



TOXICOLOGICAL REVIEW

OF

**2,2',4,4'-TETRABROMODIPHENYL
ETHER (BDE-47)**

(CAS No. 5436-43-1)

**In Support of Summary Information on the
Integrated Risk Information System (IRIS)**

December 2006

NOTICE

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U.S. Environmental Protection Agency
Washington, DC

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2,2',4,4'-TETRABROMODIPHENYL ETHER (CAS No. 5436-43-1)**

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LIST OF ABBREVIATIONS AND ACRONYMS

Ah	aryl hydrocarbon
BDE-47	2,2',4,4'-tetrabromodiphenyl ether
BMD	benchmark dose
BMDL	95% lower bound on the BMD
BMDS	benchmark dose software
BMR	benchmark response
CALUX	Chemical-Activated LUciferase gene eXpression
CAR	constitutive androstane receptor
CDD	chlorinated dibenzo-p-dioxin
CDF	chlorinated dibenzofuran
CYP450	cytochrome P-450
DHT	dihydrotestosterone
EC ₅₀	median effective concentration
ER	estrogen receptor
EROD	ethoxyresorufin O-deethylase
ESI-MS	electron spray ionization-mass spectrometry
FOB	functional observational battery
FT3	free triiodothyronine
FT4	free thyroxine
HPLC	high performance liquid chromatography
hprt	hypoxanthine-guanine phosphoribosyl transferase
IC ₅₀	median inhibitory concentration
IRIS	Integrated Risk Information System
IUPAC	International Union of Pure and Applied Chemistry
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
lw	lipid weight
MM	malignant melanoma
MROD	methoxyresorufin O-dealkylase
MUP	major urinary protein
NHL	non-Hodgkin's lymphoma
NOAEL	no-observed-adverse-effect level
PBDE	polybrominated diphenyl ether

PCB	polychlorinated biphenyl
PND	postnatal day
PROD	pentoxyresorufin O-dealkylase
PXR	pregnane X receptor
RfC	reference concentration
RfD	reference dose
T3	triiodothyronine
T4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
tetraBDE	tetrabromodiphenyl ether
TSH	thyroid stimulating hormone
TSH	thyroid stimulating hormone
TTR	transthyretin
UDPGT	uridine diphosphoglucuronosyl transferase
UF	uncertainty factor

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to exposure to 2,2',4,4'-tetrabromodiphenyl ether (BDE-47). It is not intended to be a comprehensive treatise on the chemical or toxicological nature of BDE-47.

The majority of the available toxicological information relates to the tetrabromodiphenyl congener 2,2',4,4'-tetrabromodiphenyl ether (CAS No. 5436-43-1). Toxicological information related to other congeners in the tetrabromodiphenyl ether homolog group (CAS No. 40088-47-9) is also discussed. However, this health assessment does not deal with commercial mixtures of brominated diphenyl ether homologs containing tetrabromodiphenyl ether as one of the constituents of commercial formulations.

In Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47). IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and less-than-lifetime exposure durations, and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m³) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrapulmonary or systemic effects). Reference values may also be derived for acute (≤ 24 hours), short-term (up to 30 days), and subchronic (up to 10% of average lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are derived from the application of a low-dose extrapolation procedure, and are presented in two ways to better facilitate their use. First, route-specific risk values are presented. The “oral slope factor” is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a “unit risk” is an upper bound on the estimate of risk per unit of concentration, either per $\mu\text{g/L}$ drinking water or per $\mu\text{g/m}^3$ air breathed. Second, the estimated concentration of the chemical substance in drinking water or air when associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000 is also provided.

Development of these hazard identification and dose-response assessments for 2,2',4,4'-tetrabromodiphenyl ether has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and technical panel reports that were used in the development of this assessment include the following: *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a),

Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2000a, 2005c), *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through November 2006.

2. CHEMICAL AND PHYSICAL INFORMATION

Tetrabromodiphenyl ether (CASRN 40088-47-9) is one of the possible 10 homologs of polybrominated diphenyl ethers (PBDEs). Figure 1 shows the chemical structure of tetrabromodiphenyl ether (tetraBDE). The number of possible congeners of tetraBDE is 42, with International Union of Pure and Applied Chemistry (IUPAC) numbers 40 to 81 (ATSDR, 2004). The IUPAC number and bromine substitution pattern of some congeners that have been investigated in various studies are given in Table 1.

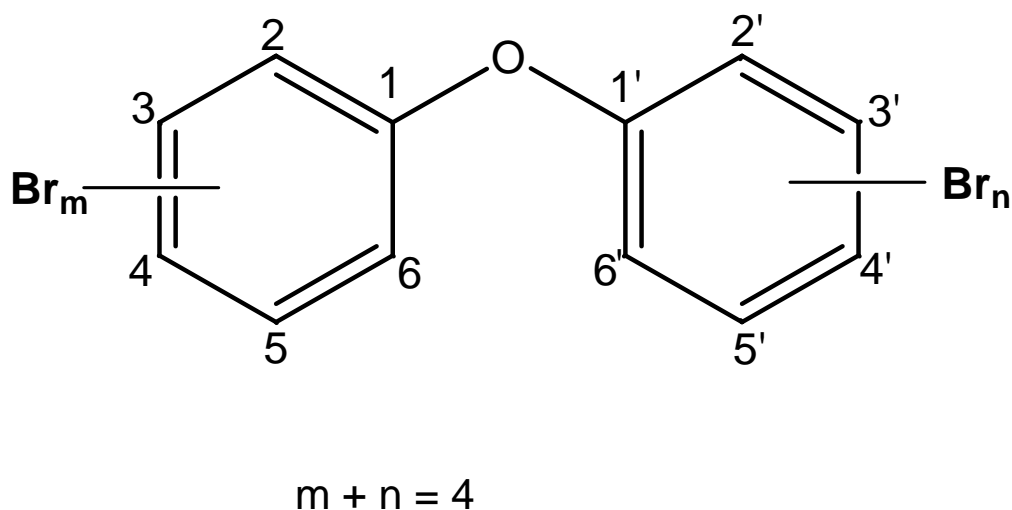


Figure 1. Chemical structure of tetrabromodiphenyl ether.

Table 1. IUPAC number and bromine substitution pattern of some tetrabromodiphenyl ether congeners

IUPAC number	Bromine substitution pattern
BDE-47	2,2',4,4'-TetraBDE
BDE-49	2,2',4,5'-TetraBDE
BDE-51	2,2',4,6'-TetraBDE
BDE-66	2,3',4,4'-TetraBDE
BDE-71	2,3',4',6-TetraBDE
BDE-75	2,4,4',6-TetraBDE
BDE-77	3,3',4,4'-TetraBDE
BDE-80	3,3',5,5'-TetraBDE

TetraBDE is found in commercial pentabromodiphenyl ether, which is usually composed of a mixture of triBDE to hexaBDE congeners. The relative proportions by weight of various PBDE congeners in the commercial pentaBDE DE-71TM are approximately pentaBDE-99, 43%; tetraBDE-47, 28%; pentaBDE-100, 8%; hexaBDE-153, 6%; and hexaBDE-154, 4%.

TriBDE-28 and -33 and tetraBDE-49 and -66 are present at about 1% or less in the formulation (Great Lakes Chemical Corporation, 2003).

The predominant PBDE congener in environmental media, biota, and human biological media is the ortho-para (2,4-) substituted congener 2,2',4,4'-tetraBDE or BDE-47 (CASRN 5436-43-1).

Physical and chemical properties of BDE-47 are listed in Table 2.

Table 2. Physical and chemical properties of 2,2',4,4'-tetrabromodiphenyl ether

Parameter	Value	Reference
Synonym	Benzene,1,1'-oxybis[2,4-dibromo-]; 2,2',4,4'-tetrabromodiphenyl ether; BDE-47	U.S. EPA (2004)
CASRN	5436-43-1	U.S. EPA (2004)
Chemical formula	C ₁₂ H ₆ Br ₄ O	U.S. EPA (2004)
Molecular weight	485.8	U.S. EPA (2004)
Vapor pressure (Pa) at 25°C	2.5 × 10 ⁻⁴	Wong et al. (2001)
Melting point (°C)	79–82	Marsh et al. (1999); Palm et al. (2002)
Solubility in water (µg/L)	11	Stenzel and Markley (1997)
Henry's Law constant (Pa m ³ mol ⁻¹) at 25°C	0.85	Cetin and Odabasi (2005)
Log octanol/water partition coefficient (K _{ow}) at 25°C	5.9–7.5	Braekevelt et al. (2003); ATSDR (2004)
Log octanol/air partition coefficient (K _{oa}) at 25°C	10.5	Chen et al. (2003)

3. TOXICOKINETICS

3.1. ABSORPTION

There are no direct studies of BDE-47 absorption in humans. The data that demonstrate human absorption come from measurements of BDE-47 in human biological media after anthropogenic exposures but do not permit estimation of route-specific uptake parameters. The data on toxicokinetics following oral exposure in animals are more complete. They include data from both single and repeat exposures in both rats and mice.

Orn and Klasson-Wehler (1998) conducted a study of radiolabeled BDE-47 in rats and mice. Adult male Sprague-Dawley rats or C57B1 male mice were given a single gavage dose of 30 $\mu\text{mol/kg}$ (approximately 15 mg/kg) of ^{14}C -labeled BDE-47 (purity 98%) dissolved in corn oil. Feces and urine were collected daily until day 5 when the animals were sacrificed. The parent compound excreted in the feces on day 1 was assumed to represent nonabsorbed dose and corresponded to approximately 6% and 8% of the administered dose in rats and mice, respectively. This suggests that over 90% of the 15 mg/kg dose was absorbed. Absorption is likely to occur by diffusion across the lipid matrix of the intestinal membrane; there may or may not be additional facilitated transport.

Building on the work by Orn and Klasson-Wehler (1998), Staskal et al. (2005) evaluated the effects of dose, route of exposure, and time on the toxicokinetics of BDE-47 by using female C57BL/6J mice. In the first part of this study, groups of six animals received single gavage doses of ^{14}C -BDE-47 (purity >97%) in corn oil at 0, 0.1, 1.0, 10, or 100 mg/kg. Urine and feces were collected daily for 5 days after dosing and analyzed for radiolabel. The percent of the dose excreted in feces was relatively consistent between dose groups on all days. In all dose groups, approximately 28% was excreted on the first day, indicating rapid absorption of BDE-47.

Studies that compare the concentrations of BDE-47 radiolabel in excreta on the first day after intratracheal, oral, intravenous, intraperitoneal, or dermal administration of 1 mg/kg to groups of 4 or 6 female mice indicate that the absorption was approximately 82% for the oral route, 91% for the intratracheal route, and 62% for the dermal route (Staskal et al., 2005). Comparison of the day-1 fecal concentration for the intravenous route to the oral results was suggestive of some biliary excretion on day 1. This would increase the absorption estimates derived from the one-day fecal excretion data after exogenous exposures by between 5 and 10%.

Sanders et al. (2006) investigated potential sex and species differences in the toxicokinetics of BDE-47. Approximately 2 to 3-month-old male and female F344 rats or B6C3F1 mice (n = 4 to 5 animals) were given by gavage a single dose of 1 $\mu\text{mol/kg}$ (approximately 0.5 mg/kg) of ^{14}C -BDE-47 (purity 99%) in corn oil. BDE-47 was absorbed from

the GI tracts within the 24-hour period after dosing as demonstrated by tissue distribution of BDE-47-derived radioactivity. An estimate of the extent of absorption of BDE-47 in rats and mice was made by comparing tissue distribution and excretion data of ¹⁴C-BDE-47. Rats absorbed about 75% of the dose while mice absorbed about 85%. Although these data are quantitatively different from the data of Orn and Klasson-Wehler (1998) discussed above, they are consistent in suggesting that intestinal absorption may be lower in rats than in mice.

3.2. DISTRIBUTION

The high K_{ow} of BDE-47 suggests a strong potential for bioaccumulation in lipid-rich tissues. This property of BDE-47 is evident from the data on distribution in humans and animals described below.

3.2.1. Human Data

The human data described below come from monitoring of PBDEs in human populations rather than from measured dosing studies. The data demonstrate that humans are exposed to PBDEs and that absorption and distribution to some tissues occurs. The data do not provide information on the quantitative aspects of absorption or the kinetics of tissue and retention. Monitoring data, described below, are available for human adipose tissue, liver, milk and blood samples and indicate a tendency for BDE-47 to distribute to these tissues. However, distribution studies have not been conducted in humans, and therefore it is not known whether BDE-47 distributes to other tissues as well. The number of samples examined in various studies and countries is small and therefore the data should not be construed as representative at the national level.

3.2.1.1. Adipose Tissue

Breast adipose samples were collected between 1996 and 1998 from 23 San Francisco Bay area women as part of a case-control study on organochlorine compounds and breast cancer (She et al., 2002). Women ranged from 28 to 62 years of age and were predominantly Caucasian and born in the United States. Pathology reports indicated 12 women had malignancies, 8 had benign tumors and 3 had ductal carcinomas in situ, a condition considered by some as transitional to malignancy. Breast adipose samples were collected during biopsy or breast surgery and were analyzed for BDE-47, pentaBDEs (BDE-99 and BDE-100), and hexaBDEs (BDE-153 and BDE-154). Mean and median concentrations of the sum of these PBDEs were 86 and 41 ng/g lipid weight (lw), respectively, the highest human levels reported so far. Concentration of BDE-47 ranged from 7 to 196 ng/g lw, with mean and median concentrations of 33 and 18 ng/g lw, respectively. Mean concentrations of individual PBDE congeners were, in

decreasing order, 33 ng/g lw BDE-47, 17 ng/g lw hexaBDE-154, 16 ng/g lw hexaBDE-153, 11 ng/g lw pentaBDE-99, and 9 ng/g lw pentaBDE-100. The highest concentrations found were therefore for BDE-47, followed by hexaBDEs and pentaBDEs, a distribution that does not follow that of the commercial pentaBDE used in the United States. There was an inverse relationship between the sum of the concentrations of these PBDEs in breast adipose tissue and age, with women younger than the median age of 48 years having significantly higher concentrations of PBDEs in adipose tissue than women older than 48. This may imply that different activities may expose different age groups more than others or that some PBDE congeners may accumulate differently with age. Five paired samples of breast and abdominal adipose tissues were also analyzed for tetra- to hexaBDEs. Abdominal and breast concentrations of PBDEs were highly correlated and of comparable magnitude.

The study of She et al. (2002) was expanded to include additional samples, collected between 1996 and 1998, from a total of 32 women in the same population group (Petreas et al., 2003). Concentration of BDE-47 ranged from 5 to 196 ng/g lw, with mean and median concentrations of 29 and 17 ng/g lw, respectively. Concentrations of other PBDEs in adipose tissue were not reported. There was a significant inverse relationship between BDE-47 concentration in adipose tissue and age, a departure from other persistent organic pollutants where exposure is driven by diet and leads to a direct correlation of concentration with age. The authors suggested that the negative association could be spurious because of the small number of samples and the presence of outliers or that it could indicate that some age groups may be exposed from dietary or nondietary sources to a greater degree than others (Petreas et al., 2003).

In a study in New York City, adipose tissue samples (n = 52) were collected in 2003–2004 from male and female patients undergoing liposuction procedures (Johnson-Restrepo et al., 2005). BDE-47 was the major congener detected, followed by the pentaBDE congeners BDE-99 and BDE-100. No significant difference was found between genders in the concentrations of PBDEs. Concentrations of PBDEs were, on average, similar to those for polychlorinated biphenyls (PCBs). PBDE concentrations did not increase with increasing age of the subjects, whereas concentrations of PCBs increased with increasing age in males but not in females. These results suggest differences between PBDEs and PCBs in their sources or time course of exposure and disposition.

In a Swedish study, samples of adipose tissue were obtained in 1994 at autopsy from one woman (age 47) and four men (ages 66–83 years) and analyzed for tri- to hexaBDEs (Guvénus et al., 2001). PBDEs were found in all samples. The mean concentration of BDE-47 in adipose tissue was 2.5 ng/g lw; mean concentration of pentaBDEs was 1.7 ng/g lw. BDE-47 constituted 40–50% of the total PBDE concentration in adipose tissue. A higher level of BDE-47 (8.8 ng/g lw) was found in an adipose tissue sample collected in 1994 from a healthy Swedish 74-year-old male (Haglund et al., 1997).

In a study in Japan, 10 human adipose samples taken from the general Tokyo population in 1970 and in 2000 were analyzed for PBDEs. Total tri- through heptaBDE median concentrations were 0.03 and 1.3 ng/g lw in 1970 and 2000, respectively. BDE-47 was the most abundant of the PBDEs analyzed, with median concentrations of 0.02 and 0.5 ng/g lw in 1970 and 2000, respectively (Choi et al., 2003).

3.2.1.2. Liver

In the study by Guvenius et al. (2001) of PBDEs in adipose tissue, liver tissue samples were also obtained in 1994 at autopsy from the same five Swedish subjects. The concentration of BDE-47 in the liver (mean 3.0 ng/g lw) was similar to that in adipose tissue. The BDE-47 concentration constituted 30–50% of the total PBDE concentration in the liver. In contrast to adipose tissues, pentaBDE was the predominant congener in the liver with a mean concentration of approximately 4 ng/g lw.

3.2.1.3. Human Milk

In a study conducted in 2002 of levels of PBDEs in human milk in the United States, 47 samples from Caucasian, African-American, and Hispanic nursing mothers 20–41 years of age and living in Texas were analyzed for 13 PBDE congeners (Schechter et al., 2003). Mean and median total concentrations of tri- through decaBDEs were 74 and 34 ng/g lw, respectively. BDE-47 was found at the highest level with maximum, mean, and median concentrations of 272, 41, and 18 ng/g lw, respectively. There was no correlation between age and level of PBDEs in human milk. Concentrations of PBDEs found in this study were substantially higher than those measured in human milk in Europe or Japan.

PBDEs were also found in breast milk samples collected in Japan and Sweden (Akutsu et al., 2003; Lind et al., 2003; Ohta et al., 2002). In all cases, BDE-47 was the major congener present. In the study by Ohta et al. (2002), there was a strong positive relationship between PBDE levels in milk from 12 Japanese nursing mothers and fish consumption. However, no such association was found in the Swedish study by Lind et al. (2003), where samples from 93 nursing mothers were analyzed.

3.2.1.4. Blood

Levels of PBDEs in the blood are representative of either recent exposures or the slow release of PBDEs from tissue stores. Median concentration of BDE-47 in serum samples collected in 1988 from 12 male blood donors in the United States was 0.63 ng/g lw (Sjodin et al., 2001). In 2000–2002 (Sjodin et al., 2004), the median concentration of BDE-47 in seven serum pools from the United States was 34 ng/g lw. The number of donors in the serum pools ranged

from 40 to 200. When the BDE-47 concentrations in archived serum samples were arrayed by collection periods, there was a progressive increase from 1985 to 1999 and then a slight decrease in 2000–2002.

BDE-47 was not present in archived serum samples collected between 1959 and 1967 from 420 women in the San Francisco Bay Area. However, samples from 50 Laotian women residents of the San Francisco Bay area collected between 1997 and 1999 had mean and median BDE-47 concentrations of 51 and 10 ng/g lw, respectively (Petreas et al., 2003).

Concentrations of PBDE congeners BDE-47, hexaBDEs (BDE-153 and BDE-154), heptaBDE (BDE-183), and decaBDE (BDE-209) were determined in blood serum from groups of 19–20 Swedish male and female subjects in the following occupational groups: hospital workers (control), clerks working full-time at computer screens, and personnel at an electronic-dismantling plant (Sjodin et al., 1999). Commercial PBDEs used as flame retardants in the electronic industry are usually decabromodiphenyl ether and to a lesser extent octabromodiphenyl ether. The median concentration of BDE-47 in serum was about the same in the controls and computer clerks (~1.5 ng/g lw) but almost double that level in the electronic-dismantling personnel. There was no correlation between plasma levels of BDE-47 with age or fish consumption (the only food evaluated in the study). Serum concentrations of all PBDE congeners in the electronic-dismantling workers decreased after vacation. The median decreases, standardized to 30 days of leave, were 14% for BDE-47, hexaBDE-153, and -154; 30% for heptaBDE-183; and 66% for decaBDE-209. These results indicate shorter half-lives of the more highly brominated diphenyl ethers.

A study was conducted in Sweden to determine the possible relationship between levels of the thyroid hormones triiodothyronine (T_3), thyroxine (T_4), and thyroid stimulating hormone (TSH) and PBDE levels in plasma of 11 subjects, aged 20 to 55 years, working at an electronic recycling facility (Julander et al., 2005). PBDEs studied were triBDE-28, tetraBDE-47, pentaBDEs 99 and 100, hexaBDEs 153 and 154, and heptaBDE-183. At the start of employment, the median level of the sum of these PBDEs was 7.2 pmol/g lw. Median levels of individual congeners in pmol/g lw were, in decreasing order, BDE-47 (2.8), BDE-153 (1.7), BDE-99 (0.81), BDE-100 (0.44), BDE-154 (0.14), BDE-28 (0.11), and BDE-183 (<0.19). No statistically significant correlation between age and plasma concentration of PBDEs was found at the start of employment. After a period of exposure of 1.5 years in sorting or dismantling, significant increases in median levels in plasma were observed for BDE-47 (5.7), BDE-28 (2.0), BDE-154 (0.29), and total PBDEs (10). All measured levels of thyroid hormones were within the normal physiological range. No relevant changes in plasma hormone levels were seen in relation to PBDE exposure within the workers participating in the study.

In Norway, pooled serum samples collected in 1998 from eight population groups of different ages (0 to >60 years) and genders were analyzed for tri-, tetra-, penta- and hexaBDEs. Each group consisted of five persons. Total concentration of these PBDEs in men older than 60 years was 5.3 ng/g lw, with BDE-47 being the most abundant congener (3.4 ng/g lw), followed by hexaBDE-153 (0.6 ng/g lw), pentaBDE-100, pentaBDE-99 and hexaBDE-154 (all at approximately 0.4 ng/g lw), and triBDE-28 (0.1 ng/g lw). The sum of plasma PBDE concentration was highest for the 0–4-year-old children (12 ng/g lw) but was about one-third lower and relatively constant for the different age groups above 4 years. Except for the 0 to 4-year-olds who seem to experience elevated exposure, there was a lack of an age-related trend of PBDEs body burden. This may be explained by the fact that PBDEs are relatively new contaminants in the environment; the time period for human exposure is therefore relatively short, and different age groups (except the 0–4 years group) may thus have experienced a similar exposure period (Thomsen et al., 2002).

3.2.1.5. Placental Transport

Twelve paired samples of maternal and cord blood collected in 2001 from women in Indiana were analyzed for tetra- to heptaBDE congeners. None of the mothers had work-related potential for exposure to PBDEs and none smoked. Median concentrations of the various PBDEs found in maternal and fetal sera are given in Table 3. BDE-47 was the most abundant congener, followed by pentaBDE-99 and pentaBDE-100. PBDEs concentrations were highly correlated between mother and fetal sera, indicating that PBDEs cross the placenta into the fetal circulation. In addition, the results indicate that all tetra- through hepta-substituted congeners have approximately the same potential to cross the placenta. There was a decreasing trend in concentration of PBDE congeners in maternal and fetal sera with increasing degree of bromination (Mazdai et al., 2003).

Samples of maternal and cord blood plasma were collected during 2000–2001 from 15 Swedish mothers (Guvenius et al., 2003). BDE-47 was the most abundant of all congeners and comparable median concentrations were found in maternal and cord blood plasma (Table 3). The levels of the higher brominated congeners, pentaBDE-99 to heptaBDE-183, were higher in maternal blood than in cord blood, indicating that the higher brominated PBDEs do not pass through the placenta to the same extent as the lower brominated congener BDE-47. This trend was not apparent in the Mazdai et al. (2003) study, where comparable levels were found in maternal and fetal sera for all PBDE congeners studied.

The concentrations of PBDEs found in maternal and fetal blood samples in Indiana women (Mazdai et al., 2003) were substantially higher than those found in Swedish women (Guvenius et al., 2003).

Table 3. Median PBDE congener concentrations in maternal and fetal sera in the United States and Sweden

PBDE congener	Maternal serum, ng/g lw		Fetal serum, ng/g lw	
	Mazdai et al. (2003) ^a	Guvenius et al. (2003) ^b	Mazdai et al. (2003) ^a	Guvenius et al. (2003) ^b
tetraBDE-47	28	0.8	25	1.0
pentaBDE-99	5.7	0.2	7.1	0.07
pentaBDE-100	4.2	0.2	4.1	0.07
hexaBDE-153	2.9	0.6	4.4	0.2
hexaBDE-154	0.3	0.04	0.7	<0.01
heptaBDE-183	0	0.06	0	0.01
Σ PBDEs	37	2.1	39	1.7

^aMazdai et al., 2002 (United States): year of sampling, 2001; number of donors, 12.

^bGuvenius et al., 2003 (Sweden): year of sampling, 2000–2001; number of donors 15.

A summary of the data described above on concentrations of PBDEs in various human biological media in the United States is given in Table 4. Median concentrations of PBDE congeners are available for human adipose tissue (Johnson-Restrepo et al., 2005; She et al., 2002), breast milk (Schechter et al., 2003) and serum (Sjodin et al., 2004; Mazdai et al., 2003). The concentration profiles in the United States of PBDEs in maternal and fetal sera, pooled serum, and human milk are similar, although these studies were conducted in different regions of the United States.

The predominant congeners found in human biological samples in the United States are tetra-, penta-, and hexaBDEs. Few measurements have been made of other PBDE congeners, such as tri-, hepta-, and decaBDE. The predominant congener found in human biological samples is BDE-47, followed by pentaBDE-99. Current median concentrations of BDE-47 found in the United States in adipose tissue, human milk, and blood are in the vicinity of 20 ng/g lw; median concentrations of the sum of PBDEs measured in human biological media are in the vicinity of 40 ng/g lw. These levels are substantially higher than the levels found in human populations in Europe or Japan.

Table 4. Median PBDE congener concentrations in human biological media in the United States (ng/g lipid weight)

	Year ^a	N ^a	BDE-47	BDE-99	BDE-100	BDE-85	BDE-153	BDE-154	BDE-183	Σ PBDE	Reference
Adipose tissue	1996–1998	23	18	7	3	—	4	6	—	41	She et al. (2002)
Adipose tissue	2003–2004	52	29	10	12	<1	<1	<1	—	75	Johnson-Restrepo et al. (2005)
Breast milk	2002	47	18	6	3	0.4	2	0.2	0.1	34	Schechter et al. (2003)
Maternal serum	2001	12	28	6	4	—	3	0.3	0	37	Mazdai et al. (2003)
Fetal serum	2001	12	25	7	4	—	4	0.7	0	39	Mazdai et al. (2003)
Serum pools	2000–2002	7 ^b	34	11	6	0.7	7	1	—	61	Sjodin et al. (2004)

^aYear = year of sampling; N = number of donors.

^bSeven serum pools with number of donors in each serum pool ranging from 40 to 200.

3.2.2. Animal Data

The animal data on BDE-47 distribution are more quantitative than the human data because they represent the distribution after deliberate dosing studies. Information is available for male and female rats and mice along with data on the impact of age at the time of exposure on tissue distribution.

In the study by Orn and Klasson-Wehler (1998), adult male Sprague-Dawley rats or male C57B1 mice were given a single gavage dose of approximately 15 mg/kg of ¹⁴C-labeled BDE-47 (see also Section 3.1). Adipose, liver, lung, kidney, brain, and plasma tissues were analyzed for ¹⁴C-BDE-47 and metabolites. After 5 days, 86% of the administered dose was retained in rat tissues, mainly stored as the parent compound in adipose tissue, which had the highest concentration of ¹⁴C on a lipid weight basis. The lung had the second highest ¹⁴C concentration on a lipid weight basis, amounting to twice the concentrations found in kidney and liver, and 10-fold the concentration in brain. ¹⁴C levels in plasma were low. In mice, 47% of the dose remained in the body after 5 days, mainly as the parent compound. On a lipid weight basis, concentrations of ¹⁴C in mice were highest in adipose and liver tissues and of similar magnitude. Kidney and lung had about half that concentration, while levels in brain were about one-tenth of the concentrations in adipose and liver tissues. As in rats, ¹⁴C levels in mice plasma were low. The low levels in brain may indicate limited transport across the blood brain barrier.

The C57BL/6 mouse tissue data from Staskal et al. (2005) are generally consistent with those of Orn and Klasson-Wehler (1998). After oral exposures, the concentration in adipose tissue (ng/g tissue) was highest. Levels in skin, liver, muscle and lung were of intermediate concentrations and those in kidney, blood, and brain were low. The concentrations roughly reflected the tissue levels of lipid and support the hypothesis that BDE-47 accumulates in lipid-rich tissues. Concentrations in all tissues increased with dose. A pronounced increase in BDE-47 tissue concentration occurred with the doses of 10 mg/kg and 100 mg/kg, especially in the adipose tissue, suggesting a propensity for bioconcentration. When tissue concentrations were examined over 21 days following administration of a 1 mg/kg gavage dose, concentrations in adipose tissue and skin peaked at three and two days, respectively; concentrations in brain and muscle peaked at 8 hours; and peak concentrations in kidney, blood, liver, and lung occurred at 3 hours. The authors proposed that distribution of BDE-47 to tissues such as kidney, liver, lung, muscle, and brain is a flow-limited process, while that for adipose tissue and skin is a diffusion-limited process.

Tissue distribution was influenced by the route of administration (Staskal et al., 2005). Some dose was retained at the site of application for the intratracheal and dermal routes. The amount found in the lung after intratracheal dosing was considerably higher than for the other routes. In the case of the skin, absorption was slow as indicated by the amounts excreted and some of the applied compound (~15%) remained at the site of administration five days after dosing.

Staskal et al. (2006a) examined the distribution of BDE-47 after repeat exposures to 10 consecutive 1.0 mg/kg-day doses (total dose 10 mg/kg). The 10th dose was radiolabeled to permit evaluation of tissue distribution. Excretion of radiolabeled compound was measured for the 5 days after administration of the radiolabeled dose. Tissue concentrations of BDE-47 5 days after the last 1 mg/kg-day dose were compared with those 5 days after a single 1 mg/kg and 10 mg/kg dose. The amounts of the radiolabel in the adipose tissue, blood, brain, and kidney were significantly higher when the 1 mg/kg dose was administered after 10 days of pretreatment than when the same dose was given to naive mice. The concentration in adipose tissue was slightly, but not significantly, higher than after the single 10 mg/kg dose (20% versus 15%), but the concentrations in the tissues other than the liver were roughly comparable. The percent in the liver was higher for a single 10 mg/kg dose than in the 10 consecutive 1.0 mg/kg-day doses.

The overall distribution of ¹⁴C-labeled BDE-47 was studied in mice, using a qualitative whole-body autoradiography technique (Darnerud and Risberg, 2006). ¹⁴C-BDE-47 (>95% purity) was administered to male and female C57BL mice by intravenous injection at 20 μmol/kg of body weight (~10 mg/kg). The animals were sacrificed at time intervals varying from 1 hour to 4 days after administration. Qualitatively, the distribution of radioactivity in mice

was characterized by a high initial uptake of radioactivity in fatty tissues. In addition, the liver, adrenal cortex, lung, ovaries, and nasal epithelium accumulated radioactivity. Intermediate radioactivity levels were initially present in the brain tissue. No radioactivity was observed in the thyroid gland. At 4 days after administration, the radioactivity concentration was weaker, indicating significant ^{14}C excretion. In the male mouse after 6 hours, the concentration of radioactivity in the testis was low; in females, labeling in the ovaries was localized to the follicular structure. At 16 days postinjection, labeling was still visible in the fat tissues, liver, lung, and adrenal cortex; elimination from the lungs seemed to be slower than elimination from the liver. Some faint labeling remained in the brain.

Sanders et al. (2006) studied the disposition of BDE-47 in male and female F344 rats and B6C3F1 mice (approximately 2 to 3 months old). Groups of 4 to 5 animals were given a single dose of $1\ \mu\text{mol/kg}$ (approximately $0.5\ \text{mg/kg}$) of ^{14}C -BDE-47 (purity 99%) in corn oil by gavage. The majority of the radiolabel in tissues of both species and sexes 24 hours postdosing was contained in adipose tissue. In male and female rats, adipose tissue contained 25% and 37% of the total dose, respectively, while in male and female mice 20% and 31% of the total dose, respectively, was present in adipose tissue. More BDE-47 derived radioactivity was contained in adipose tissue of female rats and mice than in males of the respective species. However, there were no statistically significant differences in the percent total dose or concentration of ^{14}C in adipose tissue between the female rats and mice or between male rats and mice. Other rat and mouse tissues containing more than 1% of the total dose were, in decreasing order, skin, muscle, and liver. Blood, brain, kidney, and lung contained 0.4% or less of the total dose.

In another component of the Sanders et al. (2006) study, male rats received single doses of 0.1, 1, 10, 100, or 1000 mg/kg of ^{14}C -BDE-47, and the concentration of ^{14}C in various tissues was determined 24 hours following administration. Absorption and distribution of ^{14}C to major tissues were dose-proportional. Blood contained less ^{14}C than did other tissues for each treatment group, resulting in tissue to blood ratios >1 for all tissues examined. Adipose tissue contained the highest concentration of BDE-47-derived radioactivity, about 10-fold higher than that contained in liver across the dose range.

Sanders et al. (2006) also examined the impact of repeat dosing on the disposition of BDE-47 in male rats. Doses of $0.1\ \mu\text{mol/kg}$ (approximately $0.05\ \text{mg/kg}$) of ^{14}C -BDE-47 were administered for 1, 5, or 10 consecutive days. Accumulation of radioactivity showed a linear response to time and did not appear to reach saturation in adipose and other major tissues, excluding lung, over the course of the ten daily doses. BDE-47-derived radioactivity appeared to be at or near steady state in the lung and thyroid by the fifth dose. Male mice were evaluated in a similar fashion but with a higher dose ($1\ \mu\text{mol/kg}$) and the elimination of the 5-day sacrifice. After allowing for the differences in dose, tissue burdens of BDE-47-derived radioactivity in

mice were either similar or significantly lower than those in rats 24 hours following a single dose and significantly lower in all mouse tissues, with the exception of thyroid and thymus after 10 days of dosing.

The prenatal and neonatal disposition of BDE-47 in tissues, especially the brain, is important because of the work of Eriksson et al. (2001), suggesting that there is a period in neonatal development during which young mice are vulnerable to the neurodevelopmental effects of BDE-47 exposures (see Section 4.3). For this reason, several studies have examined the impact of the timing of exposure on tissue distribution. Darnerud and Risberg (2006) studied fetal uptake in pregnant mice sacrificed 24 hours after administration of 10 mg/kg of ¹⁴C-BDE-47 on gestational day 16–17. Overall, fetal uptake was low. Radiolabel was observed in the membranes surrounding the fetus and labeling of fetal liver and intestinal contents was higher than that for surrounding tissues. Faint radiolabeling was observed in the fetal brain.

Staskal et al (2006b) examined the disposition in neonatal (postnatal day [PND] 10) and juvenile (PNDs 22, 28, and 40) C57BL/6 mice. In the first phase of this study, groups of six pups (three males and three females; one pup per litter) were orally administered 0 or 1 mg/kg BDE-47 dissolved in corn oil on PND 10. The pups were sacrificed at 3, 8, or 24 hours or 5 or 10 days after dosing. The tissue levels in the pups were compared with those from adult rats from the Staskal et al. (2005) study discussed above. The percent of dose/g brain tissue for the pups was significantly lower than in adults 3 hours after dosing and comparable to adults at 8 and 24 hours. At 5 and 10 days, the amounts in the pup brains were higher than in adult animals; the 10-day concentration in pups was higher than the 5-day value. The percent of dose/g adipose tissue peaked 24 hours after dosing and was greater in the pups than in the adults for all time points reaching statistical significance for the 8-hour, 24-hour, and 5-day time points. Levels in pup kidneys were statistically higher than in adults for all time points. Based on total body level of radiolabel, the retention of administered dose was significantly higher in pups than in adults at all time points.

The second phase of the Staskal et al. (2006b) study compared the tissue deposition of a 1 mg/kg BDE-47 dose 24 hours after dosing on PND 22, 28, or 40. Tissue levels declined as the age of the animal increased for the three time points measured. When the data for the 10-day time period were included in the comparison, the blood concentrations declined across all five time points and the 10-day levels were significantly higher than those for adults. Adipose tissue levels for the 10-day and 22-day animals were approximately equivalent and more than two times higher than the other time points. The authors hypothesized that the younger animals were less able to remove the BDE-47 and/or metabolites via a renal active transport system. Significantly higher concentration in the urine for the 40-day group and the adult animals

compared with the 22-day and 28-day groups provided support for this hypothesis. The 10-day pups were too immature for urine collection.

An *in vitro* study of BDE-47 uptake by cultured neurons and glia from neonatal Long-Evans rats was conducted by Mundy et al. (2004). The cells were exposed to 0.01 to 3.0 μM BDE-47 and incubated at 37° C for 60 minutes. The concentration of the BDE-47 in the culture's neuronal cells was 100-fold greater than the concentrations in the culture media. However, when the composition of the medium was altered with the addition of 10% horse serum, BDE-47 enrichment within the cells decreased but was still 20% above the background concentrations in the medium. The diminished uptake of the cells in the presence of serum proteins suggests probable binding to lipophilic sites on serum proteins. The uptake in the presence of serum proteins is likely to be more representative of *in vivo* conditions than uptake from buffered unsupplemented media. Uptake of BDE-47 into the neocortical cells appeared to be a diffusion controlled process driven by its lipophilicity. About 30% of the BDE-47 was not recovered and was believed to bind to the polystyrene cell culture dishes.

3.3. METABOLISM

The database on the metabolism of BDE-47 is incomplete and does not support anything other than a very rudimentary understanding of the metabolic pathway. Metabolites have been isolated from the urine and feces of both rats and mice but there are inconsistencies among studies. Although structures have been proposed for some of the isolated metabolites, structural confirmation is lacking in most cases. No human data are available.

The metabolites in excreta were analyzed by Orn and Klasson-Wehler (1998) in four adult male Sprague-Dawley rats or groups of four C57B1 male mice dosed orally with 30 $\mu\text{mol/kg}$ (approximately 15 mg/kg) of ^{14}C -labeled BDE-47. Feces and urine were collected daily until day 5 when the animals were sacrificed. Excreta were analyzed for ^{14}C -BDE-47 and metabolites. Excretion in rats was slow and amounted to less than 0.5% of the dose in urine and 14% in feces within 5 days. Approximately 3% of the administered dose was found in the feces as metabolites. Metabolites in the urine of rats were not analyzed. The mouse excreted considerably more ^{14}C , a total of 53% of the administered dose, with 33% of the dose being excreted in urine and 20% in feces by 5 days. Metabolites were also identified in the mouse feces at low concentrations; in the urine they accounted for 1% or less of the total. The mouse formed and excreted a major highly water-soluble and labile metabolite in urine that could not be identified.

The dominant compound found in the feces of rats and mice and in all tissues analyzed by Orn and Klasson-Wehler (1998) was the parent BDE-47. Metabolites covalently bound to macromolecules and lipids were noted in feces and tissues of both species. In addition, five

different hydroxylated metabolites of BDE-47, named M1–M5 after their elution order from the gas chromatography, were detected in small amounts in the feces and tissues of rats and mice. Plasma from rats and mice contained small amounts of three of these hydroxylated metabolites. Definite identification of the hydroxylated tetraBDEs could not be made because of the lack of reference compounds. Trace amounts of a possible thio-substituted BDE-47 metabolite were detected in the feces of rats and mice (Orn and Klasson-Wehler, 1998).

Marsh et al. (2006) determined the structures of the hydroxylated metabolites formed from BDE-47 in rat feces, tentatively identified as M1–M5 in the study of Orn and Klasson-Wehler (1998). Six hydroxylated tetraBDEs as well as three hydroxylated triBDEs were structurally identified. The OH-triBDEs could have formed metabolically or may have been due to debromination during sample storage prior to analysis.

Sanders et al. (2006) found that the radiolabel from BDE-47 found in rat urine was present primarily as metabolites but that in mouse urine the radiolabel was parent BDE-47 after both 1 dose and 10 consecutive doses. Metabolites were present in the feces for both species. The metabolites present in feces were increased after the 10 consecutive doses as compared to the single dose. In rats the fecal metabolites increased from 0 to $39 \pm 4\%$ of the radiolabel in the 24-hour collected fecal matter from animals receiving a single dose of BDE-47 compared with those collected 24 hours after the 10th consecutive dose. In mice, the metabolites increased from $8 \pm 8\%$ to $18 \pm 5\%$ of the radiolabel.

Based on analysis of bile collected from rats receiving $1 \mu\text{mol/kg}$ BDE-47 intravenously and exposed to γ -glutamyl transpeptidase, Sanders et al. (2006) proposed that two fecal metabolites isolated by high performance liquid chromatography (HPLC) and evaluated using positive ion electron spray ionization-mass spectrometry (ESI-MS) were glutathione conjugates formed through an arene oxide intermediate. They tentatively identified the metabolites as 5-(glutathion-S-yl)-2,2',4,4'-tetrabromodiphenyl ether and 6-(glutathion-S-yl)-2,2',4,4'-tetrabromodiphenyl ether. Two urinary metabolites from male rats treated with $1000 \mu\text{g/kg}$ were also isolated using HPLC and examined via ESI-MS. They were tentatively identified as glucuronide and sulfate conjugates of 2,4-dibromophenol.

Like Sanders et al. (2006) but unlike Orn and Klasson-Wehler (1998), Staskal et al. (2005) did not find BDE-47 metabolites in the urine of mice. Chromatography (HPLC) of the extracts from urine collected over 5 days following administration of single BDE-47 gavage doses of 0, 0.1, 1, 10, or 100 mg/kg identified only one peak, that for the parent compound. However, the results from a second study by Staskal et al. (2006c), where BDE 47 (1 mg/kg) was administered intravenously to female mice, provided different results. In this case, 40% of the radiolabel in the urine and 60% of that in the feces were metabolites. Extraction of the

metabolites from the feces, chromatographic separation, and mass spectroscopy identified three monohydroxylated metabolites.

Identification of PBDE metabolites in seals and salmon have suggested a tendency for hydroxylation to occur in the ortho position to the diphenyl ether bond (Marsh et al., 2004; Haglund et al., 1997).

Oxidation of many aromatic xenobiotic contaminants in the liver occurs through the catalytic action of the cytochrome P450 isozymes of the hepatic mixed function oxidase system. In a study by Hallgren et al. (2001), female C57BL/6N mice were administered BDE-47 (>98% purity) dissolved in corn oil once a day by gavage at 18 mg/kg/day for 14 days. Induction of hepatic microsomal phase I enzymes was measured as ethoxyresorufin *O*-deethylase (EROD), methoxyresorufin *O*-dealkylase (MROD), and pentoxyresorufin *O*-dealkylase (PROD) activities. EROD and MROD are markers for the induction of cytochrome P450 1A1 and P450 1A2 (CYP1A1/2) enzyme activity, while PROD is a marker of CYP2B enzyme activity. EROD and MROD activity were significantly increased, but PROD activity was not. The phase II enzyme uridine diphosphoglucuronosyl transferase (UDPGT) activity was not significantly induced. Although this enzyme was examined because of its role in glucuronidation of T₄, the fact that there was no induction of this enzyme suggests that the tetraBDE hydroxylated metabolites, if they are conjugated with glucuronic acid, do not require up-regulated expression of the enzyme. This is consistent with the negligible or minimal urinary excretion of metabolites.

Follow-up studies were conducted by Hallgren and Darnerud (2002) in female Sprague-Dawley rats (six/group) in order to examine the dose-response pattern of enzyme induction. Doses of 0, 1, 6, or 18 mg/kg-day BDE-47 (>98% pure) in corn oil were administered by gavage once a day for 14 days. EROD activity was significantly induced at 6 and 18 mg/kg-day, but not in a dose-dependent manner. MROD and PROD activity showed dose-dependent increases, statistically significant at 6 and 18 mg/kg-day. There was a moderate dose-dependent induction of the UDPGT activity, significant only at 18 mg/kg-day. The results from rats suggest greater involvement of CYP2B enzymes than in mice and a possibility for glucuronidation or sulfate conjugation. (As mentioned before, the authors included UDPGT in the analysis to determine if it was active in the modification of T₄ in the liver rather than as a marker for the conjugation of hydroxylated BDE-47 metabolites.)

In the study by Staskal et al. (2005), there was no induction of EROD or PROD at single doses up to and including 10 mg/kg when compared with the control. EROD was not induced by the 100 mg/kg dose, but there was a statistically significant (approximately threefold) induction of PROD activity with the 100 mg/kg dose. The results from Staskal et al. (2005) differ from those of Hallgren et al. (2001) and Hallgren and Darnerud (2002), however, their dosing over a

14-day period was more likely to have had an inducing influence on enzyme activity than a single dose of 10 mg/kg or less.

Kester et al. (2002) evaluated whether or not the human estrogen sulfotransferase and the human phenol sulfotransferase were able to conjugate sulfate from 3'-phosphoadenosine-5'-phosphosulfate to an hydroxylated tetraBDE (4-OH-3,2',4',6'-BDE) compared with hydroxylated tri- and pentaBDE congeners. The highest degree of sulfation was observed with the tetraBDE hydroxy congener for both enzymes. In the case of the estrogen sulfotransferase, sulfate conjugation of 43.1 % of the BDE was observed while the phenol sulfotransferase was able to conjugate sulfate with 30.4%. There is little evidence from the analysis of excreted metabolites that sulfate conjugation is a major metabolic process for BDE-47, the only tetraBDE congener with data on metabolites in animal studies. However, since hydroxylation appears to be a minor metabolic route, the opportunity for formation of conjugates is limited.

The available data on metabolism indicate that a considerable portion of BDE-47 is eliminated unchanged or distributed to storage compartments for periods that exceed the 5-day period evaluated by Orn and Klasson-Wehler (1998). There may be some minimal hydroxylation via CYP1A1/2 and CYP2B isozymes. Conjugations of the hydroxylated metabolites with glutathione are possible as indicated by the detection of a thiol metabolite in the feces. The metabolites in urine were not identified; however, Marsh et al. (2006) and Staskal et al. (2006c) have identified hydroxylated metabolites in the feces of rats and mice. Methylated metabolites have been identified in aquatic mammals and fish tissues but not in humans (Haglund et al., 1997). These metabolites were formed by methylation of hydroxylated metabolites.

3.4. ELIMINATION

In the study by Orn and Klasson-Wehler (1998), feces and urine were collected daily until day 5, when the male rats or mice were sacrificed. BDE-47 excreted on day 1 was assumed to represent nonabsorbed parent compounds and accounted for approximately 6% of the dose in rats and 8% in mice. Total excretion in male rats was slow, with less than 0.5% of the dose in urine and 14% in feces within 5 days. Approximately 3% of the administered dose corresponded to metabolites in the feces. The urine of rats was not analyzed for metabolites. Male mice excreted considerably more ¹⁴C—a total of 53% of the administered dose— with 33% of the dose being excreted in urine and 20% in feces by 5 days. Small amounts of five hydroxylated metabolites were also identified in the mouse feces, and there was a suggestion that there may have been a small amount of metabolite excreted in the urine. One percent of the label in the urine was found to be water soluble, while the remainder partitioned into the organic extraction solvent. Fecal excretion of metabolites and the presence of a sulfur containing metabolite in the

mouse feces suggest that a portion of the excretion may occur through the biliary route. This hypothesis received support from the autoradiography data of Darnerud and Risberg (2006) which showed radiolabeling of the bile and intestinal contents following i.v. injection of 10 mg/kg of ¹⁴C-BDE-47 to male and female C57BL mice. The data by Sanders et al. (2006) confirmed the presence of metabolites in bile collected from cannulated F344 rats in the 6-hour period after intravenous injection of 1 μmol/kg BDE-47. Radiolabel in the bile accounted for 2.8 ± 0.6% of the injected dose over the 6-hour collection period.

The differences in urinary excretion of BDE-47 were also seen in the studies of Sanders et al. (2006). Only a trace (<1%) of the oral 1 μmol/kg dose of ¹⁴C-BDE-47 was present in the urine of F344 rats. B6C3F1 mice excreted about 100-fold more of the dose in 24 hour cumulative urine than did rats (~2% in female and ~3% in males). Radiolabel in mice appeared to be parent BDE-47, while that in rats was mostly or totally BDE-47 metabolites. The percent total dose in 24-hour collected feces was similar for both species and sexes. When male mice and rats were given 10 consecutive doses of 1 μmol/kg dose of ¹⁴C-BDE-47, 0.9 ± 0.2 was collected in urine over the 24-hour period after the last dose in rats and 57 ± 3% in mice.

The cumulative concentrations excreted in the urine over 5 days accounted for about 40% of the 0.1 mg/kg and 1.0 mg/kg oral doses, about 30% of the 10 mg/kg dose, and only about 10% of the 100 mg/kg dose in the study by Staskal et al. (2005). Cumulative fecal excretion for the oral doses increased from 30% for the 0.1 mg/kg dose to 50% for the 100 mg/kg dose. There was some evidence that urinary excretion was mediated by an active transport process. Staskal et al. (2006b) measured the presence of radiolabel in urine in groups of 10 mice (5 male and 5 female drawn randomly from eight litters) exposed to doses of 1 mg/kg BDE-47 on PND 22, 28, or 40 to see if there was a change in the amount of urinary radiolabel that would support the hypothesis that renal active transport plays a role in excretion via the kidneys in mice. The time points chosen were believed to cover the period of renal transporter development. The concentration of BDE-47 in the 24-hour collected urine from the mice exposed on PND 22 and PND 28 was significantly lower than that for the adults and those exposed on PND 40; the concentration in the mice exposed on PND 40 was lower than that in adult mice, but the difference was not significant.

Staskal et al. (2005) also evaluated the impact of exposure route on BDE-47 excretion. Five days after dosing with 1 mg/kg, cumulative concentration in the urine was lowest (20%) after dermal exposure; 30% for the intratracheal, intraperitoneal, and oral routes; and about 42% for the intravenous route. Cumulative fecal excretion over 5 days was highest for the oral route (~35%) and lowest for the intravenous and intraperitoneal routes (~15%). The fecal excretion for the dermal exposure was initially very low, but by the end of 5 days it was equivalent to the intratracheal exposure (~25%).

Urinary excretion of BDE-47 appears to involve binding to major urinary protein (MUP) in both male and female mice (Staskal et al., 2006c). These proteins are synthesized in the liver, secreted into serum, and eliminated in urine. Male mice secrete more protein than females. Analysis of pooled urine samples from BDE-47 intravenously dosed female mice indicated that 98.5% was protein bound to a MUP. The binding isoform was identified as MUP-1.

Elimination half-lives of individual tetra, penta, and hexa components of commercial pentaBDE (Bromkal 70) was investigated in groups of male and female Wistar rats given a single oral dose of 300 mg/kg of Bromkal 70 dissolved in peanut oil. Groups of three animals of either sex were sacrificed on days 1, 2, 3, 4, or 7 and then once a week for 10 weeks. Perirenal fat was collected and analyzed. The half-life of a tetraBDE, presumably BDE-47, was 30 days for female rats and 19 days for male rats. The difference in half-lives between sexes was significant. It is unclear why male rats are faster eliminators of BDE-47 than female rats. The half-lives of two unspecified pentaBDE congeners were 25 and 47 days for female rats, and 25 and 37 days for male rats. Half-life of an unspecified hexaBDE congener was 91 days in female rats and 119 days in male rats. The difference in half-lives between sexes was not significant for both penta- and hexaBDEs. There seems to be an increasing trend in half-lives with increasing degree of bromination. However, as the authors indicated, the metabolic rate and therefore the elimination of PBDE may be affected by the high dose given to the animals, and half-lives of PBDEs as determined in this study may not be representative of cases where the exposure doses are lower (von Meyerinck et al., 1990). The extended half-life of the PBDEs in adipose tissue suggests a need to examine metabolite excretion for a postexposure period longer than the five days examined by Orn and Klasson-Wehler (1998).

The half-life of BDE-47 in C57BL/6J mice after a single oral exposure was biphasic (Staskal et al., 2005). The initial whole body half-life after a single oral dose of 1 mg/kg was 1.5 days and accounted for elimination of 67% of the dose. The half-life for the second elimination phase was approximately 23 days. The biphasic elimination pattern supports the hypothesis that BDE-47 has the potential to bioaccumulate in lipophilic tissues.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Limited information is available on the absorption, distribution, metabolism, and excretion of BDE-47 in experimental animals and in humans. In addition, qualitative and quantitative differences in metabolism in rats and mice have been observed (Orn and Klasson-Wehler, 1998). A model for human metabolism has not been established. Extrapolation of results from laboratory animals to humans by using physiologically based toxicokinetic models is not possible at this time.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

In the study of PBDE levels in breast adipose tissue of 23 California women, described in Section 3.2.1 (She et al., 2002), there was no correlation between disease status (malignancies, benign tumors, or ductal carcinomas in situ) and total PBDE concentration in breast adipose tissues.

The possible relationship between high dietary exposure to persistent organohalogen compounds through consumption of fatty fish from the Baltic Sea and selected hormone levels in adult men was investigated (Hagmar et al., 2001). Blood samples were drawn from 110 men, aged 23 to 79 years and consuming varying amounts of fish (0 to 32 meals per month), for analysis of plasma levels of BDE-47 and several other organohalogen compounds (PCBs, hydroxy-PCBs, p,p'-DDT, p,p'-DDE and hexachlorobenzene). Plasma levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, thyrotropin or thyroid stimulating hormone (TSH), free triiodothyronine (FT₃), free thyroxine (FT₄), total T₃ and T₄, and total testosterone were analyzed. Median, 90th and 10th percentile plasma levels of BDE-47 for the 110 men were 1.0, 5.2, and 0.1 ng/g lw, respectively, indicating substantial interindividual variations in plasma levels. Plasma levels of BDE-47, as well as for the other organohalogen compounds studied, were highly correlated with the estimated fish consumption. After age adjustment, there was a weak negative correlation between plasma levels of TSH and BDE-47. There was no correlation between levels in plasma of BDE-47 and plasma levels of LH, prolactin, FT₃, FT₄, and total T₃ or T₄.

Adipose tissue levels of BDE-47 were measured in 42 male or female cancer patients (19 with non-Hodgkin's lymphoma [NHL] and 23 with malignant melanoma [MM]) and in 27 controls without a diagnosis of cancer. The mean concentration of BDE-47 was 5.1 ng/g lw for the 27 controls, 13.0 ng/g lw for the NHL patients, and 4.8 ng/g lw for the MM subgroup. The authors recognized that, due to the small size of the study groups, the correlation between levels of BDE-47 in adipose tissue and NHL should be regarded as hypothesis-generating and further studies are needed (Hardell et al., 1998).

To assess whether PBDEs may be detrimental to neurodevelopment, Mazdai et al. (2003) determined concentrations of PBDEs and total and free serum T₄ and T₃ in human fetal and maternal sera. Twelve paired maternal and cord blood samples were obtained from women 18 to 37 years old, presenting in labor at an Indiana hospital. The PBDE congeners and their concentrations measured in fetal and maternal serum samples are given in Table 3. There was no

relationship between infant birth weight and PBDE concentrations. No birth defects were documented. Thyroid hormones were assayed in 9 of the 12 sample pairs. There was no correlation between total PBDEs and T₃ or T₄ concentrations (total or free). The authors cautioned that the sample size may have been too small to detect an association between serum concentrations of PBDEs and thyroid hormone levels.

In the study of Julander et al., 2005 (see also Section 3.2.1.4), no correlation was found between PBDE levels in plasma of Swedish workers involved in an electronic recycling facility and changes in thyroid hormone levels (T₃, T₄, TSH). As in the Mazdai et al. (2003) study, the study population was small (11 workers), and, in addition, levels of individual PBDE congeners in plasma in the exposed population may have been too low, about 10-fold smaller than in the Mazdai study, to detect any association.

In summary, the available limited human studies do not permit any conclusions to be made concerning a possible association between exposure to PBDEs or BDE-47 and adverse health outcome in humans.

4.2. LESS-THAN-LIFETIME AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

Inhalation toxicological studies of BDE-47 in experimental animals are not available.

4.2.1. Acute, Short-term, and Subchronic Studies

4.2.1.1. Mice

The ability of BDE-47 to alter thyroid hormone and vitamin A levels as well as microsomal enzyme activities in mice was compared with that of the commercial pentaBDE Bromkal and PCBs (Hallgren et al., 2001). Groups of female C57BL/6 mice were administered BDE-47 (>98% purity) dissolved in corn oil, once a day by gavage at 0 (n = 12) or 18 mg/kg-day (n = 8) for 14 days. The animals were evaluated for body-weight gain during the exposure period. Blood samples were collected prior to sacrifice and analyzed for TSH, and total and free T₄. The terminal weights of liver, thymus, and spleen were recorded. In addition, the activities of Phase I and Phase II enzymes in the liver tissues were assayed as were the levels of extractable vitamin A (retinol and retinyl esters). Body-weight gains were not affected and no overt signs of toxic effects were seen in the study. The liver somatic index (liver weight/body weight) was significantly increased from control, but no statistically significant differences were found in thymus or spleen somatic indices. BDE-47 significantly decreased plasma free and total T₄ levels, the effects being more pronounced for free T₄, believed to be the most direct indicator of thyroid status. In contrast to T₄ levels, plasma TSH was not significantly changed, which could be due to the short-term nature of the study. The decrease in concentration of T₄

was highest for PCBs, followed by BDE-47 and commercial pentaBDE Bromkal. Hepatic vitamin A levels were not significantly changed, which could be due to the short-term nature of the study. Vitamin A was included in the assessment because it is transported by the same protein complex as T₄.

Induction of microsomal phase I enzymes was measured as EROD, MROD, and PROD activities in the study by Hallgren et al. (2001) (see also Section 3.3). EROD and MROD activity were greatest after exposure to PCBs, followed by Bromkal, but were also significantly increased in the BDE-47 treated group; PROD activity was significantly induced by PCBs but not by BDE-47 or Bromkal treatment. The phase II enzyme UDPGT activity that glucuronidates T₄ for excretion in bile was not significantly induced by any of the compounds tested.

4.2.1.2. Rats

The effects of BDE-47 on thyroid hormone levels were examined in rats (Hallgren and Darnerud, 2002). Female Sprague-Dawley rats (six/group) were administered by gavage doses of 0, 1, 6, or 18 mg/kg-day BDE-47 (>98% pure) in corn oil, once a day for 14 days. Plasma total and free T₄ and TSH were measured at the end of the study. In order to test possible mechanisms for the alterations of thyroid hormones, the induction of UDPGT activity, morphological effects on the thyroid epithelia, and ex vivo binding of ¹²⁵I-thyroxine to the plasma thyroid hormone transporter transthyretin (TTR) were studied. In addition, microsomal phase I enzyme activities were also assayed (EROD, MROD, and PROD). Induction of these enzymes would suggest metabolic transformation of BDE-47, and this could affect the levels of circulating T₄, as the produced metabolites may have effects on T₄ homeostasis by replacing T₄ at TTR binding sites (Hallgren and Darnerud, 2002). No signs of clinical toxicity were seen in the study, and liver thyroid somatic indices and body-weight gains were unaffected. No effects were seen on thyroid morphology at any dose. Plasma levels of FT₄ showed a decreasing trend that was significant only at 18 mg/kg-day (61% of control). Plasma levels of total T₄ showed the same pattern of reduction as the free hormone, but the effects were less pronounced and not significant at any dose.

In contrast to T₄ levels, plasma levels of TSH were not changed at any dose. The ex vivo binding of ¹²⁵I-T₄ to TTR was significantly reduced at 18 mg/kg-day. EROD activity was significantly induced at 6 and 18 mg/kg-day, but not in a dose-dependent manner. MROD and PROD activity showed dose-dependent increases, statistically significant at 6 and 18 mg/kg-day. Treatment with BDE-47 resulted in a moderate dose-dependent induction of the UDPGT activity, significant only at 18 mg/kg-day but the increase in UDPGT activity did not correlate well with the decrease in T₄ levels.

As a possible mechanism behind the thyroid hormone effects, the authors noted that the observed degree of thyroid hormone reduction after BDE-47 exposure coincided with a decrease

in the ex vivo binding of $^{125}\text{I-T}_4$ to the plasma thyroid hormone transport protein TTR and with induction of the microsomal phase I enzymes EROD, MROD, and PROD. These observed effects match the hypothesis that the T_4 decrease is chiefly due to disturbances in serum transport, caused by binding of in vivo formed BDE-47 metabolites to TTR. It is hypothesized that the lack of response on serum TSH levels to the reduction in T_4 levels is due to BDE-47 and/or its metabolites mimicking thyroid hormones and possibly binding to thyroid hormone receptors in the pituitary, thereby blocking TSH release (Hallgren and Darnerud, 2002). Free T_4 was found to be the most sensitive indicator of imbalance in thyroid hormone status in this study.

4.2.2. Chronic Studies

Chronic toxicity/carcinogenicity studies of BDE-47 are not available.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

Reproductive toxicity studies are not available for BDE-47.

Eriksson et al. (2001) conducted a neurobehavioral study in adult male mice following neonatal exposure to BDE-47. Single doses of 0, 0.7, or 10.5 mg/kg of BDE-47 (>98% purity) in a 20% fat emulsion (1:10 egg lecithin to peanut oil) were administered by gavage to male NMRI mice on PND 10, a period of rapid brain growth and increased susceptibility in neonatal mice.¹ Male mice serving as controls received 10 mL/kg of the 20% fat emulsion. Spontaneous motor behavior was tested at ages 2 and 4 months in groups of eight male mice, randomly selected from three to four different litters, and the mice were tested once only. Spontaneous motor behavior was measured for a 60-minute period, divided into three 20-minute periods, at both doses. Spontaneous motor behavior tests used measured locomotion (horizontal movement), rearing (vertical movement), and total activity (all types of vibration within the test cage [i.e., those caused by mouse movement, shaking/tremors, and grooming]). In order to study time-dependent changes in habituation (2-month-old vs 4-month-old mice), data from the spontaneous motor behavior tests were used. A habituation ratio, was calculated between the performance periods 40–60 minutes and 0–20 minutes for each of the three different variables: locomotion, rearing, and total activity. An habituation ratio was used to analyze alteration in habituation of 2-month-old and 4-month-old treated mice, within each treatment group, in comparison with their respective controls. Swim maze performance, a measure of learning and

¹ Eriksson et al. (2002) investigated whether behavioral disturbances observed in adult mice following neonatal exposure to pentaBDE-99 are induced during a defined neonatal brain developmental window of unique biological susceptibility. Mice were exposed to 8 mg/kg BDE-99 on PND 3, 10, or 19. Adult mice exposed on PND 10 showed more pronounced effects on spontaneous motor behavior than mice exposed on PND 3. No changes in spontaneous motor behavior were seen in adult mice exposed on PND 19.

memory ability, was tested in groups of 16–18 mice, randomly selected from three to four different litters, at age 5 months given the high dose of BDE-47 (10.5 mg/kg). There were no clinical signs of dysfunction in the treated mice throughout the experimental period nor were there any significant deviations in body-weight gain in the BDE-47 treated mice compared with the vehicle-treated mice.

Control mice showed habituation (i.e. a decrease in locomotion, rearing and total activity) in response to the diminishing novelty of the test chamber over the three 20-minute test periods. For all three spontaneous motor behavior variables (locomotion, rearing, and total activity), 2-month-old mice receiving 10.5 mg/kg BDE-47 displayed significantly less activity than controls during the first 20-minute period (hypoactivity) but were significantly more active than controls during the third 20-minute period (hyperactivity). The aberrations in spontaneous motor behavior were more pronounced in 4-month-old mice than in 2-month-old mice, indicating worsening with increasing age. In mice given 10.5 mg/kg BDE-47, the habituation capability was significantly reduced in 4-month-old mice compared with 2-month-old mice for all three variables (locomotion, rearing, and total activity). Performance of 5-month-old mice in the swim maze learning/memory test was not affected at any dose. The no-observed-adverse-effect level (NOAEL) in this study was 0.7 mg/kg and the lowest-observed-adverse-effect level (LOAEL) was 10.5 mg/kg for changes in spontaneous motor behavior and decreased habituation capability in adult male mice, worsening with increasing age.

4.4. OTHER STUDIES

4.4.1. Receptor Site Interactions

There is considerable evidence from studies of PCBs, chlorinated dibenzo-*p*-dioxins (CDDs) and chlorinated dibenzofurans (CDFs) that halogenated aromatic compounds exert an influence on cells by interacting with membrane receptor sites and activating cellular transcription factors. Transcription factor complexes then initiate DNA synthesis, allowing the cell to respond to the extracellular signal by producing a series of messenger RNAs (mRNAs) that in turn produce a variety of proteins. This process is termed signal transduction. The structural similarities between PBDEs and PCBs suggest that PBDEs might activate both the aryl hydrocarbon (Ah) receptor site and the estrogen receptor (ER) site. Based on the data from the well-studied PCBs, CDDs, and CDFs, the activation of these receptor sites is associated with immunotoxicity, reproductive effects, and carcinogenesis (Klaassen, 1996, pp. 47-49, 373-376) at all endpoints of interest for PBDEs.

4.4.1.1. Aryl Hydrocarbon Receptors

The transcription of the genes for CYP1A1, 1A2, and 1B1 is linked to a signal transduction cascade that is initiated by activation of the Ah receptor by an appropriate ligand.

The CYP1 family of enzymes is highly conserved in mammals and is responsible for the oxidative metabolism of a variety of planar and near-planar compounds (Lewis et al., 1998). The CYP1 family of enzymes metabolically activates and metabolizes polycyclic aromatic hydrocarbons and aromatic amines as well as PBDEs. Many substrates for the CYP1 family enzymes are also Ah receptor ligands. Differences in Ah receptor affinity are correlated to variations in CYP1 inducibility. Receptor site affinity has been shown to reflect potency and the potential for a xenobiotic to cause adverse health effects.

Chen et al. (2001) studied the affinity of several PBDE congeners for rat hepatic Ah receptor through competitive binding assays and determined their ability to induce hepatic CYP450 enzymes by means of EROD assays (a biomarker for CYP1A1/2 induction) in chick and rat hepatocytes, in liver cell lines from rainbow trout, and in rat and human tumor-cell lines. TetraBDE congeners (BDE-47, -49, -66, -71, -75, and -77) had Ah receptor binding affinities approximately 10^{-3} to 10^{-5} that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and none of the receptor/tetraBDE complexes was found to bind with an oligonucleotide containing the nuclear dioxin response element (the segment of DNA that is activated by dioxin-stimulated transcription factors). The binding of the tetraBDE did not seem to be influenced by whether or not the phenyl rings were coplanar. The authors hypothesized that the large atomic volume of bromine distorts the Ah binding site so that the coplanarity of the rings is less important in Ah binding than it is for the PCBs.

Quantitative measures of EROD induction were reported for tetraBDE-47, -66 and -77. EROD induction was strongest in all cell lines for BDE-77, although its relative induction potency in the different cell cultures was approximately 10^{-3} to 10^{-4} that of TCDD. Induction of EROD in the rodent cell lines was slightly lower than that for the human cell line. BDE-66 was a very weak inducer in rat hepatocytes and inactive in the other cell lines. BDE-47 did not act as an EROD inducer in any cell line. These structurally related tetraBDEs congeners were therefore found to have differing responses in the in vitro test systems studied, and all were considerably less potent than TCDD, a strong Ah activator (Chen et al., 2001).

Peters et al. (2006) examined the interaction of tetraBDE-47 and -77 as well as other PBDEs on the Ah receptor in cultured liver cells from four healthy cynomolgus monkeys (three males; one female), using EROD activation as a biomarker for receptor activation. Both compounds were weak Ah agonists when co-exposures of TCDD and PBDEs were tested, as evidenced by a decrease in the activation caused by TCDD alone. The impact of the PBDEs was receptor localized rather than through inhibition of the enzyme since no EROD inhibition occurred if TCDD exposure preceded the PBDE exposure. Environmentally relevant concentrations of PBDEs (1 to 10 μ M) were evaluated. There was variability in the response of

the four monkeys, likely reflecting individual differences in the animals. Planar BDE-77 was a stronger agonist than nonplanar BDE-47.

Using hepatocyte cultures from Sprague-Dawley rats, Chen and Bunce (2003) investigated whether PBDE congeners, including tetraBDEs, act as Ah receptor agonists or antagonists at sequential stages of the Ah receptor signal transduction pathway leading to CYP1A1. These issues are environmentally relevant because of the strong rank-order correlation among strength of Ah receptor binding, CYP1A induction, and toxicity for many halogenated aromatic compounds.

There were four components to this study (Chen and Bunce, 2003): (1) the binding of the PBDE congener to the Ah receptor, (2) the binding of the receptor/PBDE complex to an oligonucleotide segment of the dioxin response element, (3) the induction of EROD, and (4) the production of CYP1A mRNA and CYP1A protein. The tetraBDE congeners evaluated in the study were BDE-47, -49, -71, -75, and -77.

TetraBDE-77 was the most active of this group when compared to TCDD. It was moderately active in dioxin response element binding and induced responses of both CYP1A1 mRNA and CYP1A1 protein equivalent to the maximal response of TCDD in primary Sprague-Dawley rat hepatocytes, although at concentrations three to five orders of magnitude greater than TCDD. When tested in combination with TCDD, BDE-77 tended to enhance the activity of a nonsaturating concentration of TCDD and slightly inhibit a saturating TCDD concentration.

The environmentally prominent congener BDE-47 was inactive at all stages of signal transduction, suggesting that current concentrations of BDE-47 in biota contribute negligibly to dioxin-like toxicity compared with other environmental contaminants, such as PCBs and TCDD. BDE-47 did not have an additive relationship with a nonsaturating TCDD concentration and acted as an antagonist in combination with a saturating TCDD concentration.

TetraBDE congeners BDE-49, -71, and -75 showed consistently low activity while the tetraBDE congener BDE-66 had moderate activity in this study. The authors concluded that the tetraBDEs contribute minimally to the Ah-mediated toxicity of halogenated aromatic hydrocarbons at the present time but cautioned that this may change as the concentrations of PCBs decline and those for the brominated compounds increase.

Somewhat different results from those observed by Chen and Bunce (2003) were found for tetraBDE-77, using a different assay. Villeneuve et al. (2002) used H4IIE-luc (luciferase) recombinant rat hepatoma cells. The cells were grown in culture well-plates and then exposed to concentrations of 2 to 500 ng/mL BDE-47 or BDE-77. Luminescence was measured and compared to the maximum response observed with a 1500 picomolar TCDD standard (%TCDD max). A positive response was defined as any response that was greater than three standard deviations above the mean value for the control. The lack of a response for tetraBDE-47 and -77

was interpreted as a lack of activation of the Ah receptor. The BDE-47 results are consistent with those of Chen and Bunce (2003). The Chen and Bunce (2003) results for BDE-77 differed in that they were suggestive of moderate binding and activation of the Ah receptor. The difference in results may suggest that the Ah receptors in H4IIE-luc cells are less sensitive measures of Ah binding to tetraBDEs than the cell lines used by Chen and Bunce (2003).

Sanders et al. (2005) used an in vivo approach to study Ah receptor site activation by several PBDE congeners, including BDE-47 and tetraBDE-80, a tetraBDE congener with a greater potential than BDE-47 to achieve a planar conformation. Groups of F344 male rats (three/group), 10–12 weeks old, were dosed by gavage once daily for three days with BDE-47 (99% pure) in corn oil at 0, 1, 10, or 100 $\mu\text{mol/kg-day}$ or 10 $\mu\text{mol/kg-day}$ of BDE-80 (>98% pure). The animals were sacrificed 24 hours after receiving the last dose. The liver was removed and RNA from a 100 g liver sample was isolated, converted to its complementary DNA, and amplified by using the polymerase chain reaction. The resultant DNA samples were then analyzed to determine the expression of the CYP4501A1, a protein linked to Ah receptor activation.

BDE-47 had a significant effect on the level of CYP1A1 (2.4 times the vehicle-treated controls) only at 100 $\mu\text{mol/kg-day}$ (49 mg/kg-day) making it a weak activator of the Ah receptor. BDE-80 had only a weak effect on 1A1 expression despite its more planar conformation. When the 1A1 expression from BDE-47 was compared to that for pentaBDE-99 and hexaBDE-153, the impact on the Ah receptor seemed to be correlated to the levels of polybrominated dibenzofurans in each congener, which in turn correlated with increased bromine content of the congeners.

The results from this study confirm in vitro data, suggesting that tetraBDEs are, at best, weak activators of the Ah receptor. These results also raise the possibility that brominated dibenzofuran impurities identified in the congeners studied may, in some cases, have confounded the results from other studies.

4.4.1.2. Estrogen Receptors

Studies have also been conducted to evaluate the interaction between PBDEs and the estrogen receptor sites. Activation of estrogen receptors induces cell division in female reproductive organs, mammary glands, and liver. Receptor-induced mitogenic activity has been linked to tumor formation in the affected organs (Klaassen, 1996, p. 48).

The in vitro estrogenic and antiestrogenic potencies of seventeen PBDEs including five tetraBDEs (BDE-47, -51, -71, -75, and -77) and three hydroxylated PBDEs (HO-PBDEs), were investigated in a human T47-breast-cancer cell line based on ER-dependent luciferase reporter gene expression. The modified T47 D cells that contained ER α and ER β receptors were trypsinized and seeded in 96 well plates for the ER-CALUX (Chemical Activated Luciferase

gene Expression) assay. After allowing for cell growth, the wells were exposed to solutions containing the test compounds or estradiol and were incubated. The luciferase activity was measured with a luminometer. BDE-51, -71, and -75 showed estrogenic potencies in the assay with concentrations leading to 50% induction (median effective concentration [EC₅₀]) of 3.1, 7.3, and 2.9 μM, respectively, in comparison to the EC₅₀ value of 1.0 × 10⁻⁵ μM for estradiol. These tetraBDEs were thus 300,000 to 700,000 less potent than estradiol. TetraBDE-47 and -77 did not show any estrogenic activity in the ER-CALUX assay (Meerts et al., 2001).

Several hydroxylated derivatives of PBDEs were also evaluated in the CALUX assay described above (Meerts et al., 2001). 2,4,6,5'-Tetrabromo-4'-hydroxyBDE [or 2-bromo-4-(2,4,6-tribromophenoxy)phenol], a T₃-like hydroxylated-BDE, had a maximum luciferase induction nearly the same as that of estradiol but at concentrations 100,000 times higher. Antiestrogenic potency was determined in the ER-CALUX assay by treating T47D.Luc cells with various concentrations of PBDEs in the presence of estradiol. The five tetraBDEs (BDE-47, -51, -71, -75, -77) and the T₃-like hydroxylated-BDE compound did not show antiestrogenic activity.

Villeneuve et al. (2002) examined the ability of 10 different PBDEs, including tetraBDE-47 and -77 (99% purity), to initiate ER-mediated gene expression in vitro. At concentrations up to 500 ng/mL, BDE-47 and BDE-77 failed to induce ER-mediated gene expression in MVLN recombinant human breast carcinoma cells, using a luciferase response element for detection. Overall, the PBDEs tested were found to be 50,000 times less potent than estradiol for inducing ER-mediated gene expression.

Villeneuve et al. (2002) also studied the ability of PBDEs to displace steroid hormones from serum proteins. At concentrations up to 833 ng/mL, the PBDEs tested in this study did not show an appreciable capacity for displacing ³H-steroids from carp serum proteins that had been stripped of hormones before testing. Unlabeled estradiol and testosterone also had a limited effect on displacing the radiolabeled ligands, suggesting limited sensitivity of assay with carp serum.

Segura-Aguilar et al. (1997) examined a different aspect of the impact of organohalogen compounds on estrogenic hormones. Rather than studying the ability of PBDEs to bind to the estrogen receptor site, they examined the impact of BDE-47 on induction of the enzymes responsible for the oxidation of estradiol at the 2- and 4-ring positions. It has been suggested that the carcinogenic properties of estradiol are related to its hydroxylation at these sites, resulting in the formation of a catecholestradiol, which can be oxidized to an *o*-quinone derivative. The effect of BDE-47 on the induction of 2- and 4-hydroxylation of estradiol was studied in male and female rat liver microsomes. A significant 2.5-fold increase in the enzymatic activity of the enzymes that catalyze 4-hydroxylation of estradiol was found in liver microsomes of BDE-47-treated male rats, while this activity was nearly unchanged in female rat

liver microsomes. Hydroxylation of estradiol at the 2-position was also found to be increased in male rat liver microsomes (1.6-fold above that of controls) while this activity was decreased in female rat liver microsomes. The authors suggested that the increase in 4-hydroxylation of estradiol activity in male rat liver microsomes treated with BDE-47 may be considered a risk factor in the development of estradiol-dependent tumors in men. The CYP450 form that catalyzes 4-hydroxylation has not been identified; aromatase (CYP19) has been proposed as the enzyme responsible for the 2-hydroxylation of estradiol.

A third aspect of the possible impact of PBDEs on estrogen (estradiol) was investigated by Kester et al. (2002). In this instance, the authors studied the effect of hydroxylated PBDEs on the activity of the human sulfotransferases that metabolically inactivate estrogen. Inhibition of the sulfotransferases would increase the half-life of estradiol and facilitate increased opportunities for receptor site stimulation. In this study, the human sulfotransferase that is active in liver, endometrium, mammary gland, and testes was incubated with varying concentrations of 4-hydroxyPBDE congeners. Tri-, tetra-, and pentaBDE hydroxy congeners were evaluated using concentrations of up to 1000 nM. All three compounds tested acted as inhibitors of the enzyme. The tetraBDE hydroxy congener (4-OH-2',3,4',6'-BDE) was the least effective of the three tested compounds. The 1000 nM concentration reduced the enzyme activity by about 40%. The most active congener was the pentaBDE hydroxy congener, which caused approximately 90% inhibition at the highest concentration. A Lineweaver-Burk analysis of the penta-compound data suggests that the competition was noncompetitive (i.e., the interaction with the enzyme did not involve the active site).

In summary, the mechanistic studies of the ER and Ah receptor indicate that the activities of the tetraBDEs are much lower than the activities of dioxin and PCBs. TetraBDE-77 appears to be the most active with the Ah receptor, and most PBDEs appear to be weak antagonists for the Ah receptor rather than agonists. Receptor-site mediated activity via the ER site appears to be minimal for the tetraBDEs.

4.4.1.3. Androgen Receptors

DE-71, a commercial pentaPBDE mixture, was found by Stoker et al. (2004) to delay puberty and suppress the growth of androgen-dependant tissues in male Wistar rats exposed to doses of 30 or 60 mg/kg during the peri-pubertal period but not to doses of 0 or 3 mg/kg. In order to examine which components of the mixture might be responsible for the observed effects, androgen receptor binding by several of the individual congeners found in DE-71 was examined in vitro (Stoker et al., 2005). The assays of the individual congeners examined competitive binding of BDE-47 (98% pure) in the presence of a tritium-labeled androgen agonist (R1881) by using ventral prostate cytosolic extracts along with an assay in an MDA-kb2 cell line containing the human androgen receptor and a transfected luciferase reporter element.

In the assay with the ventral prostate extract, 0.001, 1.6, 3.3, 16.7, or 33 μM concentrations of BDE-47 were incubated in the presence of 1.0 nM R1881 and 10 μM of an agent to block the progesterone and glucocorticoid receptors. Under these conditions, BDE-47 was shown to be a competitive inhibitor for the binding of R1881. The approximate median inhibitory concentration (IC_{50}) for BDE-47 was 16.7 μM .

In the assay using the MDA-kb2 cell line, BDE-47 was introduced at concentrations of 10 pM, 10 nM, 1 μM , or 5 μM in the presence of 0.1 nM of the receptor agonist dihydrotestosterone (DHT). BDE-47 demonstrated a concentration-dependant antiandrogenic activity in this assay with a 50% decrease in DHT activity at the 5 μM concentration. BDE-99 was inactive in the MDA-kb2 assay.

4.4.1.4. Other Receptors

The study of CYP450 mRNA expression in rat liver by Sanders et al. (2005) (Section 4.4.1.1) found that expression of CYP2B and CYP3A was up-regulated by BDE-47 in F344 rats to a greater extent than that of CYP1A1, a biomarker for the activation of the Ah receptor. CYP2B and CYP3A are respective biomarkers for activation of the constitutive androstane receptor (CAR) and pregnane X receptor (PXR). The authors concluded that these results indicated that BDE-47 activated the CAR and PXR to a greater extent than the Ah receptor. In the case of BDE-47, the effect on CAR was greater than that on PXR. The CAR and PXR receptors are classified as orphan receptors. They are both involved in the metabolism of xenobiotics and are stimulated by phenobarbital. The CAR receptor is also involved with steroid metabolism. The impact of BDE-47 on these receptors is similar to the impact of noncoplanar PCBs on the same receptors, however, the implication of activation of CAR and PXR are not well known.

4.4.2. Thyroid Effects

Because PBDEs and particularly BDE-47 have some structural similarity to the thyroid hormone T_4 , it has been suggested that they may interfere with thyroid hormone transport by competitively binding with TTR, one of the thyroid hormone-binding transport proteins in plasma of vertebrate species. The possible interference of several tetraBDEs with T_4 -TTR binding was investigated in an in vitro competitive binding assay, using human TTR and ^{125}I -labeled T_4 as the displaceable radioligand. The five tetraBDE congeners evaluated (BDE-47, -51, -71, -75, and -77) did not compete with T_4 -TTR binding (Meerts et al., 2000).

Meerts et al. (2000) also tested these five tetraBDEs, before and after incubation with differently induced hepatic microsomes to examine the ability of their hydroxylated metabolites to displace thyroxine from TTR. The tetraBDEs were individually incubated with liver

microsomes prepared in the presence of phenobarbital (a CYP2B inducer), β -naphthoflavone (a CYP1A inducer), or clofibrate (a CYP4A3 inducer). Incubation of the tetraBDEs with CYP2B-enriched rat liver microsomes resulted in the formation of metabolites that were able to displace $^{125}\text{I-T}_4$ from TTR. The metabolites of the tetraBDEs-47, -51, -75, and -77 were able to displace more than 60% of the $^{125}\text{I-T}_4$ from TTR. Only tetraBDE-71 showed a lower ability to displace $^{125}\text{I-T}_4$ from TTR (20-60%). T_4 -TTR displacement by tetraBDEs after incubation with liver microsomes enriched with CYP1A or CYP4A3 was much lower. BDE-47 was inactive after treatment with both the CYP1A and CYP4A enriched microsomes and tetraBDE-51 was inactive after the treatment with the CYP1A enriched microsomes. TetraBDEs are therefore able to compete with T_4 -TTR binding only after metabolic conversion by induced rat liver microsomes, suggesting an important role for hydroxylation. The relevance of this observation for humans has yet to be resolved. Thyroxine-binding globulin, rather than TTR, is the major thyroxine-binding protein in humans.

4.4.3. Immunotoxicity

The immunotoxic potential of BDE-47 was assessed in mice (Thuvander and Darnerud, 1999). Groups of C57BL/6 female mice were given BDE-47 (>98% purity) by gavage in corn oil at 0 or 18 mg/kg-day for 14 days. The number of mice in the control and treated groups were 12 and 8, respectively. No signs of clinical toxicity or difference in body weights due to BDE-47 treatment were observed. Liver weights were significantly increased in comparison with control animals, indicating induction of hepatic enzymes. There was a tendency towards lower spleen and thymus weights, but the changes were not statistically significant. A pronounced and statistically significant decrease in the number of splenocytes (by approximately 25%) was observed. The decrease in splenocyte numbers was reflected in statistically significant decreased numbers of CD45R, CD4, and CD8 cells in the spleens of treated mice compared with controls. The number of thymocytes was not statistically different from controls, and no alterations in the proportion of the CD4 and CD8 lymphocyte subpopulations were found in thymus of mice exposed to BDE-47. No effect was seen on the in vitro production of immunoglobulin (Ig)G in supernatants from pokeweed-stimulated splenocyte cultures from mice exposed to BDE-47.

Mitogen-induced DNA synthesis and IgG synthesis by human lymphocytes were examined after exposure to BDE-47 ($\geq 98\%$ purity) in vitro, in order to determine the immunotoxic potential of this substance. Human peripheral lymphocytes were isolated from blood donated by 15 healthy females. The lymphocytes were cultured and utilized to assay radiolabeled deoxythymidine uptake in response to pokeweed mitogen stimulation. In addition, the supernatants from the culture media were examined for the presence of immunoglobulin by

using an anti-human IgG from goats. No effects on mitogen-induced proliferation or IgG synthesis were observed after exposure of cells to 10^{-9} to 10^{-5} M BDE-47 (Fernlof et al., 1997).

4.4.4. Genotoxicity

Helleday et al. (1999) examined the effects of BDE-47 at concentrations of 0 to 40 $\mu\text{g/mL}$ in two in vitro V79 Chinese hamster cell-line assays, Sp5 and SDP8, for intragenic recombination at an endogenous locus in mammalian cells. The Sp5 and SDP8 clones exhibit spontaneous partial duplication of the *hprt* (hypoxanthine-guanine phosphoribosyl transferase) gene, resulting in a nonfunctional Hprt protein. These mutants revert spontaneously back to a functional *hprt* gene phenotype by recombination with a frequency of 10^{-5} reversions/cell generation. This frequency can be increased by exposure to chemicals that are mutagenic.

Results from this study indicate that BDE-47 is weakly recombinogenic in the SPD8 cell line assay with up to a 1.8-fold increase at 40 $\mu\text{g/mL}$ but not recombinogenic in the Sp5 cell line. This difference in assay results may be due to different levels of sensitivity and mechanisms between the Sp5 and SPD8 cell lines. Based on these results, BDE-47 appears to be weakly mutagenic at best in mammalian cells. Additional studies are necessary to determine the mutagenic potential of BDE-47.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

4.5.1. Oral

Alterations of behavioral parameters, namely impaired motor functions and decreased habituation capability worsening with age, have been shown to occur in adult male mice neonatally exposed to BDE-47 (Eriksson et al., 2001). The *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a) consider that an agent that produces detectable adverse neurotoxic effects in experimental animals will pose a potential hazard to humans. These adverse neurotoxic effects include behavioral, neurophysiological, neurochemical, and neuroanatomical effects. Accordingly, the behavioral disturbances seen in adult mice neonatally exposed to BDE-47 in the Eriksson et al. (2001) study raise concerns about possible neurobehavioral effects in children and adults.

BDE-47 has been found in human milk, maternal and cord blood, and adipose tissues. Concentrations found are high in all human biological samples in the United States, relative to those of other countries. Fetuses and infants are exposed to BDE-47. Whether such exposures constitute a health risk for adverse neurodevelopmental effects in these population groups is not known at this time. An association between prenatal or neonatal exposures to BDE-47 and neurobehavioral dysfunction in humans has not been established.

4.5.2. Inhalation

No data are available on the toxicity of BDE-47 by the inhalation route of exposure.

4.5.3. Mode-of-Action Information

Exposure of mice and rats to BDE-47 by the oral route resulted in reduction of serum total and free thyroid hormone levels, however, no changes in plasma TSH concentrations were seen (Hallgren and Darnerud, 2002; Hallgren et al., 2001). It is known that thyroid hormones are essential for normal brain development in humans and that hypothyroidism during fetal and early neonatal life may have profound adverse effects on the developing brain (Morreale de Escobar et al., 2000; Haddow et al., 1999). However, the limited available human data do not indicate that BDE-47 affects thyroid hormone levels (Julander et al., 2005; Mazdai et al., 2003) and thyroid hormone levels and behavioral activity were not co-measured in the study in mice of Eriksson et al. (2001).

Decrease in thyroxine levels following exposure of rats to BDE-47 is believed to be chiefly due to disturbances in serum transport caused by binding of in vivo formed BDE-47 metabolites to the plasma thyroid hormone transport protein transthyretin (TTR) (Hallgren and Darnerud, 2002). Hydroxylated tetraBDE metabolites have been shown in vitro to compete with thyroxine for binding with high affinity to TTR (Meerts et al., 2000). Staskal et al. (2006b) hypothesized that structural similarities between thyroid hormones and the hydroxylated BDE metabolites could reduce distribution of thyroid hormone to the brain through competition for the same transporters. Limited renal excretion of hydroxylated BDE-47 metabolites in the early postnatal period increases the BDE-47 metabolite body burden, favoring the BDE-47 metabolite transport over that of the thyroid hormones. At present there are no data to support this hypothesis.

Induction of the liver microsomal phase I enzyme (EROD, MROD, and PROD) activities were significantly increased in mice and rats treated with BDE-47. Induction of these enzymes suggests metabolic transformation of BDE-47 to hydroxy-metabolites that affect thyroxine homeostasis. The data from Meerts et al. (2000) suggest that CYP2B is more active than CYP1A in producing the hydroxylated metabolites. Hallgren and Darnerud (2002) hypothesized that hydroxylated metabolites of BDE-47 can displace T₄ from its binding sites on TTR, leading to the observed reduction in thyroid hormone levels.

Phase II enzymes activity (UDPGT) was moderately increased in mice and rats treated with BDE-47 but it is doubtful that such changes could have a role in the metabolic transformation of BDE-47 or the observed reduction in thyroxine levels (Hallgren and Darnerud, 2002; Hallgren et al., 2001). Glucuronidated conjugates have not been identified in either feces or urine.

Despite data suggesting possible thyroid hormone involvement in the neurodevelopmental impact of BDE-47 on the habituation response in male mice exposed to a single dose on PND 10, there are no mode-of-action data that link thyroid hormones to the observations of Eriksson et al. (2001). Thyroid hormone levels and behavioral activity were not co-measured in the study in mice of Eriksson et al. (2001). However, impaired development of the cholinergic system during the postnatal “brain growth spurt” period has been offered as another plausible hypothesis for the observed neurodevelopmental impact of the penta- and hexa-PBDEs on adult responses to cholinergic agents (Viberg et al., 2005, 2004a, 2003a). Ankarberg (2003) determined that there appears to be a critical window of vulnerability in development of the cholinergic system during postnatal development. In mice this period occurs in the first few weeks after birth with a peak at PND 10 (Viberg et al., 2003a). The resulting deficit in cholinergic receptor is irreversible and could cause a hypoactive response to exposure to cholinergic stimulants in adulthood.

Studies of tetraBDE interactions with the aryl hydrocarbon and estrogen receptors indicate that these compounds are much less potent than dioxins and PCBs (Chen and Bunce 2003; Villeneuve et al., 2002; Chen et al., 2001).

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Epidemiological studies of exposure to BDE-47 and cancer occurrence in humans are not available. Animal chronic toxicity/carcinogenicity studies have not been conducted for BDE-47. BDE-47 demonstrated low or no recombinogenic potential in two in vitro Chinese hamster cells assays. Additional in vitro or in vivo studies are not available to determine the genotoxic potential of BDE-47.

There is “inadequate information to assess the carcinogenic potential” of BDE-47 (U.S. EPA, 2005a,b).

4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.7.1. Possible Childhood Susceptibility

A population subgroup is susceptible if exposure occurs during a period of sensitivity as observed in Eriksson et al. (2001) with adult mice exhibiting effects following neonatal exposure to BDE-47. The neonatal stage is a period of rapid development of the nervous system and is considered a critical window of development. The animal model indicates a potential for concern for early lifetime exposure (i.e., fetal or infant exposure) to the chemical. The evidence of neuron and glial cell BDE-47 accumulation in newborn rats (Mundy et al., 2004) as well as the identification of BDE-47 in human maternal and cord serum, milk, and children’s serum (Mazdai et al., 2003; Schecter et al., 2003; Thomsen et al., 2002) implies animals and humans

are exposed to and accumulate BDE-47 during a period of rapid development of the brain, a critical window of development, indicating a potential for susceptibility. Whether such exposure constitutes a health risk for adverse neurodevelopmental effects in infants and children is not known at this time because of the limited toxicological database for BDE-47. An association between prenatal or neonatal exposures to BDE-47 and neurobehavioral dysfunction in humans has not been established.

4.7.2. Possible Gender Differences

Studies on BDE-47 are not available to determine whether susceptibility to BDE-47 differs in male and female humans or experimental animals. However, the half-life of BDE-47 in females was about one-third greater than in males in one study in rats (von Meyerinck et al., 1990). This could potentially make females more sensitive to the effects of BDE-47 than males. However, the dose used in the study was relatively high and the results may not be applicable to low-dose environmental exposures.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

The subchronic studies (Section 4.2.1) in mice (Hallgren et al., 2001) and rats (Hallgren and Darnerud, 2002) measured hepatic mixed function oxidase system enzyme activities and plasma thyroid hormone levels following exposure to BDE-47. Changes in the activity of the mixed function oxidase system enzymes often accompany exposure to xenobiotic compounds, and changes in thyroid hormone levels are not suitable endpoints for dose-response assessment.

The only study suitable for dose-response assessment is the neurobehavioral study of Eriksson et al. (2001). In this study, male NMRI mice were administered 0, 0.7, or 10.5 mg/kg of BDE-47 on PND 10, and effects on spontaneous motor behavior were investigated in adult mice at 2 and 4 months of age. Pair-wise testing between adult mice exposed on PND 10 and control groups indicated significant changes in the habituation ratio calculated from three behavior variables (locomotion, rearing, and total activity) in mice exposed to 10.5 mg/kg and evaluated at 2 and 4 months of age. Disruption of spontaneous motor behavior, manifested as a hyperactive condition and decreased habituation capability, was also seen to worsen with increasing age. The habituation ratio for total activity (ratio between the performance periods 40–60 minutes and 0–20 minutes for total activity) in 2-month-old mice was 11.7, 15.9, and 50.6 for the control, 0.7, and 10.5 mg/kg dose groups, respectively; the habituation ratio for total activity in 4-month-old mice was 13.1, 16.2, and 79.7, respectively, indicating that the capability of the animals to habituate to a new environment decreased with increasing BDE-47 dose and with age. Based on these effects, the LOAEL in this study was 10.5 and the NOAEL 0.7 mg/kg.

There are several concerns regarding the design of the Eriksson et al. (2001) study. The protocol was unique and did not conform to health effects test guidelines for neurotoxicity screening battery or developmental neurotoxicity studies (U.S. EPA, 1998b). Supporting data that exposure occurred during the period of maximum vulnerability of the developing mouse brain come from a study of pentaBDE, which demonstrated that vulnerability of adult mice to the neurodevelopmental effects occurs during a narrow phase of neonatal brain development (Eriksson et al., 2002). The dosing regimen did not include gestation and lactation exposure (U.S. EPA, 1998b); only single doses were given. In some respects, the observation that effects occurred with such limited dosing argues for the importance of this study. While the study design appears to have been conducted during a developmental window of susceptibility, it is not adequate to determine the effect of longer dosing. Translating the implications of these data to more traditional dosing regimens is problematic, particularly with regard to evaluating the

implications of in utero and postnatal exposure. Another concern is that, based on the data provided in the published report, more than one pup per litter was used for the behavioral testing (eight mice were randomly selected from three to four different litters in each treatment group). Increasing the number of samples from each litter may bias the analyses towards false positives, and the observed neurobehavioral effects may be attributable to non treatment-related differences in pups born to a single dam.² Another concern regarding the study design was the limited number of neurobehavioral parameters that were assessed. The absence of a full functional observational battery (FOB) limits the ability to correlate the reported effects with other FOB parameters. This would be helpful in gauging the reliability of the limited parameters that were measured. As indicated in the *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), it is assumed that an agent that produces detectable adverse neurotoxic effects in experimental animal studies will pose a potential hazard to humans. For BDE-47, in the absence of human evidence, data from experimental animal studies are used as the basis for the RfD.

While study design limitations cloud the utility of this study, several additional considerations support the use of these data. Acute exposure to a highly lipophilic and long half-life chemical, such as BDE-47, will result in exposure that lasts much longer than just acutely. In addition, there are a wide variety of brain structures that have very limited critical windows during development. These short critical windows translate to susceptible periods of exposure that can be very short. Therefore, even chronic exposures may lead to developmental neurotoxicity via disruption of developmental events that take place during a short critical window of development (Rice and Barone, 2000).

The concept that exposure during critical periods of development can induce functional neurological effects later in development has been demonstrated with structurally related PBDE congeners, including penta-, hexa-, and decaBDEs (Kuriyama et al., 2005; Viberg et al., 2005, 2004a,b, 2003a,b, 2002; Ankarberg, 2003; Branchi et al., 2002; Eriksson et al., 2002, 2001).

Taken together, these considerations support the use of the Eriksson et al. (2001) study as the critical study for deriving the RfD for BDE-47.

5.1.2. Methods of Analysis

The RfD for BDE-47 was derived by applying the benchmark dose (BMD) approach to the data on habituation response to BDE-47 exposure collected by Eriksson et al. (2001). In

² Eriksson et al. (2005) evaluated in 18 different litters the use of the litter (n = 9) or randomly selected individuals (n = 9, 3 mice randomly selected from 3 different litters) as a statistical unit in development toxicology in the neonate. In this study of mice neonatally exposed to BDE-99, there was no statistical difference whether the litter or the randomly selected individuals were used as the statistical unit, indicating that multiple sampling from the same litter is unlikely to affect the LOAEL.

the case of motor activity, there is no specific change that is generally regarded as indicative of an adverse response. In the absence of some idea of the level of response to consider adverse, the benchmark response (BMR) selected was for a change in the mean equal to one control standard deviation (SD) from the control mean. In addition to employing the standard BMR corresponding to a change of 1 control SD from the mean, BMRs associated with a change in mean response equivalent to 0.5 and 1.5 times the control SD were also used in order to evaluate the impact of BMR selection on model-derived BMDs and their 95% lower bounds (BMDLs). Because the best fitting dose-response model is linear, any change in the BMR will yield a change in the resulting BMD or BMDL that is directly proportional to this change in BMR. More specifically, relative to the BMD and BMDL derived by using a BMR associated with 1 SD change, the BMD and BMDL resulting from a BMR associated with a 0.5 SD change were 50% lower, while the BMD and BMDL resulting from a BMR associated with a 1.5 SD change were 50% higher.

The continuous habituation (locomotion, rearing, and total activity) data were modeled by using the linear, polynomial, and power models. Habituation ratios for total activity in 2- and 4-month old male mice were the most suitable endpoints for developing a point of departure (POD).

Based on all the BMD and BMDL estimates from the continuous models that provided an adequate fit, the lowest BMD and BMDL were obtained from the linear model for decreased total activity habituation in 4-month-old mice. The estimated BMD_{1SD} is 0.47 mg/kg and the $BMDL_{1SD}$ is 0.35 mg/kg.

Data sets used and details of the BMD modeling results are presented in Appendix A.

5.1.3. RfD Derivation

Using benchmark dose modeling, the $BMDL_{1SD}$ of 0.35 mg/kg for decreased total activity habituation in 4-month-old male mice exposed to BDE-47 on PND 10 (Eriksson et al., 2001) was selected as the point of departure for the RfD. To calculate the RfD, a total uncertainty factor (UF) of 3000 was applied: 10 for extrapolating animal data to humans (UF_A interspecies variability), 10 for susceptible human subpopulation (UF_H interhuman variability), 3 for extrapolating from subchronic to chronic exposure (UF_S) and 10 to account for a deficient database (UF_D). The rationale for application of the UFs is described below.

A default UF_A of 10 was applied to account for the extrapolation of laboratory animal data to humans. No information was available to support a change from the default.

A default UF_H of 10 was applied to account for variation in sensitivity among the members of the human population (intraspecies or interhuman variability). This factor accounts for humans who may be more sensitive than the general population to exposure to BDE-47. It is

known that thyroid hormones are very important for normal brain development in humans and that hypothyroidism during fetal and early neonatal life may have profound adverse effects on the developing brain.

A UF_S of 3 was used for extrapolating effects seen in a single exposure neurodevelopmental study to a lifetime exposure. Exposure on PND 10 occurred during a period of rapid brain development in mice. Brain development does not continue at an equivalent rate across the lifespan and is more quiescent during adult life stages. There are a wide variety of brain structures that have very limited critical windows during development. These short critical windows translate to susceptible periods of exposure that are very short in duration. Therefore, even chronic exposures may lead to developmental neurotoxicity via disruption of developmental events that take place during a short critical window of development. On the basis of this rationale, it is not necessary to make a 10-fold adjustment for exposure duration. Uncertainties regarding the effects of exposures during the prenatal period, extended postnatal exposures, and latent expression of early postnatal changes in the brain are addressed as a component of the database uncertainty factor.

A UF_D of 10 was used to account for database uncertainty. The available oral database for BDE-47 lacks prenatal developmental neurotoxicity studies and reproductive toxicity studies.

Application of a total uncertainty factor of 3000 to the $BMDL_{1SD}$ of 0.35 mg/kg results in a reference dose for BDE-47 of 1.2×10^{-4} mg/kg-day or 0.1 μ g/kg-day.

For a NOAEL/LOAEL approach to the derivation of the RfD, a total uncertainty factor of 3000 is applied to the NOAEL of 0.7 mg/kg for neurodevelopmental effects identified in the Eriksson et al. (2001) study, giving a reference dose for BDE-47 of 2.3×10^{-4} mg/kg-day or 0.2 μ g/kg-day.

5.1.4. Previous RfD Assessment

The tetrabromodiphenyl ether congener BDE-47 has not been previously assessed in IRIS. However, a health assessment of the tetrabromodiphenyl ether homolog group (CASRN 40088-47-9) was previously entered in the IRIS database in 1990 (U.S. EPA, 1990). Information was not available to derive an RfD or RfC or to assess the carcinogenic potential of the tetrabromodiphenyl ether homolog group.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

No data are available for deriving a reference concentration for BDE-47.

5.3. CANCER ASSESSMENT

Data are not available to assess the carcinogenic potential of BDE-47.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

BDE-47 (CASRN 5436-43-1) is a component of the commercial pentabromodiphenyl ether flame retardant. BDE-47 has been found in human milk, adipose tissue, and blood. As a result, fetuses and infants are exposed to BDE-47.

No data are available regarding the potential toxicity of BDE-47 in exposed humans via the oral route. However, the available animal data indicate that the nervous system is a sensitive target organ. Changes in spontaneous motor behavior have been identified as the critical endpoint of concern in adult male mice following neonatal oral exposure to BDE-47 (Eriksson et al., 2001). Since fetuses and infants are exposed to BDE-47 via maternal/cord blood and human milk, such exposure may constitute a health risk for adverse neurodevelopmental effects in these population groups.

There are no studies of the potential carcinogenicity of BDE-47 in humans or experimental animals. Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a,b), there is “inadequate information to assess carcinogenic potential” of BDE-47.

6.2. DOSE RESPONSE

The RfD for BDE-47 of 0.1 $\mu\text{g}/\text{kg}\text{-day}$ was calculated from a $\text{BMDL}_{1\text{SD}}$ of 0.35 mg/kg for effects on spontaneous motor behavior in adult mice. A total UF of 3000 was used: 10 for interspecies variability, 10 for interindividual variability, 3 for extrapolation from single to lifetime exposure, and 10 for data base deficiencies.

No data are available regarding the potential toxicity of BDE-47 in exposed humans via the oral route, and no suitable toxicokinetic or toxicodynamic models have been developed to reduce uncertainty in extrapolating from mice to humans.

The extent of variability in susceptibility to BDE-47 among humans is unknown, representing another important area of uncertainty in the RfD. However, subpopulations expected to be more susceptible to BDE-47 toxicity are fetuses, infants, and children. Chronic studies relevant to BDE-47 toxicity have not been performed in experimental animals.

The principal study for the RfD (Eriksson et al., 2001) examined a number of behavioral parameters in adult male NMRI mice that had been neonatally exposed to BDE-47 and tested two doses, administered in a single day, using a limited number of animals. Aside from this

study, the oral database is sparse. No information is available for the testing of BDE-47 in assays of reproductive toxicity.

The overall confidence in the RfD assessment of BDE-47 is low.

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APPENDIX A. BENCHMARK DOSE MODELING FOR BDE-47

The data on habituation response to BDE-47 exposure collected by Eriksson et al. (2001) were modeled using EPA's benchmark dose software (BMDS). A benchmark response (BMR) equal to one estimated control standard deviation (1.0 SD) was used. The continuous habituation data were modeled by using the linear, polynomial, and power models. The Hill model was not used because there were not enough dose groups—only three dose groups were available. BMDS version 1.3.2 was used to run the power model, and version 1.4 beta was used for linear and polynomial models. This was done due to errors in the BMDS version 1.3.2 that are corrected in the 1.4 beta version. The continuous models in the version 1.3.2 of BMDS calculate incorrect degrees of freedom for *p*-value determination in some situations, and this problem appears to be corrected in version 1.4 beta of BMDS. Thus, version 1.4 beta was used as the best available for linear and polynomial models. The Power model in the version 1.4 beta of BMDS occasionally fails to optimize model fitting where version 1.3.2 succeeds; therefore, the Power model in version 1.3.2 was used in the data modeling even though this version of the Power model provides incorrect chi-square residuals and, in some cases, possible incorrect degrees of freedom for goodness-of-fit analysis. The errors in the degrees of freedom in the Power model were corrected manually if they occurred.

Locomotion, rearing, and total activity habituation in male mice were modeled. The test for equal variances across dose groups failed for all data sets modeled, and so the standard deviation was modeled as a power function of the mean. The variance model adequately (i.e., goodness-of-fit *p*-value > 0.10) predicted the observed variances of all endpoints.

Results

The model results that provided an adequate fit to the mean responses for any endpoint are summarized in Table 1. Although the power model was also fit to the data, there are too many parameters in this model to compute a *p*-value for goodness of fit to 3 data points. Although the full quadratic polynomial model also has too many parameters to compute *p*-values, they can sometimes be successfully computed because model parameters hit boundaries, increasing available degrees of freedom. For example, the parameters of the polynomial model are commonly required to be non-negative, and the linear term may be driven to zero by the optimizer when the response in the second dose group is at or below the control value. This frees one degree of freedom because the linear term is essentially fixed at zero, allowing *p*-values to be computed.

Table A-1. Adequate BDE-47 dose-response models

Endpoint	Model	Age (months)	AIC	P-value	BMD 1.0 SD	BMDL 1.0 SD
Locomotion	Polynomial (2)	4	86	0.12	1.6	1.4
	Linear	2	88	0.15	0.54	0.39
Total Activity	Polynomial (2)	2	88	0.15	0.54	0.39
	Linear	4	109	0.50	0.47	0.35

Based on all the BMD and BMDL estimates from the continuous models that provided an adequate fit, the lowest BMD and BMDL were obtained from the linear model for total activity in 4-month-old mice. Figure 1 shows the dose-response curve corresponding to the recommended model for total activity. Detailed BMD_{1SD} model output file is presented below. The estimated BMD_{1SD} is 0.47 mg/kg, and the BMDL_{1SD} is 0.35 mg/kg.

Total Activity Habituation in Four-Month-Old Male and Female Mice

Linear Model
 BMR = 1.0 SD

```
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Polynomial Model. (Version: 2.3; Date: 6/21/2005)
Input Data File: S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\ETT.(d)
Gnuplot Plotting File: S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\ETT.plt
Tue Jul 19 14:53:30 2005
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~~~~~  
 BMDS MODEL RUN  
 ~~~~~

The form of the response function is:

$$Y[\text{dose}] = \text{beta}_0 + \text{beta}_1 * \text{dose} + \text{beta}_2 * \text{dose}^2 + \dots$$

Dependent variable = MEAN

Independent variable = mg/kg

Signs of the polynomial coefficients are not restricted

The variance is to be modeled as $\text{Var}(i) = \text{alpha} * \text{mean}(i)^\rho$

Total number of dose groups = 3

Total number of records with missing values = 0

Maximum number of iterations = 250

Relative Function Convergence has been set to: 1e-008

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha = 54.3774
 rho = 0
 beta_0 = 12.4332
 beta_1 = 6.40183

Parameter Estimates

95.0% Wald Confidence Interval

Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
alpha	0.237688	0.292585	-0.335769	0.811145
rho	1.43117	0.368555	0.70882	2.15353
beta_0	12.5842	0.8509	10.9164	14.2519
beta_1	6.34765	0.389564	5.58412	7.11119

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	beta_0	beta_1
alpha	1	-0.97	-0.053	0.079
rho	-0.97	1	0.053	-0.08
beta_0	-0.053	0.053	1	-0.32
beta_1	0.079	-0.08	-0.32	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	8	13.1	2.86	12.6	2.99	0.489
0.7	8	16.2	4.25	17	3.71	-0.631
10.5	8	79.7	11.7	79.2	11.1	0.118

Model Descriptions for likelihoods calculated

Model A1: $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \sigma^2$

Model A2: $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3: $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \alpha * (\mu(i))^\rho$

Model R: $Y_i = \mu + e(i)$
 $\text{Var}\{e(i)\} = \sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	d.f.	AIC
A1	-58.348999	4	124.697999
A2	-50.056259	6	112.112518
A3	-50.282658	5	110.565316
fitted	-50.515177	4	109.030355
R	-95.277304	2	194.554607

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)

Test 2: Are Variances Homogeneous? (A1 vs A2)

Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

(Note: When $\rho=0$ the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	$-2 \cdot \log(\text{Likelihood Ratio})$	Test df	p-value
Test 1	90.4421	4	<.0001
Test 2	16.5855	2	0.0002503
Test 3	0.452798	1	0.501
Test 4	0.465039	1	0.4953

The p -value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p -value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

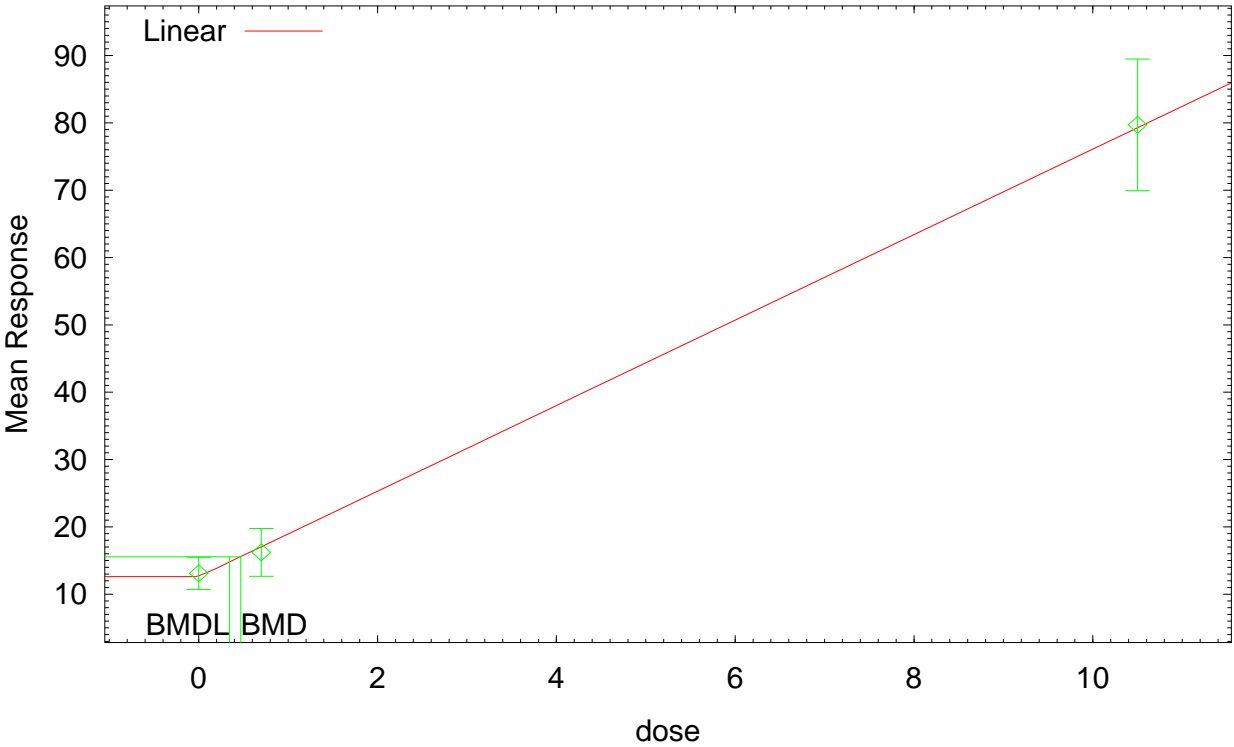
The p -value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p -value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect	=	1
Risk Type	=	Estimated standard deviations from the control mean
Confidence level	=	0.95
BMD	=	0.470339
BMDL	=	0.345373

Linear Model with 0.95 Confidence Level



15:21 08/08 2005

Figure 1. Linear dose-response for total activity habituation ratio in four-month-old mice exposed to BDE-47.