al. 1978a; Meserve et al. 1992; Norris et al. 1975a, 1975b; NTP 1983; Sepkovic and Byrne 1984; Sleight et al. 1978; Werner and Sleight 1981). Therefore, serum levels of T_4 and/or other thyroid hormones are potential biomarkers of effects for PBBs. Additional studies could better characterize thyroid effects of PBBs in humans and develop specific correlations between levels and duration of exposure and alterations in serum T_4 and T_3 levels, including information on the specific amount of change in the biomarkers associated with a demonstrably adverse effect. These potential biomarkers are not specific to PBBs because PBDEs and other antithyroid agents can have similar effects.

Caffeine has been used as a potential tool to characterize exposure and/or effect of PBBs (Lambert et al. 1990). In this test, caffeine is used as a metabolic probe of cytochrome P-450 isozymes activity from the CYP1A family, which is significantly induced by PBBs in animals (Safe 1984). The caffeine breath test (CBT) is primarily useful for detecting induction of CYP1A2 activity in human liver. Because the

induction of CYP1A enzymes is AhR mediated, the test has been used as a marker for exposure to PCBs, CDDs, and CDFs (Lambert et al. 1992). A volunteer population of 50 Michigan subjects with previously high serum PBB levels and 50 with undetectable or low serum levels was compared to a control population not exposed to PBBs (Lambert et al. 1992). Two tests were conducted, the CYP1A2-dependent caffeine 3-N-demethylase activity was monitored by the CBT, and 7-N-demethylase activity was monitored by the caffeine urinary metabolite ratio (CMR). PBB-exposed subjects had higher CBT values ($p<0.02$) than urban nonsmokers, but the values were comparable to those of urban smokers. The correlation between serum PBB levels and the CBT value was poor ($r^2=0.2$). The CMR value in PBB-exposed subjects was also higher than that of urban nonsmokers ($p<0.05$); there was no correlation between serum PBB levels and CMR values. Generally, smokers have higher CBT values than nonsmokers due to the presence of polycyclic aromatic hydrocarbons (PAH) in tobacco smoke, which induce CYP1A (Kotake et al. 1982).

Many reports have been published regarding possible associations between PBB exposure and adverse health effects in populations from the state of Michigan. An early study compared the health status of people on quarantined farms with people in nonquarantined farms in the same area (Humphrey and Hayner 1975). The results showed no pattern of differences between the groups. Moreover, no abnormalities of heart, liver, spleen, or nervous system that could be related to PBB exposure were found in physical examinations. A follow-up study examined the prevalence of selected symptoms in groups of varying potential exposure 4 years after exposure (Landrigan et al. 1979). In general, symptoms were more prevalent in two self-selected groups and were least prevalent in the group composed of chemical workers. No positive associations were found between serum PBB concentrations and symptom frequencies; yet a third group of studies reported an increased incidence of symptoms in Michigan farmers relative to a group of control Wisconsin farmers (Anderson et al. 1978a, 1978b, 1978c, 1979). As observed in other epidemiology studies, self-selected groups, which had lower PBB concentrations in serum, reported a high incidence of symptoms, compared to randomly selected groups. No specific biomarker of effect could be identified in the Michigan contamination episode. Furthermore, the prevalence of the reported symptoms had no consistent relationship to the extent or types of exposure, and most objective clinical measurements have failed to show a significant relationship to PBB exposure (Fries 1985a). Additional information regarding biomarkers for effects can be found in OTA (1990) and CDC/ATSDR (1990). For a more detailed discussion of the health effects caused by PBBs see Section 5.2.

Polybrominated Diphenyl Ethers. 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. The thyroid is a critical target for lower brominated PBDEs in animals. As discussed in Sections 5.2.2.2, Endocrine Effects, exposure to commerical penta- and octaBDE products caused thyroid changes in rats and mice, particularly reduced serum T_4 levels (Darnerud and Sinjari 1996; Fowles et al. 1994; Hallgren and Darnerud 1998; WIL Research Laboratories 1984; Zhou et al. 2001, 2002). The potential for induction of thyroid effects by decaBDE appears to be low in comparison to penta- and octaBDE mixtures due to toxicokinetic and toxicity differences. In particular, lower brominated BDE congeners preferentially accumulate in the body compared to decaBDE, apparently due to their greater partitioning and retention in lipid-rich tissues and lower rates of metabolism and elimination (Hardy 2002b; Morck et al. 2001, 2003), and decaBDE only affected the thyroid in animals following lifetime exposure to dose levels several orders of magnitude higher than effect levels of penta- and octaBDE in acute-duration studies (NTP 1986; Zhou et al. 2001).

Serum T_4 may not be a reliable biomarker in humans because the extent that PBDEs affect circulating levels of T_4 (or T_3) possibly varies with species. As discussed in Section 5.5.3, Animal-to-Human Extrapolations, humans are generally regarded as less sensitive than rats to thyroid effects of PBDEs due to differences in the binding of T_4 to serum transport proteins. In particular, in humans, the binding of T_4 to thyroxine binding globule (TBG) protects the hormones from metabolism and excretion, resulting in relatively long serum half-lives. Rats lack a high-affinity binding protein (i.e., TBG) and consequently have lower serum levels of T_4 due to increased availability of the hormone for metabolism and elimination. The clearance of T_4 in rats is enhanced by the induction hepatic microsomal enzymes, particularly UDP-glucuronyl transferase, which catalyzes the conjugation of free T_4 and enhances its excretion in the bile. Effects of PBDEs on thyroid status via induction of hepatic enzymes are therefore unlikely to occur in humans. However, because PBDE-induced decreases in T_4 appear to involve multiple mechanisms in addition to increased thyroid hormone metabolism (Zhou et al. 2001), serum T_4 level is a potential biomarker of effects for lower brominated PBDEs in humans. Additional studies would be useful to characterize the applicability of the biomarker to humans and develop possible correlations between levels and duration of exposure and changes in serum T_4. This biomarker is not specific to PBDEs because other antithyroid agents can have similar effects. Additionally, screening of newborn children for hypothyroidism is a widely accepted and legislatively mandated practice (LaFranchi 1999; Landenson et al. 2000).

5.9 INTERACTIONS WITH OTHER CHEMICALS

Polybrominated Biphenyls. PBBs are potent inducers of liver and kidney P-450 enzymes (MFO) (Haake et al. 1985; Halvorson et al. 1985; Shepherd et al. 1984), and as such, they could potentially enhance or decrease the toxicity of any substance that is metabolized by the P-450 system. PBBs are thought to potentiate the hepatotoxicity and nephrotoxicity of halogenated hydrocarbons and other substances by inducing P-450s that biotransform them to more toxic metabolites (Ahmadizadeh et al. 1984; Kluwe and Hook 1978; Kluwe et al. 1978, 1979, 1982; Kuo and Hook 1982; Roes et al. 1977). In these studies, rats and/or mice were given diets containing FireMaster BP-6 that provided doses of 0.13–13 mg/kg/day for periods of 10–28 days prior to intraperitoneal challenge with the halogenated hydrocarbons. Nephrotoxicity was assessed by measuring kidney weights and the levels of blood urea nitrogen, and by the accumulation of *p*-aminohippurate and/or tetraethylammonium (TEA) in renal cortical slices. Hepatotoxicity was assessed by relative liver weights and by levels of SGPT and/or SGOT. In most cases, exposure to PBBs alone did not affect the parameters of nephrotoxicity in animals. However, exposure to PBBs alone usually caused increased relative liver weights and elevated levels of SGOT and SGPT. Pre-exposure to PBBs increased the hepatotoxicity and nephrotoxicity of chloroform (Ahmadizadeh et al. 1984; Kluwe and Hook 1978; Kluwe et al. 1978) and carbon tetrachloride (Kluwe et al. 1979, 1982) and the nephrotoxicity of trichloroethene and 1,1,2-trichloroethane (Kluwe et al. 1978, 1979). PBB pretreatment in dietary studies also potentiated the nephrotoxicity of the antibiotic, cephaloridine, in rats (Kuo and Hook 1982).

Pretreatment with PBBs also potentiated the lethality of chloroform, carbon tetrachloride, and 1,1,2-trichloroethane by decreasing the LD_{50} values (Kluwe et al. 1978, 1979) and the lethality of bromobenzene by decreasing the time to death (Roes et al. 1977) in mice after challenge with the halogenated hydrocarbon. In contrast, pretreatment of mice with PBBs in dietary studies increased the LD_{50} value of 1,2-dibromo-3-chloropropane (DBCP) but had no effect on the LD_{50} value of 1,2-dibromoethane (EDB) (Kluwe et al. 1981). Also, PBBs were found to reverse the depletion of nonprotein sulfhydryls (e.g., glutathione) caused by DBCP and EDB in the livers and kidneys of mice, suggesting that PBB exposure protected the mice from the lethality of DBCP by making glutathione more available for conjugation with the toxic metabolites.

No potentiation of toxicity was found when rats were co-exposed to diets containing PBB and mirex, photomirex, or kepone, compared with toxicity elicited by each of these substances alone (Chu et al. 1980).

Polybrominated Diphenyl Ethers. No specific information was located regarding interactions between PBDEs and other chemicals. PBDEs are a group of 209 congeners that display biological activity involving different potential mechanisms, and share some toxicological properties with structurally similar polyhalogenated aromatic compounds, particularly PBBs, PCBs, polychlorinated- and poly-brominated dibenzo-*p*-dioxins (PCDDs and PCBDs), and chlorinated and brominated dibenzofurans (PCDFs and PBDFs). However, although these chemicals are structurally similar in two dimensions, PBDEs differ from the other classes on a three-dimensional basis. In particular, as discussed Section 5.5.2 Mechanisms of Toxicity, the influences of the ether bridge in the PBDE molecule preclude clearly classifying the congeners as either dioxin-like (coplanar) or non-dioxin-like (non-coplanar). This has implications for both dioxin-type toxicities (mediated by the AhR pathway) and non-dioxin-type effects. For example, structure-activity studies of PBDEs have shown that binding affinities and induction of AhR-mediated responses are very weak or negligible (Chen et al. 2000; Meerts et al. 1998), suggesting that there is a low potential for effects and interactions involving Ah-receptor-mediated mechanisms (e.g., induction of hepatic CYP1A oxygenases and Phase II enzymes, body wasting, thymic atrophy, and porphyria). Because of the diversity of PBDE activities involving Ah-receptor-independent mechanisms (e.g., induction of CYP2B and CYP3A oxygenases, induction of changes in brain dopamine levels, and disruption of calcium homeostasis), or possibly both Ah-receptor-dependent and -independent mechanisms (e.g., liver hypertrophy, disruption of steroid hormone homeostasis, thyroid hormone homeostasis, disruption of immune functions), there is a large potential for PBDEs to alter the toxicity of other chemicals, or other chemicals to alter the toxicity of PBDEs. For example, lower brominated PBDE mixtures and congeners are inducers of hepatic microsomal enzymes (Carlson 1980a, 1980b; Fowles et al. 1994; Hallgren et al. 2001; Zhou et al. 2001, 2002) and therefore could potentially enhance or decrease the toxicity of any substance that is metabolized by the P-450 system.

5.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to PBBs and PBDEs than will most persons exposed to the same level of PBBs and 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 PBBs and PBDEs, or compromised function of organs affected by PBBs and PBDEs. Populations who are at greater risk due to their unusually high exposure to PBBs and PBDEs are discussed in Section 8.7, Populations with Potentially High Exposures.

Information was located on a small part of the U.S. population that might be unusually susceptible to PBBs and PBDEs. As indicated in Section 5.4.4.2, breast milk constitutes the most important route of excretion of PBBs in lactating females, and this is likely to be the case for lower brominated PBDEs as well. DecaBDE has not been reported in breast milk and is unlikely to accumulate in the milk due to poor absorption and rapid elimination in the feces (Hardy 2002b). Therefore, women with high body burdens of PBBs and/or lower brominated PBDEs who breast-feed may be placing their infants at a higher risk of potential health effects. However, in most cases, the benefits of breast-feeding are expected to outweigh any risk to infants from exposure to these chemicals in the maternal milk.

Experiments in animals and model simulations in humans have shown that reduction in body fat markedly decreases the elimination half-life of PBBs (Domino et al. 1982; Tuey and Matthews 1980). For example, when a dose of 0.1 mg/day for 10 months was simulated in humans, the excretion rate in a lean individual was estimated at 10.2 µg/day; overweight individuals had an excretion rate of 4.1 µg/day. The cumulative excretion was 51% of the dose in lean subjects compared to 20.7% in overweight subjects. These data indicate that overweight individuals may be at higher risk because they store PBBs for a longer time than lean subjects. On the other hand, because lean individuals have a smaller fat compartment, their body tissues will contain higher concentrations of PBB than those in subjects with more fat who received the same exposure (Tuey and Matthews 1980); thus, leaner individuals may be more vulnerable to short-term effects than fatter individuals. Because of this phenomenon, a sudden reduction in body fat, such as that which could occur during dieting, may cause a redistribution of PBBs to potential target organs, which would also increase the potential for adverse health effects to such individuals.

Pregnant women and developing infants and fetuses should be viewed as possibly sensitive populations for exposure to PBBs and lower brominated PBDEs as they are for other thyroid hormone disrupting chemicals (Glinoer 1990; McDonald 2002; Morreale de Escobar et al. 2002). 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). The potential of lower brominated PBDEs to disrupt maternal, fetal, and newborn thyroid hormone levels is demonstrated by the Zhou et al. (2001, 2002)

studies of pentaBDE (technical mixture) in rats, as discussed in Section 5.2.2.2 (Endocrine Effects subsection). The potential for induction of thyroid and neurodevelopmental effects by decaBDE is low in comparison to commercial penta- and octaBDE mixtures due to toxicokinetic and toxicity differences, as discussed in .Section 5.7 (Children's Susceptibility).

People with exposure to anti-thyroid drugs (e.g., lithium), thyroid disease, or otherwise compromised thyroid function might have a more pronounced response to PBBs and 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 B), could be considered more susceptible to health effects of PBBs and PBDEs. However, the potential for induction of thyroid effects by PBDEs in humans appears to be low, based on mechanistic data as discussed Section 5.5.3 (Animal-to-Human Extrapolations).

5.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to PBBs and 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 PBBs and PBDEs. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. Specific treatment methods have not been developed for exposures to PBBs or PBDEs.

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 PBBs and PBDEs. There is no indication of hazards associated with the treatments. The methods are particularly appropriate for trying under conditions of acute exposure, but PBBs and 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.

5.11.1 Reducing Peak Absorption Following Exposure

Ingested PBBs and PBDEs are absorbed by the gastrointestinal tract of humans and animals (see Section 5.4). Although there are no specific recommendations for clinical treatment of acute intoxication from ingested PBBs and 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 1992). 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 et al. 1991), but it is unclear if rice bran would be of benefit in poisoned humans.

The detection of PBBs and PBDEs in the serum and fat of people who were occupationally exposed to these chemicals indicates that PBBs and 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 PBBs or 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 1992). 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.

5.11.2 Reducing Body Burden

PBBs and lower brominated PBDEs tend to accumulate in lipid-rich tissues and are slowly metabolized and eliminated from the body (see Section 5.4). DecaBDE differs from the lower brominated PBDEs in that it is unlikely to preferentially accumulate in body tissues due to poor oral absorption and metabolism and rapid elimination in the feces (Hardy 2002b). Several methods to enhance the elimination of PBBs from the body have been examined in animals and are applicable to PBDEs, although the relevance of the methods is questionable because it is unclear whether current tissue levels are a health concern for the

general population. 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 5.8.1, a rapid breakdown of fat, as might occur in dieting, might lead to a transient increase in PBB and 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). 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 PBBs or PBDEs from adipose tissue is not recommended in individuals with hepatic or renal disease (Lemesh 1992).

5.11.3 Interfering with the Mechanism of Action for Toxic Effects

There are no known methods for interfering with the mechanism of action of PBBs or PBDEs. Although the mechanism of action of PBBs and PBDEs is not completely understood, experimental evidence accumulated in recent years 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 ultimately to enhancement of the CYP1A1 gene expression (see Section 5.5). It appears, therefore,

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 an AhR activator (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) (Bannister et al. 1989). Therefore, the potential for interfering with the mechanism of AhR-mediated effects of PBBs after exposure has occurred is largely untested. For PBDEs, the ether bridge precludes congeners from readily adopting the coplanar (dioxin-like) conformation characteristic of AhR ligands (see Section 5.5.2 Mechanisms of Toxicity). Because PBDE congeners are not clearly classifiable as either dioxin-like (coplanar) or non-dioxin-like (non-coplanar), AhR antagonists might not effectively antagonize effects of PBDEs.

PBBs and PBDEs may also cause toxicity by other mechanisms of action. For example, some PBB congeners can be metabolized to reactive arene oxides (Kohli and Safe 1976; Kohli et al. 1978) that may alkylate critical cellular macromolecules and result in injury. PBDE-induced decreases in thyroid T_4 hormone, which can affect neurobehavioral development, are likely to involve multiple mechanisms (see Section 5.5.3). 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 5.5.2). 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 (Kodavanti 2003; Kodavanti and Derr-Yellin 2001, 2002; Smolnikar et al. 2001; Wiegand et al. 2001), effects on dopamine uptake into synaptic vesicles and synaptosomes (Mariussen and Fonnum 2002, 2003; Mariussen et al. 2003a) and cholinergic functions (Viberg et al. 2001b, 2002a, 2002b), and/or free radical-induced neuronal death (Reistad et al. 2002). Clinical interventions designed to interfere with the aforementioned mechanisms have yet to be developed.

5.12 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 PBBs and 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 PBBs and 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.

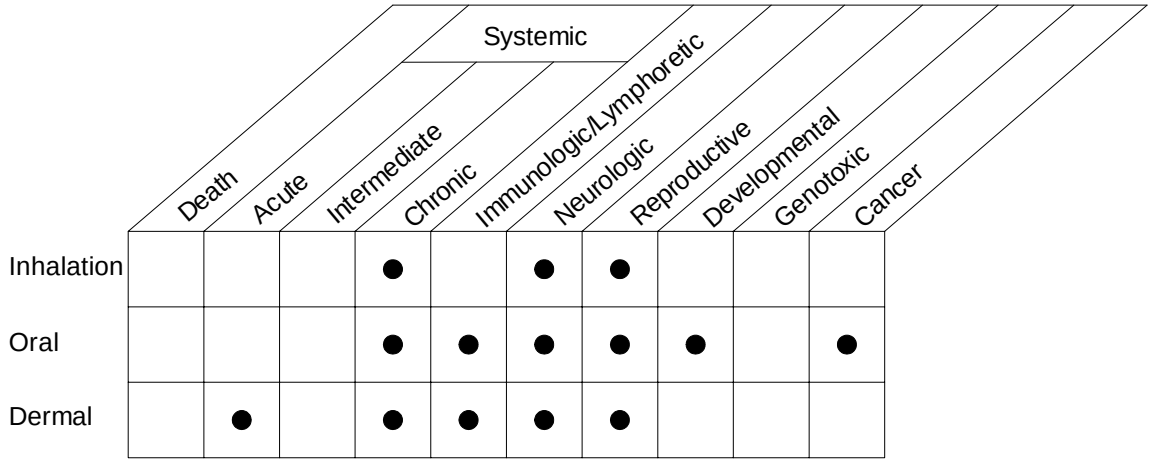
5.12.1 Existing Information on Health Effects of PBBs and PBDEs

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to PBBs and PBDEs are summarized in Figures 5-6 and 5-7. The purpose of this figure is to illustrate the existing information concerning the health effects of PBBs and 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 (Agency for Toxic Substances and Disease Registry 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.

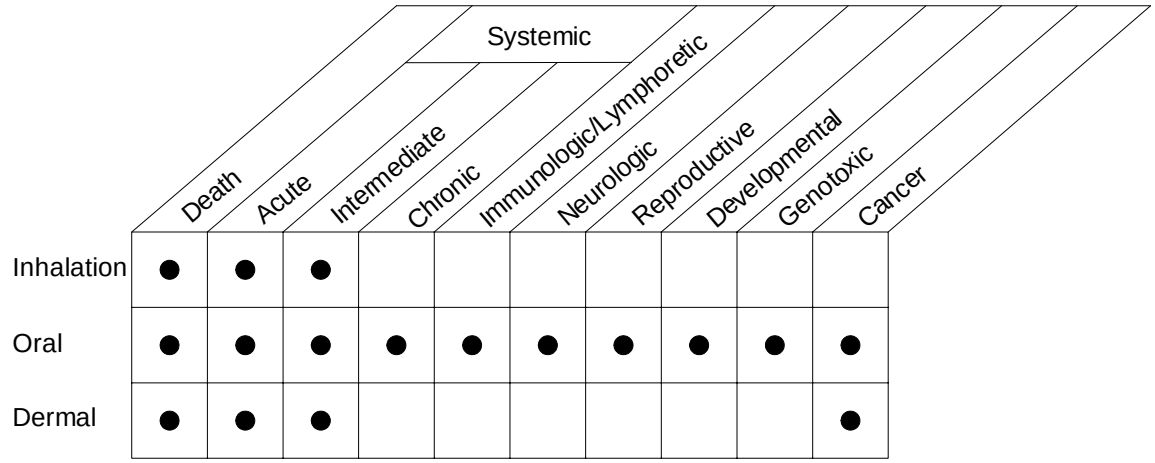
As indicated in Figure 5-6, information is available regarding systemic, immunological, neurological, developmental, reproductive, and carcinogenic effects of PBBs in humans. The information on effects in humans is derived from the Michigan contamination episode that involved chronic-duration oral exposure to contaminated food and from occupational exposure data in which it was assumed that exposure was predominantly through skin contact, although inhalation exposure cannot be ruled out.

Information on health effects in animals is extensive and available for all effect categories, but is nearly completely limited to oral exposure studies, which appears to reflect experimental practicality and concern for what is thought to be the most prevalent and likely route of environmental exposure.

Figure 5-6. Existing Information on Health Effects of PBBs



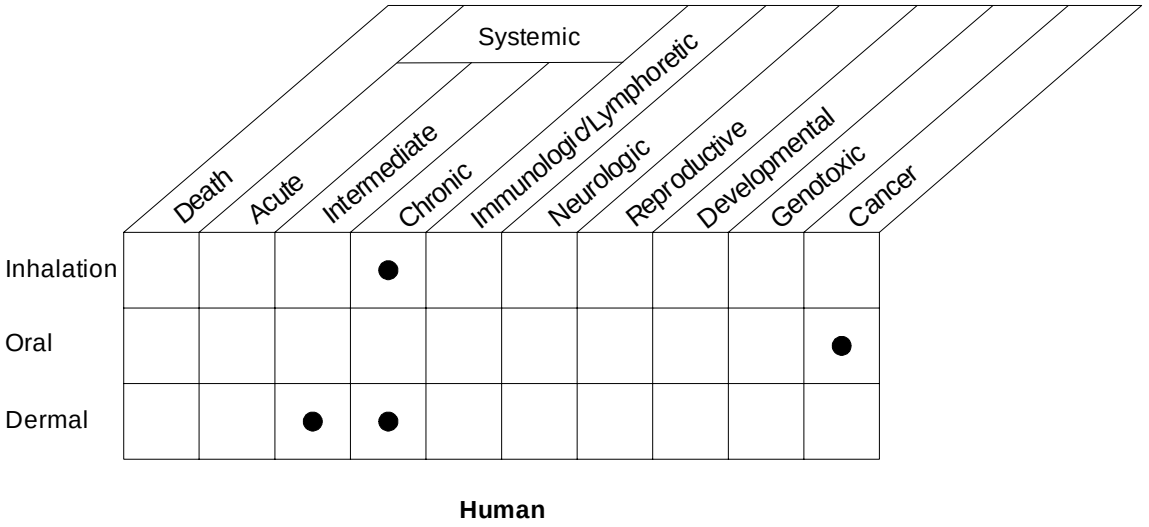
Human



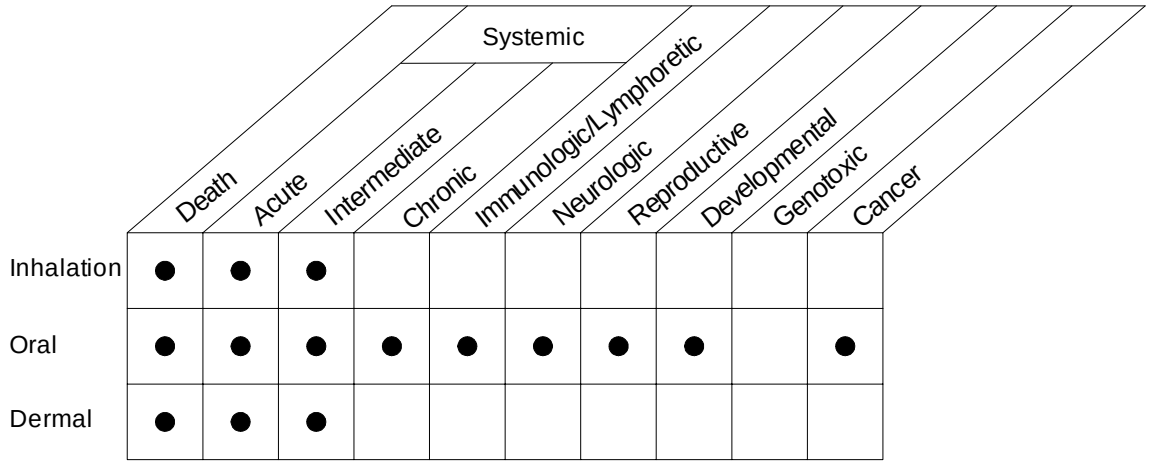
Animal

● Existing Studies

Figure 5-7. Existing Information on Health Effects of PBDEs



Human



Animal

● Existing Studies

A limited amount of information is available on the systemic and carcinogenic effects of PBDEs in humans (Figure 5-7). Information on health effects of PBDEs in animals is available for all effect categories but, like PBBs, is mainly limited to oral exposure studies in animals. In general, the health effects of PBDEs are less adequately studied than for PBBs (and PCBs), and a prominent limitation of the PBDE database is a lack of adequate human studies.

5.12.2 Identification of Data Needs

Acute-Duration Exposure.

Polybrominated Biphenyls. The hepatotoxicity of PBBs in rats and mice is reasonably well characterized for acute-duration oral exposure (Bernert et al. 1983; Corbett et al. 1975; Gupta and Moore 1979; Gupta et al. 1981; Kimbrough et al. 1978b, 1980, 1981; Lee et al. 1975a, 1975b; Norris et al. 1975a; Raber and Carter 1986; Waritz et al. 1977). Effects on body weight in rats and mice and on the thyroid in rats are also well documented (Allen-Rowlands et al. 1981; Corbett et al. 1978; Fraker 1980; Gupta and Moore 1979; Kimbrough et al. 1981), and thyroid effects occurred at doses as low as those causing liver effects. Insufficient acute data exist to definitely establish if the thyroid effects are more critical than effects in the liver, but extensive data on thyroid effects from longer term studies and the functional nature of the changes suggest that this is the case and justify using a thyroid effect as the basis for an acute oral MRL. Acute oral studies in other species would clearly establish the most sensitive target and species for acute exposure. Tests with monkeys, guinea pigs, and mink would be informative because intermediate- and chronic-duration studies indicate that these species are more sensitive than the rat and that endocrinological effects are particularly sensitive end points.

Information on toxic effects of acute-duration exposure to PBBs by routes other than oral are limited to data on hepatic, renal, dermal, and ocular effects of inhalation and dermal exposure in rats or rabbits (Millischer et al. 1980; Needham et al. 1982; Norris et al. 1975a; Waritz et al. 1977), but these data may not be reliable due to study limitations and possible delayed lethality. Limitations in the animal database include inadequate reporting (e.g., numbers of animals not reported), limited number of exposure levels, and lack of studies of PBB mixtures likely to be most toxic (i.e., Firemaster PBBs). Quantitative data for inhalation and dermal absorption of PBBs are lacking. Studies of inhalation and dermal absorption following exposure to soil containing PBBs (i.e., bioavailability studies) would be useful for assessing risk at a hazardous waste site. Further studies identifying target organs and examining the dose-response

relationship following acute inhalation and dermal exposure to PBBs would also be informative, although exposure via soil and acute toxicosis is not likely to ever be a concern.

Polybrominated Diphenyl Ethers. Acute-duration studies have documented effects of PBDEs mainly on the liver and thyroid of orally exposed rats and mice (Argus Research Laboratories 1985a; Carlson 1980a, 1980b; Darnerud and Sinjari 1996; Fowles et al. 1994; Hallgren et al. 2001; IRCD 1974, 1975a, 1975b; Life Science Research Israel Ltd. 1987; NTP 1986; WIL Research Laboratories 1986; Zhou et al. 2001, 2002). The available data on lower brominated PBDEs indicate that the thyroid is a particularly sensitive target of acute oral exposure and justify using thyroid effects as the basis for an acute oral MRL, but acute effects of lower brominated PBDEs on the liver are not as well characterized as thyroid effects. Other studies indicate that immunosuppression and neurobehavior are important and potentially critical health end points for acute exposure to lower brominated PBDEs that need to be further investigated (see discussions of data needs for Immunotoxicity and Neurotoxicity). Studies in other species 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 lower brominated PBDEs..

Data on decaBDE are insufficient for derivation of an acute-duration oral MRL. Information is available on effects of acute oral exposure to decaBDE on body and liver weights, microsomal enzyme induction in the liver, and serum thyroid levels in weanling rats (Carlson 1980b; NTP 1986; Zhou et al. 2001), but the database is limited by lack of LOAELs and/or sufficiently sensitive end points for estimation of an MRL.

Acute-duration inhalation exposure toxicity studies of decaBDE were not located. The inhalation database for acute-duration exposure to lower brominated PBDEs is essentially limited to two 14-day unpublished industry-sponsored studies of octaBDE in rats (Great Lakes Chemical Corporation 1978, 2000). Liver and nasal effect levels were identified in these studies, but MRL estimation is precluded by inconsistencies between the studies and a lack of information on thyroid hormone levels. Additional dose-response studies are needed to provide an adequate basis for derivation of an acute inhalation MRL for lower brominated PBDEs.

Intermediate-Duration Exposure.

Polybrominated Biphenyls. The preponderance of toxicity data for PBBs are available from animals exposed to FireMaster FF-1 or FireMaster BP-6 in the diet or by gavage in intermediate-duration studies (Akoso et al. 1982a, 1982b; Allen et al. 1978; Allen-Rowlands et al. 1981; Aulerich and Ringer 1979;

Byrne et al. 1987, 1988; Castracane et al. 1982; Darjono et al. 1983; Gupta and Moore 1979; Gupta et al. 1981; Kasza et al. 1978a, 1978b; Ku et al. 1978; Lambrecht et al. 1978; Lee et al. 1975a, 1975b; Loose et al. 1981; McCormack et al. 1978; Norris et al. 1975a; NTP 1983; Ringer et al. 1981; Sepkovic and Byrne 1984; Sleight and Sanger 1976; Sleight et al. 1978; Waritz et al. 1977; Werner and Sleight 1981). Studies have been performed with various species (rats have been tested most extensively), and there is evidence indicating that monkeys, guinea pigs, and mink may be the most sensitive. The liver, skin, stomach, and thyroid are unequivocal targets, but existing studies do not identify NOAELs for toxic effects in these organs in rats and/or more sensitive species. Hematologic changes indicative of anemia are consistently reported effects in various species, but the relative importance of these effects is not known. Evidence suggests that the LOAELs for thyroid effects in rats and hepatic effects in guinea pigs are similar (Akoso et al. 1982b; Sleight and Sanger 1976), but reproductive and developmental effects occurred in monkeys at a lower dosage. The serious nature of the developmental toxicity (fetal death) precludes derivation of an intermediate-duration oral MRL. Additional intermediate-duration dose-response studies determining NOAELs for the most sensitive end points, as well as the most sensitive species, would be useful for possible MRL derivation. Studies addressing interspecies differences could help to better characterize the relative sensitivity of monkeys and humans, particularly the possibility that monkeys are more sensitive than humans, as indicated by the high reproductive/developmental toxicity of PBBs in this species that has not been noted in PBB-exposed workers or the Michigan cohort. These studies could also help elucidate the toxicological significance of effects in the thyroid and other endocrine organs, particularly since the reproductive effects may be related to endocrine imbalance.

Limited information is available on effects of PBBs in animals by inhalation or dermal exposure for intermediate durations (Millischer et al. 1980; Norris et al. 1975a; Waritz et al. 1977). Some inhalation data are available for octabromobiphenyl and decabromobiphenyl mixtures and some dermal data are available for octabromobiphenyl mixture, but intermediate-duration inhalation and dermal studies of FireMaster PBBs have not been performed. Studies of FireMaster FF-1 or FireMaster BP-6 would be particularly useful because these are likely to be the most toxic PBBs based on oral data and due to their higher content of potentially toxic congeners. Although the octabromobiphenyl mixture inhalation data are limited by numbers of animals, dose levels, and end points, and only one species (rat) was tested in the octabromobiphenyl mixture and decabromobiphenyl inhalation studies, it appears that these PBB mixtures are not highly toxic. Due to the inadequacies of the octabromobiphenyl mixture data and lack of any information on inhalation toxicity of the likely more potent FireMaster mixtures, there is insufficient basis for deriving an intermediate inhalation MRL. Although intermediate-duration inhalation studies of FireMaster PBBs would be particularly relevant to MRL derivation, they may not be practical due to the

low volatilization potential of PBBs. The intermediate-duration dermal studies of octabromobiphenyl mixtures revealed some skin irritation in rabbits but no sensitization in guinea pigs. Additional studies could corroborate the potential for dermal irritation by PBBs and are relevant because the skin is a route of concern for exposure at or near hazardous waste sites. Intermediate-duration inhalation and dermal exposure studies of PBB-contaminated soil (e.g., bioavailability studies) that identify thresholds would be especially useful for risk assessment at a hazardous waste site.

Polybrominated Diphenyl Ethers. Available intermediate-duration oral studies in animals indicate that the liver and thyroid are the main systemic targets of repeated exposures to lower brominated PBDEs as shown by effects that mainly include enlargement and histological alterations in both organs and changes in serum levels of thyroid hormones, particularly decreases in serum T_4 (Carlson 1980a; IRDC 1976, 1977; WIL Research Laboratories 1984; Zhou et al. 2001, 2002). Hepatic effects occurred at the lowest LOAEL and were used as the basis for an intermediate oral MRL, but thyroid effects occurred at doses as nearly as low as those causing liver effects and the data are insufficient to clearly characterize liver effects as more critical than effects in the thyroid. Studies designed to identify NOAELs for octa-, and pentaBDE could provide a better basis for an intermediate duration MRL, as well as help to ascertain the most appropriate PBDE mixture on which to base a chronic MRL for lower brominated PBDEs. 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. As indicated in the Comparative Toxicokinetics section below, specific evidence is needed to determine whether PBDEs are likely to affect thyroid function in humans, or if humans are less sensitive to these effects than rats. 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.

A commercial decaBDPE product (97.34% DBDPO, 2.66% nonaBDE and octaBDE) was administered to groups of 25 mated female Sprague-Dawley rats by gavage in corn oil in daily doses of 0, 100, 300, or 1,000 mg/kg/day on gestation days 0 through 19 in a GLP-compliant study (Hardy et al. 2002). Each female was sacrificed on gestation day 20 and necropsied. End points included maternal clinical observations, maternal body weight/weight gain and food consumption, maternal gravid uterine and liver weights, maternal gross lesions, total number of corpora lutea, uterine implantations, early and late resorptions, viable and nonviable fetuses, and fetal weight and sex. Fetuses were examined grossly (all fetuses), evaluated for skeletal/cartilaginous malformations and ossification variations (approximately

half of each litter), and evaluated for visceral malformations (remaining fetuses). No treatment-related effects on any maternal or fetal endpoints were observed, indicating that 1,000 mg/kg/day was the NOAEL for maternal and developmental toxicity.

One intermediate-duration oral toxicity study has been conducted for high purity decaBDE. In this study, multiple dose levels of a commercial decaBDE product (94–97% pure) was fed to rats and mice for 13 weeks (NTP 1986). Comprehensive histological examinations were performed, but were limited to the control and high-dose groups, and no hematology, clinical chemistry, or urine indices, or thyroid hormone levels were evaluated. There were no compound-related clinical signs, deaths, body weight or food consumption changes, gross pathology, or histopathology, indicating that the highest tested doses in rats and mice (8,000 and 9,500 mg/kg/day, respectively) were intermediate-duration NOAELs for systemic toxicity. A developmental toxicity study in rats identified a NOAEL of 1,000 mg/kg/day (Hardy et al. 2002). Because doses of decaBDE higher than 1,000 mg/kg/day have not been tested for developmental toxicity, and the NTP (1986) study indicates that this dose is also a NOAEL for systemic toxicity, the 1,000 mg/kg/day developmental toxicity NOAEL was used to derive the MRL. Additional systemic and developmental toxicity studies could provide an indication of NOAEL/LOAEL thresholds and possibly provide a better basis for an 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 2001). 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.

Chronic-Duration Exposure and Cancer.

Polybrominated Biphenyls. Information on chronic systemic toxicity of PBBs in animals is limited to an oral bioassay showing hepatic, gastric, hematologic, and/or thyroid effects in rats and mice (NTP 1992), and a study showing effects on skin, stomach, and body weight in two monkeys (Allen et al. 1979; Lambrecht et al. 1978). Although limited by the number of studies and species, the available chronic animal data corroborate the results of intermediate-duration studies with respect to the observed effects. Additional studies would be necessary to determine the most sensitive animal target organ and species for chronic exposure and to provide a basis for an MRL, as serious hepatic changes as well as weight loss, decreased survival, and developmental effects occurred at the lowest tested dosages. Because PBBs are no longer being produced, exposure is most likely to occur at a contaminated waste site. Therefore, chronic studies examining the effects of PBB-contaminated soil following oral, inhalation, and dermal

exposure (i.e., bioavailability studies) would be particularly useful. Evaluations of the thyroid would be particularly informative because intermediate-duration animal studies indicate that the thyroid may be a particularly sensitive target organ.

There is sufficient evidence that commercial hexabromobiphenyl mixtures (FireMaster FF-1) are hepatocarcinogenic in rats and mice following acute, intermediate, and/or chronic exposure (Groce and Kimbrough 1984; Kimbrough et al. 1978b; NTP 1983, 1992). Additional animal studies could provide useful information on interspecies differences and carcinogenesis of other PBB mixtures.

Polybrominated Diphenyl Ethers. 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 is needed to 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.

Information on the chronic oral toxicity data of lower brominated PBDEs is available from a study in which 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. 1975b). 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 pentaBDE and/or octaBDE are needed to 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.

Polybrominated Biphenyls. No information is available regarding potential genotoxic effects of PBBs in exposed humans. PBB mixtures or congeners were not genotoxic in any of the prokaryotic or eukaryotic animal systems tested. These include *in vitro* assays with *S. typhimurium* and *E. coli* bacteria (Haworth et al. 1983; Millischer et al. 1980; NTP 1983; Rossman et al. 1991), a host-mediated assay with *S. typhimurium* (Millischer et al. 1980), and *in vitro* assays with hamster cells (Galloway et al. 1987; Kavanagh et al. 1985; Williams et al. 1984), rat liver cells (Kavanagh et al. 1985; Williams et al. 1984), mouse liver and lymphoma cells (Myhr and Caspary 1991; Williams et al. 1984), and human fibroblasts (Williams et al. 1984). PBBs also were inactive in *in vivo* unscheduled DNA synthesis assays with rat and mouse hepatocytes (Mirsalis et al. 1985, 1989) and in a micronucleus test with mice (Millischer et al. 1980). However, only some of these studies tested commercial PBB mixtures (Kavanagh et al. 1985; Millischer et al. 1980; Myhr and Caspary 1991; NTP 1983; Rossman et al. 1991; Williams et al. 1984). Additional studies of commercial mixtures could more fully characterize the genotoxic potential of PBBs, and provide information regarding differences in potencies of different mixtures and the sensitivities of different organisms. Cytogenic analysis of human populations exposed to PBBs in occupational settings, or exposed by consumption of food contaminated with PBBs, might make it possible to more adequately assess the genotoxic potential of these compounds in humans.

Polybrominated Diphenyl Ethers. A limited amount of information has been published on the genotoxicity of PBDEs. Cytogenetic examination of bone marrow cells showed no increase in aberrations in maternal and neonatal rats following maternal oral exposure to a 77.4% decaBDE mixture (containing 21.8% nonaBDE and 0.8% octaBDE) for 90 days prior to mating and during mating, gestation, and lactation (Norris et al. 1973, 1975a). *In vitro* assays found that decaBDE did not induce gene mutations in bacteria (*S. typhimurium*) or mammalian cells (mouse lymphoma L5178Y cells), and did not induce sister chromatid exchange or chromosomal aberrations in Chinese hamster ovary cells (NTP 1986). *In vitro* exposure to the single congeners 2,2',4,4'-tetraBDE (BDE 47), 3,4-diBDE (BDE 12), and 2-monoBDE (BDE 1) caused increased recombinogenic activity in Chinese hamster SPD8 and Sp5V79 cells (Helleday et al. 1999). Although the weight of available evidence indicates that decaBDE is not genotoxic, studies using lower brominated mixtures and a wider variety of assay types would help to better characterize the genotoxic potential of PBDEs.

Reproductive Toxicity.

Polybrominated Biphenyls. A limited amount of information is available regarding reproductive effects in humans after exposure to PBBs. No evidence for PBBs-related effects on sperm counts, motility, or sperm morphology was found in a group of male Michigan workers exposed to PBBs by inhalation or dermal contact (Rosenman et al. 1979). No relationship was found between serum levels of PBBs and the frequency and duration of lactation in women exposed during the Michigan contamination episode (Thomas et al. 2001).

Although no alterations in fertility or litter size were observed in mink fed PBB-containing diets prior to breeding and during pregnancy (Aulerich and Ringer 1979; Ringer et al. 1981) or in the F_1 or F_2 generations of female F_0 rats fed PBB-containing diets during postimplantation gestation through weaning (McCormack et al. 1981), implantation was completely blocked in 40–67% of female rats exposed by gavage to PBBs between GDs 0 and 14 (Beaudoin 1979). Additionally, a lengthening of the menstrual cycle and prolonged implantation bleeding with decreased serum progesterone were observed in two of seven female monkeys fed a PBB-containing diet prior to and during pregnancy (Allen et al. 1979; Lambrecht et al. 1978). The dosage causing these reproductive effects in monkeys was the lowest tested in any intermediate-duration study of PBBs. In addition, alterations of male reproductive organs in rats (Gupta and Moore 1979) and in a monkey (Allen et al. 1978) have been observed after intermediate-duration exposure to lethal oral doses of PBBs. Histopathological alterations were not observed in male or female reproductive organs after intermediate- or chronic-duration, oral exposure of rats or mice to nonlethal doses of PBBs (NTP 1983, 1992). The animal data suggest that PBBs may cause adverse effects on reproductive organs and their function(s) and that reproductive organ functions during the early phases of pregnancy may be particularly sensitive to PBBs. Additional studies in animals exposed by oral and other routes, including multi-generation studies with pre-breeding exposure to assess effects on fertility in both males and females, might help to further identify the reproductive processes affected by PBBs and to determine the dose-response relationships. Studies elucidating the NOAEL region and relative susceptibility of sensitive species (e.g., monkeys) to reproductive and developmental effects would be particularly useful, as these data could enable derivation of an intermediate oral MRL.

Polybrominated Diphenyl Ethers. Information on the reproductive toxicity of PBDEs is limited to a single one-generation oral study of decaBDE in rats that found no exposure-related functional effects (Dow Chemical Co. 1975; Norris et al. 1975a, 1975b). Evaluation for histologic effects on reproductive organs in the available studies of PBDEs has generally reported no detectable effects. Tests of octaBDE and/or pentaBDE, particularly second-generation studies designed to assess effects on fertility in both

sexes, would better characterize the reproductive toxic potential of PBDEs and assure the adequacy of the intermediate oral MRL.

Developmental Toxicity.

Polybrominated Biphenyls. No studies were located regarding developmental effects in humans or animals after inhalation or dermal exposure to PBBs. Studies of fetal mortality rates in Michigan (Humble and Speizer 1984) and of physical and neuropsychological development in Michigan children exposed *in utero* or in early infancy during the peak of the 1973 contamination episode (Schwartz and Rae 1983; Seagull 1983; Weil et al. 1981) did not conclusively correlate the ingestion of PBBs with developmental effects. Oral acute-, intermediate-, and chronic-duration studies of FireMaster FF-1 or FireMaster BP-6 in several species have reported fetotoxic and developmental effects, including embryolethality or increased mortality among nursing young (Allen et al. 1979; Beaudoin 1977, 1979; Groce and Kimbrough 1984; Lambrecht et al. 1978; Luster et al. 1980), fetal malformations (Beaudoin 1977; Corbett et al. 1975; Waritz et al. 1977), growth retardation in offspring (Allen et al. 1979; Aulerich and Ringer 1979; Corbett et al. 1975; Groce and Kimbrough 1984; Lambrecht et al. 1978; McCormack et al. 1981; Meserve et al. 1992; Ringer et al. 1981), liver effects in offspring (Moore et al. 1978; Werner and Sleight 1981), and performance deficits in tests of operant behavior in offspring (Henck and Rech 1986; Tilson 1992). A limited amount of data is available for octabromobiphenyl and decabromobiphenyl mixtures, which indicates that these PBBs are less developmentally toxic than FireMaster FF-1 or FireMaster BP-6 (Millischer et al. 1980; Waritz et al. 1977). Because FireMaster FF-1 caused developmental effects in monkeys at the lowest dosage tested in any study of PBBs, a chronic oral MRL could not be calculated; studies determining developmental NOAELs in sensitive species, therefore, would be particularly relevant. Additional studies regarding inhalation or dermal exposure to PBBs might help to determine whether or not the developmental toxicity of PBBs is route-specific. Studies on the mechanism(s) of action of PBBs in different animal species may provide a better understanding of the physiological and biochemical basis for the developmental toxicity of PBBs and a better basis for extrapolating from animal data in the evaluation of the hazard presented by PBBs to the development of human fetuses and children.

Polybrominated Diphenyl Ethers. Oral developmental toxicity studies have shown no evidence of teratogenicity of deca-, octa-, and pentaBDE in rats and rabbits, although fetotoxic effects, including skeletal ossification variations at maternally toxic doses, have occurred with lower brominated mixtures (Argus Research Laboratories 1985b; Breslin et al. 1989; Dow Chemical Co. 1975, 1985; Hardy et al. 2002; Life Science Research Israel Ltd. 1987; Norris et al. 1975a, 1975b; WIL Research Laboratories

1986). Effects of gestational exposure to lower brominated PBDEs included minimally increased post-implantation loss in rats and increased skeletal variations in rabbits at octaBDE doses as low as 10 and 15 mg/kg/day, respectively (Argus Research Laboratories 1985b; Breslin et al. 1989; Life Science Research Israel Ltd. 1987). Exposure to decaBDE at level as high as 1,000 mg/kg/day (highest tested dose) caused no fetal or maternal effects in rats (Hardy et al. 2002). The available evidence appears to adequately show that teratogenicity and fetal toxicity is not a critical effect of concern for either lower brominated PBDEs or decaBDE. However, there is increasing evidence that the developing nervous system is a sensitive target of particular PBDE congeners, including decaBDE, as shown by impairments in tests of spontaneous motor behavior and learning and memory in adult mice exposed early in life (Branchi et al. 2001, 2002; Eriksson et al. 1999, 2001a, 2002a; Viberg et al. 2001a, 2001b, 2002a, 2002b, 2003a) (see neurotoxicity data needs section).

Immunotoxicity.

Polybrominated Biphenyls. Information regarding immunological effects of PBBs in humans is equivocal. Some groups of investigators reported altered lymphocyte transformation responses in subjects accidentally exposed to PBBs through contaminated food (Bekesi et al. 1978, 1985; Roboz et al. 1985). Other investigators could not confirm this in the same populations (Landrigan et al. 1979; Silva et al. 1979). Carefully designed follow-up studies of these populations would provide valuable information regarding possible immunological effects of PBBs. Additional research on the binding of PBBs with different plasma fractions could be fruitful, since it appears that on a per cell basis in exposed subjects, there is ≈100-fold excess of PBB in white cell fractions, compared to the erythrocyte fraction (Roboz et al. 1980, 1985). Acute oral data in rats and mice provided information regarding histopathology of the thymus, spleen, and lymph nodes (Fraker 1980; Fraker and Aust 1978; Gupta et al. 1981). Data from oral intermediate-duration studies in experimental animals suggest that the immune system may be one of the most sensitive targets for PBBs (Farber et al. 1978; Fraker 1980; Vos and van Genderen 1973, 1974). PBBs decreased the resistance of mice to infection by reducing antibody production (Loose et al. 1981), decreased the responsiveness of lymphocytes to mitogenic stimulation in rats and mice (Luster 1978, 1980) and pigs (Howard et al. 1980), altered thymus weight in rats (NTP 1983), and caused thymus atrophy in dogs (Farber et al. 1978), guinea pigs (Vos and van Genderen 1973), and cattle (Moorhead et al. 1977). No studies were located regarding the immunological effects of PBBs in animals after inhalation or dermal exposure. Due to the relatively low vapor pressure of PBBs, inhalation is not a predominant route of exposure. Additional oral studies using a battery of immunological tests would be useful to further define the immunological effects of PBBs.

Polybrominated Diphenyl Ethers. Information regarding the immunosuppressive potential of PBDE mixtures is essentially limited to evidence from acute-duration oral studies in animals exposed to relatively high doses of pentaBDE. The 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 exposed to 36 mg/kg/day pentaBDE for 14 days (Darnerud and Thuvander 1998). Other 14-day studies in mice found no changes in natural killer cell activity at ≤72 mg/kg/day (Fowles et al. 1994) or numbers of splenic and thymic lymphocyte subsets at ≤36 mg/kg/day (Darnerud and Thuvander 1998), although 18 mg/kg/day of the single congener 2,2',4,4'-tetraBDE (BDE 47) caused significantly reduced numbers of total lymphocytes and CD4+, CD8+, and CD45R+ subtypes in the spleen (Darnerud and Thuvander 1998). In a 2-year study, NTP (1986) reported that exposure to 2,240 mg/kg/day of decaBDE in the diet resulted in a significant increase in splenic fibrosis; while not a measure of effects on immune function, it does indicate a treatment-related effect on an immune tissue. Additional oral studies using a battery of immunological tests and a lower range of doses would serve to better characterize the immunotoxic potential of PBDEs.

Neurotoxicity.

Polybrominated Biphenyls. One study was located regarding neurological effects in humans after inhalation and/or dermal exposure to PBBs (Brown et al. 1981). No studies were located regarding neurological effects in animals after inhalation or dermal exposure to PBBs. Although neurological symptoms were reported with some frequency by certain residents of Michigan who were likely to have consumed PBB-contaminated food, several studies of Michigan residents (including workers in PBB manufacturing who presumably were exposed predominately by inhalation and dermal contact) found no statistically significant associations between levels of PBBs in serum or fat (from oral or dermal exposure to PBBs) and frequencies of subjectively reported neurological symptoms or performance on neuro-psychological tests (Anderson et al. 1978c, 1979; Barr 1980; Brown and Nixon 1979; Brown et al. 1981; Landrigan et al. 1979; Stross et al. 1981; Valciukas et al. 1978, 1979). Studies of the neuropsychological development of children exposed *in utero* or in early infancy, likewise, were inconclusive in establishing an association with PBB exposure (Schwartz and Rae 1983; Seagull 1983; Weil et al. 1981). Subtle effects in neurobehavioral tests were found in rodents, including decreased motor activity (Geller et al. 1979; Tilson and Cabe 1979) and hind limb weakness (Cabe and Tilson 1978) after intermediate-duration, oral exposure and performance deficits in tests of learning behavior in the offspring of female mice and female rats exposed during gestation and lactation (Henck and Rech 1986; Henck et al. 1994; Tilson 1992). Histopathological alterations of brain or spinal nerve tissue revealed no abnormalities in rats or

mice after intermediate- or chronic-duration oral exposure (NTP 1983, 1992). Periodic neurobehavioral testing of animals exposed to PBBs at multiple doses for chronic durations would be useful for determining if longer-term exposure leads to more severe neurological effects than those observed with intermediate-duration exposures.

Polybrominated Diphenyl Ethers. A limited amount of information is available on neurological effects of commercial PBDE mixtures. No clinical signs of neurotoxicity or neurohistopathology were observed in rats or mice exposed to commercial decaBDE in 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. A commercial pentaBDE mixture was evaluated for several behavioral end points in offspring of rats that were perinatally exposed to 1–100 mg/kg/day by gavage on GD 6 through PND 21 (MacPhail et al. 2003; Taylor et al. 2002, 2003). Evaluation of the offspring as adults showed no alterations in motor or sensory development as assessed by motor activity, habituation, and auditory startle response, although suggestive decreases in fear conditioning were observed.

Neurobehavioral effects of individual PBDE congeners were evaluated in mice that were exposed during perinatal and/or early postnatal periods to 2,2',4,4'-tetraBDE (BDE 47), 2,2',4,4',5-pentaBDE (BDE 99), 2,2',4,4',5,5'-hexaBDE (BDE 153), or 2,2',3,3',4,4',5,5',6,6'-decaBDE (BDE 209) (Branchi et al. 2001, 2002; Eriksson et al. 1999, 2001a, 2002a; Viberg et al. 2001a, 2001b, 2002a, 2002b, 2003a). Most of these studies used similar single oral dose experimental designs and evaluated spontaneous motor behavior and swim maze performance at 2–6 months of age. The findings collectively indicate that the nervous system is a target of particular PBDE congeners during a defined critical phase of neonatal brain development, as shown by mild impairments in spontaneous motor behavior and learning and memory in older mice. One study used a different experimental design in which mice were exposed to BDE 99 from GD 6 to PND 21, and were evaluated using a variety of somatic (body weight gain, hair growth, day of eyelid and ear opening, day of incisor eruption) and neurobehavioral (righting reflex, forelimb stick grasping reflex, forelimb placing reflexes, negative geotaxis, screen grasping and climbing, pole grasping, ultrasonic vocalizations, homing test) end points during PNDs 2–22, as well as spontaneous activity endpoints on PNDs 22–120 (Branchi et al. 2001, 2002). Findings were suggestive of delayed sensorimotor development and altered spontaneous behavior.

Additional oral studies using comprehensive neurobehavioral test batteries are needed to better characterize the potential for PBDEs to cause neurotoxic effects in humans. Based on the limited available information (predominantly study abstracts), studies evaluating effects of exposure to lower-brominated PBDEs and decaBDE commercial mixtures during critical postnatal periods of brain development would be particularly useful.,

Epidemiological and Human Dosimetry Studies.

Polybrominated Biphenyls. Epidemiology studies of people exposed by ingesting PBB-contaminated food as a result of the 1973 Michigan PBB contamination episode or who were exposed occupationally in the manufacture or distribution of PBBs have not provided conclusive evidence that detectable effects have occurred as a result of exposure to PBBs (Anderson et al. 1978c, 1979; Barr 1980; Henderson et al. 1995; Hoque et al. 1998; Humble and Speizer 1984; Landrigan et al. 1979; Thomas et al. 2001; Valciukas et al. 1978, 1979). Clinical examinations, including neuropsychological, liver function, and sperm count testing, of people who may have experienced the highest exposures did not conclusively identify particular effects or clinical signs associated with exposure (Brown and Nixon 1979; Brown et al. 1981; Rosenman et al. 1979; Schwartz and Rae 1983; Seagull 1983; Stross et al. 1981; Weil et al. 1981). No relationship was found between serum levels of PBBs and the frequency and duration of lactation in women exposed during the Michigan contamination episode (Thomas et al. 2001). A relationship between increasing serum levels of PBBs and increasing risk of breast cancer was indicated in case-control studies of women exposed during the Michigan episode (Henderson et al. 1995; Hoque et al. 1998), but the results are only suggestive due to factors such as small number of cases, insufficient information on known breast cancer risk factors, and confounding exposures to other organochlorine chemicals. The evidence for an association between breast cancer and PBBs is inconclusive and warrants further study. Continued monitoring of the Michigan cohort for prevalence of other types of cancer as the cohort ages are also of interest, because lifetime and short-term exposure to PBBs are known to cause cancer in animals, and the residence time of PBBs in the body is expected to be long. If human exposure to PBBs is found to be occurring at a hazardous waste site, the nearby population should be studied for both exposure and effect data.

Polybrominated Diphenyl Ethers. A limited amount of epidemiological information is available for PBDEs. Plasma levels of various organohalogen compounds, including the congener 2,2',4,4-tetraBDE, as well as serum hormone levels (free and total T_3 and T_4, TSH, free testosterone, follicle-stimulating hormone, lutenizing hormone, and prolactin), were analyzed in 110 men who consumed fatty fish from the Baltic Sea (Hagmar et al. 2001). There was a weak negative correlation between 2,2',4,4'-tetraBDE

(BDE 47) and plasma TSH after age adjustment, but BDE 47 could not explain more than 10% of the variance in TSH. No clear association was found between risk of non-Hodgkin's lymphoma and adipose tissue levels of 2,2'4,4'-tetraBDE in a case-control study of 77 Swedish men and women (Hardell et al. 1998; Lindstrom et al. 1998). 2,2'4,4'-TetraBDE was used as a marker for total PBDE exposure in both of these studies. No epidemiological data on PBDEs are available for non-oral exposure routes.

Animal data raise particular concern for effects of PBDEs on the thyroid as well as possible immunological, neurodevelopmental, and carcinogenic effects of exposure. Epidemiological investigations are needed to better characterize the potential for PBDEs, both the lower PBDEs and decaBDE, to induce these and other kinds of effects as well as the relationship between PDBE body burden and the observed effects. 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. Limitations that are likely to constrain the epidemiological investigations, such as unmeasured PBDE exposure concentrations and lack of controls for confounding co-exposures, should be addressed.

Biomarkers of Exposure and Effect.

Exposure.

Polybrominated Biphenyls. PBBs are stored primarily in adipose tissue and are present in serum and human milk of exposed populations. Several studies have shown that serum and adipose PBB levels are biomarkers of exposure (Blanck et al. 2000b; Brilliant et al. 1978; Humphrey and Hayner 1975; Lambert et al. 1990; Landrigan et al. 1979; Rosen et al. 1995; Wolff et al. 1982). It has been proposed that measurement of PBB levels in adipose tissue may be a more reliable prediction of body burden than serum levels because of the high adipose/serum PBB partition ratio (Anderson 1985). However, once a stable correlation between adipose/serum levels has been characterized, serum levels are a better choice for surveillance and monitoring (Anderson 1985). Further studies on the predictive value of levels of PBB (particularly congeners) in serum and adipose tissue in individuals exposed to PBBs for acute, intermediate, and chronic durations would provide valuable information that could lead to early detection of PBB exposure.

A potential biomarker of exposure to PBBs is related to their effect on the thyroid gland. Effects in exposed workers included increased serum thyrotropin, low or borderline low serum T_4, and increased thyroid antimicrosomal antibody titers (Bahn et al. 1980), and effects in exposed rats included reduced levels of serum T_4 and T_3 (Akoso et al. 1982b; Allen-Rowlands et al. 1981; Byrne et al. 1987; Gupta and Moore 1979; Kasza et al. 1978a; Meserve et al. 1992; Norris et al. 1975a, 1975b; NTP 1983; Sepkovic and Byrne 1984; Sleight et al. 1978; Werner and Sleight 1981). Additional studies could better characterize thyroid effects of PBBs in humans and develop specific correlations between levels and duration of exposure and alterations in serum hormone levels.

Polybrominated Diphenyl Ethers. PBDEs also 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 are needed to provide time-trend data and to verify findings that PBDE levels have been exponentially increasing in breast milk during the past 25 years (Norén and Meironyté 1998, 2000). Studies on the predictive value of levels 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 kept carefully in mind when designing studies evaluating PBDE exposure.

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.

Polybrominated Biphenyls. There are no specific biomarkers of effects for PBBs. Numerous studies have attempted to correlate serum and adipose PBB levels with an array of symptoms and health

complaints in PBB exposed subjects from the state of Michigan (Anderson et al. 1978a, 1978b, 1978c, 1979; Bekesi et al. 1978; Humphrey and Hayner 1975; Landrigan et al. 1979; Stross et al. 1979). Thus far, no significant correlation has been found. Continued follow-up studies on the Michigan cohort would provide information on effects that may have a long latency, such as cancer. Elevated levels of two cytochrome P-450I-dependent enzymes were observed among PBB exposed subjects, relative to controls (Lambert et al. 1990). The thyroid is a sensitive target for PBBs and characteristic changes include reduced serum levels of T_4 and other thyroid hormones (Akoso et al. 1982b; Allen-Rowlands et al. 1981; Byrne et al. 1987; Gupta and Moore 1979; Kasza et al. 1978a; Meserve et al. 1992; Norris et al. 1975a, 1975b; NTP 1983; Sepkovic and Byrne 1984; Sleight et al. 1978; Werner and Sleight 1981), indicating that they are potential biomarkers of effect. Levels of CYP enzymes and thyroid hormones, however, are not specific for PBB exposure. Further studies designed to identify specific biomarkers of effects of PBBs would facilitate medical surveillance leading to early detection of potentially adverse health effects and possible treatment. Congener-specific analysis may be useful for characterizing dioxin-like health effects.

Polybrominated Diphenyl Ethers. Biomarkers that could be used to characterize health effects caused specifically by PBDEs have not been identified. The thyroid is a critical target for PBDEs in animals, as discussed in Section 3.2.2.2, Endocrine Effects, and serum T_4 is a potential biomarker of effect for these chemicals in humans. Although this biomarker is not specific to PBDEs because other antithyroid agents can have similar effects, changes in T_4 could be considered to indicate potential impairment of health.

Absorption, Distribution, Metabolism, and Excretion.

Polybrominated Biphenyls. There are no quantitative data regarding absorption in humans via the inhalation route, but data from occupationally exposed individuals and individuals who ingested food contaminated with PBBs suggest that exposure by the oral or dermal route may lead to considerable accumulation of PBBs in tissues (Anderson et al. 1978c; Eyster et al. 1983; Landrigan et al. 1979). The animal data indicate that the main component of a commercial PBB mixture (2,2,'4,4',5,5'-hexa-bromobiphenyl) is efficiently absorbed by the oral route (Matthews et al. 1977; Tuey and Matthews 1980). Data regarding absorption after inhalation exposure was limited to a single study (Waritz et al. 1977). There are no data regarding absorption via the dermal route. No studies were located in which several doses of different PBB congeners were administered by the inhalation, oral, and dermal routes, and for various exposure periods. Such studies could provide information on the relationship between bromination patterns and absorption efficiency and rates of absorption by the different routes of exposure.

In addition, studies with different PBB mixtures could help determine possible interaction effects among congeners that could affect absorption.

Distribution data are limited to qualitative information derived from cases of accidental ingestion of food contaminated with PBBs, cases of occupational exposure via dermal contact (Eyster et al. 1983; Landrigan et al. 1979) and autopsy reports (Miceli et al. 1985). These data suggest that PBBs distribute preferentially to tissues with high fat content regardless of the route of exposure. Data derived from oral administration of PBBs to animals indicate that PBBs are distributed first to liver and muscle and then to adipose tissue where they are stored (Domino et al. 1982; Lee et al. 1975; Matthews et al. 1977). Little information regarding distribution of PBBs could be drawn from the limited number of studies in animals administered PBBs by the inhalation or dermal routes. Additional well-conducted studies by these routes of exposure would provide useful information regarding possible route-dependent distribution patterns. Studies regarding distribution through the placenta after inhalation and dermal exposure were not available.

Data regarding biotransformation of PBBs in humans are limited to individuals who accidentally consumed food contaminated with PBBs or who were exposed to PBBs in the workplace (Wolff and Aubrey 1978; Wolff et al. 1979a). The use of human cell systems in culture might be considered a useful addition to whole animal studies for studying the metabolic fate of PBBs. There are studies regarding the metabolism of some PBB congeners after oral administration to rats (Sparling et al. 1980), rabbits (Kohli et al. 1978), and pigs (Kohli and Safe 1976). However, the PBBs mainly studied were monobromo-biphenyls and dibromobiphenyls, which are only trace components of FireMaster mixtures. Therefore, studies on the *in vivo* metabolism of the main components of commercial PBB mixtures would provide valuable information regarding the metabolic disposition of highly brominated congeners. A limited amount of information is available on the metabolism of PBBs in farm animals (e.g., dairy cows, chickens). This is a data gap because people exposed to PBBs during the Michigan PBB contamination episode were predominately exposed by consuming products of farm animals. Although information regarding metabolism after inhalation or dermal exposure is lacking, there is no evidence to suggest that other pathways would operate after exposure by these routes.

Studies regarding urinary or fecal excretion of PBBs in humans were not located; however, elimination of PBBs through maternal milk is well documented (Brilliant et al. 1978; Eyster et al. 1983; Jacobson et al. 1984; Landrigan et al. 1979). Fecal excretion of unabsorbed PBBs appears to be the main route of elimination of highly brominated congeners after oral exposure (Matthews et al. 1977; Norris et al. 1975a;

Rozman et al. 1982), whereas polar derivatives formed by lower brominated congeners appeared to be excreted mainly in the urine (Kohli and Safe 1976; Kohli et al. 1978; Sparling et al. 1980). Although data regarding excretion in animals after inhalation and dermal exposure were not located, there is no reason to believe that results from additional studies would reveal different patterns of excretion.

Polybrominated Diphenyl Ethers. A limited amount of data is available on the toxicokinetics of PBDEs. There are data gaps in a number of areas, particularly for octaBDE and pentaBDE commercial mixtures and the tetra and hexa congeners that are most prevalent in the environment. Quantitative absorption studies could corroborate the conclusions on oral uptake in animals that are based on elimination and excretion data. 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.

Comparative Toxicokinetics.

Polybrominated Biphenyls. The data suggest that there are qualitative differences in the toxicokinetic disposition of PBBs among humans and among animal species (Wolff and Aubrey 1978; Wolff et al. 1979a). However, these differences appear to be highly dependent on the specific congener or mixture studied. In general, all species absorb PBBs, with varying efficiency, and accumulate PBBs in tissues rich in fat. Once absorbed, PBBs are distributed in a biphasic manner in all examined animal species (Domino et al. 1982; Ecobichon et al. 1983; Matthews et al. 1977). No studies were located that provide information regarding differences or similarities in metabolic disposition of PBBs between humans and animals. Limited data in humans indicate that fecal excretion of PBB residues occur (Eyster et al. 1983). Experimental data in animals suggest that the rate and extent of PBB elimination in the urine and feces are dependent on the degree and pattern of bromination (Kohli et al. 1978; Matthews et al. 1977; Sparling et al. 1980). Analysis of the excreta of humans exposed in the workplace and near hazardous waste sites would provide information regarding biotransformation and elimination kinetics in humans. In addition, similar target organs have been identified across animal species, but the database is not complete enough for identifying a most sensitive species. Although the toxicological data in humans are limited and inconclusive, adverse immune effects observed in humans (Bekesi et al. 1978) have also been observed in rats, mice, and pigs (Howard et al. 1980; Luster et al. 1978, 1980) suggesting that any of these species may represent a suitable animal model for humans. The only reported PBPK model for PBBs describes the distribution and body burden of the major component of FireMaster mixtures, 2,2',4,4',5,5'-hexa-bromobiphenyl (BB 153), in the rat (Tuey and Matthews 1980). The serum mean half-life of 6.5 years

predicted using this model is shorter than half-life values of approximately 12–29 years estimated using human sera data from the Michigan PBB cohort (Blanck et al. 2000b; Lambert et al. 1990; Rosen et al. 1995); one possible reason for this difference is differences in body fat between humans and laboratory rodents resulting in a different distribution of the administered compound. This indicates a need for an improved PBPK model for extrapolating animal data to humans and/or for studies designed to produce data for improving the performance of PBPK analyses.

Polybrominated Diphenyl Ethers. Insufficient data are available to determine whether qualitative differences in the toxicokinetic disposition of PBDEs exist between humans and animals and among animal 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.

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. The main basis for this opinion seems to be studies showing that PBDEs affect binding of thyroid hormones to transthyretin (TTR), the primary transport protein in rats. Because TTR is not the major transport protein in humans, the findings have been interpreted as evidence that humans will be less sensitive than rats to thryoid effects of PBDEs. As discussed in Section 5.5.3, the greater sensitivity of rats 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 thyroid binding globulin (TBG) in rats. Whereas TTR is the major thyroid hormone binding protein in rats, TBG is the main binding protein in humans and most other mammals. Specific evidence is needed to determine whether PBDEs are likely to affect thyroid function in humans, or if humans are less sensitive to these effects than rats.

Methods for Reducing Toxic Effects.

Polybrominated Biphenyls. The mechanism by which PBBs enter the blood stream in humans is not known; consequently, there are no established methods for reducing absorption. Studies in experimental animals that could identify substances that prevent or delay absorption and that do not represent a toxic risk *per se* would be valuable. There are no established methods for reducing body burden in humans, but studies in animals and model simulations in humans indicate that reducing body fat markedly increases elimination of PBBs (Domino et al. 1982; Tuey and Matthews 1980). The effect of reduction of body fat (e.g., by dieting and exercising) in PBB-exposed humans has not been fully researched.

The mechanism of toxic action of PBBs is not completely understood and no methods exist to block the toxic response due to exposure to PBBs. A more complete characterization of the cytosolic AhR protein, to which some PBB congeners are thought to bind, 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 PBBs would also be valuable.

Polybrominated Diphenyl Ethers. 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. Types of studies that could address these data gaps and possibly provide information on reducing toxic effects of PBDEs are discussed in the preceding subsection on PBBs.

Children's Susceptibility.

Polybrominated Biphenyls. Information on health effects of PBBs in children is available from several studies of the Michigan feed contamination episode. A 1976 study of Michigan children likely to have been exposed to PBBs found that the number of subjectively reported symptoms of ill health, including several symptoms of neurological effects, did not increase with increasing serum PBB levels, but rather decreased; general neurological examinations did not reveal a pattern of abnormality among the Michigan children (Barr 1980). Studies of fetal mortality rates in Michigan (Humble and Speizer 1984) and of physical and neuropsychological development in Michigan children exposed during the contamination episode (Schwartz and Rae 1983; Seagull 1983; Weil et al. 1981) did not conclusively correlate the ingestion of PBBs with effects. Neurobehavioral alterations have been observed in rats following gestational and lactational exposure to PBBs (Henck and Rech 1986; Henck et al. 1994; Tilson 1992). Other effects in offspring of rats exposed to PBBs during gestation and lactation include decreased serum levels of thyroid hormone levels (Meserve et al. 1992; Werner and Sleight 1981). These effects in offspring are consistent with evidence that the thyroid gland is an unequivocal target of PBBs in adult animals. No information was located on possible immunological effects of PBBs in children, and data in adult humans are limited and largely inconclusive (Bekesi et al. 1978; Landrigan et al. 1979; Roboz et al. 1985; Silva et al. 1979; Stross et al. 1981), but exposure to PBBs caused altered immune responses in a variety of animal species, which suggests that children may also be affected. Continued assessment of children exposed to PBBs during the Michigan contamination episode, with particular emphasis on

evaluation of cognitive abilities, thyroid function, and immune competence, would help to better assess the susceptibility of children to PBBs.

Polybrominated Diphenyl Ethers. No information was located on health effects of PBDEs in newborn or older children. Thyroid and neurobehavioral alterations have been observed in animals following pre- and/or postnatal exposure to commercial PBDE mixtures and single PBDE congeners, indicating that these are potential effects of concern in exposed children. Serum levels of thyroid T_4 and T_3 hormones were reduced in offspring of rats that were orally exposed to pentaBDE during gestation and lactation and in rats exposed as weanlings (Zhou et al. 2002). Alterations in spontaneous locomotion behavior and learning and memory ability were observed in mice that were tested at 2 months of age and as adults (4 months) following neonatal exposure to single low oral doses of the congeners 2,2',4,4'-tetraBDE (BDE 47) and 2,2',4,4',5-pentaBDE (BDE 99) (Eriksson et al. 1998, 1999, 2001a, 2002a). Effects were observed in adult mice treated at either 3 or 10 days of age, but not at 19 days of age, suggesting that there was a critical window for the induction of behavioral disturbances (Eriksson et al. 1999, 2002a). Additional studies are needed to 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. No information is available regarding the immunosuppressive potential of PBDEs in children or young animals, indicating that studies of immune competence in developing animals would also help to more fully assess children's susceptibility to PBDEs.

Child health data needs relating to exposure are discussed in Section 8.8.1 Identification of Data Needs: Exposures of Children.

5.12.3 Ongoing Studies

Ongoing studies that are relevant to health effects of PBBs and PBDEs, as identified in the Federal Research in Progress database (FEDRIP 2002) and the websites of various U.S. government agencies, are listed in Table 5-9.

The Great Lakes Chemical Corporation is collaboratively working with Health Canada to assess potential effects of pentaBDE using a three-tier study approach (Biesemeier 2004). The first tier study, a 28-day repeated oral (gavage) toxicity study in rats, has been completed, but the draft report was not available as of June 2004. The basis of this study was mainly to serve in selecting gavage dose levels for the second

Table 5-9. Ongoing Studies on the Health Effects of PBBs and PBDEs

Investigator	Affiliation	Research description	Sponsor	Source
Anderson HA	Wisconsin Department of Health and Family Services, Madison, Wisconsin	To characterize human exposures to PCBs, PBDEs, and DDT from Great Lakes fish consumption and to determine mechanisms by which PBDEs in Great Lakes fish may act separately or synergistically with PCB exposure to impair human thyroid function	EPA	EPA 2004d
Hammock B	University of California, Davis, California	Support for testing hypotheses regarding the association of PBBs, PBDEs, and other known xenobiotic immunotoxicants and neurotoxicants with autism	NIEHS/EPA[a]	FEDRIP 2002
Hites RA and Bigsby M	Indiana University, Bloomington, Indiana	Compare the concentration of PBDEs in infants and analyze sediments and fish in the Great Lakes	EPA	EPA 2004d
Huwe JK	Agricultural Research Service, Fargo, North Dakota	Development of effective remediation procedures for PBDEs and other persistent organic pollutants in animal tissues and their environment	USDA	FEDRIP 2003
Karmaus W et al.	Michigan State University, East Lansing, Michigan	An effort to determine if exposure to halogenated organic compounds (including PBBs and PBDEs) via breastfeeding creates a risk to the immune system of the child	EPA	EPA 2004d
Ludewig G	University of Kentucky, Lexington, Kentucky	A multispecies approach to analyze the toxic effects of PBDEs on organs and development	EPA	FEDRIP 2002
Marcus M	Emory University, Atlanta, Georgia	Investigation of the effect PBBs have on pubertal development, reproductive health, and ovarian function	NIEHS	FEDRIP 2003
Omann GM	Department of Veterans Affairs, Washington, DC	The immunotoxicological potential of PBDEs found in the environment will be assessed using fish immune cells in an *in vitro* model	Department of Veteran Affairs	FEDRIP 2003
Palmer BD	University of Kentucky Medical Center, Lexington, Kentucky	Multidisciplinary/multispecies investigation of end points and mechanisms of action for PBDEs and PCDEs, including structure-activity relationships for endocrine disruption	NIEHS/EPA[a]	FEDRIP 2002
Raymer JH et al.	Research Triangle Institute, Research Triangle Park, North Carolina	Development of a PBPK animal model for PBDEs to estimate fetal exposure in humans and to determine the validity of the model by creating/using new analytical methods using cord blood and meconium	EPA	EPA 2004d

Table 5-9. Ongoing Studies on the Health Effects of PBBs and PBDEs

Investigator	Affiliation	Research description	Sponsor	Source
Robertson L	University of Kentucky Medical Center, Lexington, Kentucky	Laboratory synthesis of pure PBDE and PCDE congeners and their metabolites	NIEHS/EPA[a]	FEDRIP 2002
Sikka HC	State University of New York College at Buffalo, Buffalo, New York	Disposition and metabolism of PBDEs in fish	NOAA[b]	FEDRIP 2002
Trosko JE	Michigan State University, East Lansing, Michigan	Epigenic effects of PBBs and other environmental toxicants on cellular communication pathways	NIEHS/EPA[a]	FEDRIP 2002
Vos J	Bilthoven, Netherlands	Risk assessment of brominated flame retardants for human health and wildlife	EU	EU 2004
Willett LB	Ohio State Universities, Wooster, Ohio	Develop methods to monitor the occurrence of PBBs and other xenobiotics in the environment of cattle; create methods that will reduce or eliminate exposure of cattle and the food products they produce to these xenobiotics; determine mechanisms by which xenobiotics are transported, bound, and mobilized *in vivo*; describe the pharmacokinetics; and to study target organ modifications in cattle caused by xenobiotic chemicals that result in cellular or metabolic alterations	Department of Agriculture	FEDRIP 2003

[a]NIEHS/EPA Superfund Basic Research Program
[b]National Sea Grant College Program

DDT = dichlorodiphenyltrichloroethane; EPA = U.S. Environmental Protection Agency; EU = European Union; NIEHS = National Institute of Environmental Health Sciences; NOAA = National Oceanic and Atmospheric Administration; PCB = polychlorinated biphenyls; PBPK = physiologically based pharmacokinetic; USDA = U.S. Department of Agriculture

tier study, a one-generation reproductive study in rats, of which the draft protocol is currently being written (as of June 2004). This protocol is being drafted with a pharmacokinetic segment, but it has not yet been confirmed whether it will be retained in the final study design. The results of the one-generation study will be used to select gavage dose levels for the third tier study, a two-generation reproductive study in rats with endocrine, developmental neurobehavioral, and other end point segments. The entire program is not expected to be completed until near the end of 2005.

NTP is in the process of designing a study to assess the toxicity of tetraBDE (NTP 2004).