



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)**

June 2008

U.S. Environmental Protection Agency
Washington, DC

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**CONTENTS—TOXICOLOGICAL REVIEW OF
2,2',4,4'-TETRABROMODIPHENYL ETHER (CAS No. 5436-43-1)**

LIST OF TABLES	v
LIST OF FIGURES	v
LIST OF ACRONYMS	vi
FOREWORD	viii
AUTHORS, CONTRIBUTORS, AND REVIEWERS.....	ix
1. INTRODUCTION	1
2. CHEMICAL AND PHYSICAL INFORMATION.....	3
3. TOXICOKINETICS	5
3.1. ABSORPTION	5
3.2. DISTRIBUTION.....	6
3.2.1. Human Data.....	6
3.2.1.1. Adipose Tissue.....	7
3.2.1.2. Liver.....	8
3.2.1.3. Human Milk.....	8
3.2.1.4. Blood.....	9
3.2.1.5. Placental Transport	11
3.2.2. Animal Data	13
3.3. METABOLISM.....	17
3.4. ELIMINATION.....	22
3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS.....	24
4. HAZARD IDENTIFICATION.....	25
4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS.....	25
4.2. SHORT-TERM, SUBCHRONIC, AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION.....	26
4.2.1. Oral Studies	26
4.2.1.1. Short-term and Subchronic Studies.....	26
4.2.1.2. Chronic Studies	28
4.2.2. Inhalation Studies	28
4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES.....	29
4.4. OTHER DURATION- OR ENPOINT-SPECIFIC STUDIES.....	30
4.4.1. Receptor Site Interactions	30
4.4.1.1. Aryl Hydrocarbon Receptors	31
4.4.1.2. Estrogen Receptors	34
4.4.1.3. Androgen Receptors.....	36
4.4.1.4. Other CYP-Inducing Receptors	36
4.4.2. Thyroid Effects.....	37
4.4.3. Immunotoxicity	38
4.4.4. Genotoxicity	38
4.5. SYNTHESIS OF MAJOR NONCANCER EFFECTS	39

4.5.1. Oral.....	39
4.5.2. Inhalation.....	39
4.5.3. Mode-of-Action Information.....	39
4.6. EVALUATION OF CARCINOGENICITY	41
4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES	42
4.7.1. Possible Childhood Susceptibility.....	42
4.7.2. Possible Gender Differences	42
5. DOSE-RESPONSE ASSESSMENTS	43
5.1. ORAL REFERENCE DOSE (RfD).....	43
5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification	43
5.1.2. Methods of Analysis.....	44
5.1.3. RfD Derivation	45
5.1.4. Previous RfD Assessment	47
5.2. INHALATION REFERENCE CONCENTRATION (RfC).....	47
5.3. CANCER ASSESSMENT	47
6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE.....	48
6.1. HUMAN HAZARD POTENTIAL.....	48
6.2. DOSE RESPONSE	48
7. REFERENCES	49
APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION	A-2
APPENDIX B. BENCHMARK DOSE MODELING FOR BDE-47	B-1

LIST OF TABLES

Table 2-1. IUPAC number and bromine substitution pattern of some tetraBDE congeners	3
Table 2-2. Physical and chemical properties of 2,2',4,4'-tetraBDE	4
Table 3-1. Median PBDE congener concentrations in maternal and fetal sera in the U.S. and Sweden.....	12
Table 3-2. Median PBDE congener concentrations in human biological media in the U.S.	13
Table 4-1. Receptor interaction studies of tetraBDE congeners.....	31
Table B-1. Habituation ratio data for total activity in four-month-old male mice exposed to BDE-47.....	B-2
Table B-2. BMD _{1SD} and BMDL _{1SD} estimates based on BMDS continuous dose-response models that did not show significant lack of fit for habituation ratios based on locomotion and total activity in male mice exposed to BDE-47.....	B-2

LIST OF FIGURES

Figure 2-1. Chemical structure of tetraBDE.....	3
Figure 3-1. Proposed metabolic pathway for BDE-47.	18
Figure B-1. Linear model fit to habituation ratio data for total activity in four-month-old male mice exposed to BDE-47.....	B-5

LIST OF ACRONYMS

Ah	aryl hydrocarbon
AIC	Akaike Information Criterion
ATSDR	Agency for Toxic Substances and Disease Registry
BDE-47	2,2',4,4'-tetrabromodiphenyl ether
bdNA	branched DNA
BMD	benchmark dose
BMDL	95% lower bound of the BMD
BMDS	benchmark dose software
BMR	benchmark response
CALUX	Chemical-Activated LUciferase gene eXpression
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service Registry Number
CDD	chlorinated dibenzo-p-dioxin
CDF	chlorinated dibenzofuran
cDNA	complementary DNA
CYP-450	cytochrome P-450
DNA	deoxyribonucleic acid
EC₅₀	median effective concentration
ER	estrogen receptor
EROD	ethoxyresorufin O-deethylase
ESI-MS	electron spray ionization-mass spectrometry
FOB	functional observational battery
heptaBDE	heptabromodiphenyl ether
hexaBDE	hexabromodiphenyl ether
HO-PBDE	hydroxylated PBDE
HPLC	high performance liquid chromatography
<i>hprt</i>	hypoxanthine-guanine phosphoribosyl transferase
IC₅₀	median inhibitory concentration
IgG	immunoglobulin G
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
MCT	monocarboxylate transporter
MM	malignant melanoma
mRNA	messenger RNA
MROD	methoxyresorufin O-dealkylase
MRP	multidrug resistance-associated protein
MUP	major urinary protein
NHL	non-Hodgkin's lymphoma

NOAEL	no-observed-adverse-effect level
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
pentaBDE	pentabromodiphenyl ether
PND	postnatal day
POD	point of departure
PROD	pentoxyresorufin O-dealkylase
PXR	pregnane X receptor
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SD	standard deviation
SXR	steroid X receptor
T₃	triiodothyronine
T₄	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
tetraBDE	tetrabromodiphenyl ether
triBDE	tribromodiphenyl ether
TSH	thyroid-stimulating hormone
TTR	transporter 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 chronic 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 on the tetrabromodiphenyl ether homolog group (CAS No. 40088-47-9) 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 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 addition to BDE-47, IRIS health assessments have also been prepared for three other polybrominated diphenyl ether congeners: pentaBDE-99, hexaBDE-153, and decaBDE-209. These four congeners, for which toxicological studies suitable for dose-response assessments were available, are the ones most commonly found in the environment and human biological media.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. 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).

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Joyce Morrissey Donohue, Ph.D.
Office of Water, Office of Science and Technology
Health and Ecological Criteria Division
U.S. Environmental Protection Agency
Washington, DC

AUTHORS

Joyce Morrissey Donohue, Ph.D.
Office of Water, Office of Science and Technology
Health and Ecological Criteria Division
U.S. Environmental Protection Agency
Washington, DC

Hend Galal-Gorchev, Ph.D.
Office of Water, Office of Science and Technology
Health and Ecological Criteria Division
U.S. Environmental Protection Agency
Washington, DC

Mary Manibusan
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

OFFICE OF RESEARCH AND DEVELOPMENT CO-LEAD

Samantha J. Jones, Ph.D.
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

REVIEWERS

This document has been reviewed by EPA scientists, interagency reviewers from other federal agencies, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the Toxicological Review of 2,2',4,4'-Tetrabromodiphenyl ether (BDE-47).

INTERNAL EPA REVIEWERS

Ted Berner, M.S.
National Center for Environmental Assessment
Office of Research and Development

Linda Birnbaum, Ph.D.
Experimental Toxicology Division
National Health and Environmental Effects Research Laboratory
Office of Research and Development

Kevin Crofton, Ph.D.
Neurotoxicology Division
National Health and Environmental Effects Research Laboratory
Office of Research and Development

Karen Hogan, M.S.
National Center for Environmental Assessment
Office of Research and Development

Tammy Stoker, Ph.D.
Reproductive Toxicology Division
National Health and Environmental Effects Research Laboratory
Office of Research and Development

EXTERNAL PEER REVIEWERS

Ahmed E. Ahmed, Ph.D.
University of Texas Medical Branch

Richard J. Bull, Ph.D.
MoBull Consulting

Lucio G. Costa, Ph.D.
University of Washington

Ralph Kodell, Ph.D.
University of Arkansas for Medical Sciences

Deborah Rice, Ph.D. (chair)
State of Maine

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 other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed 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 are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

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 may be derived. 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 may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per µg/m³ air breathed.

Development of these hazard identification and dose-response assessments for BDE-47 has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference*

Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (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 2007.

2. CHEMICAL AND PHYSICAL INFORMATION

Tetrabromodiphenyl ether (tetraBDE) (CASRN 40088-47-9) is one of the possible 10 homologs of polybrominated diphenyl ethers (PBDEs). Figure 2-1 shows the chemical structure of tetraBDE. The number of possible congeners of tetraBDE is 42, with International Union of Pure and Applied Chemistry (IUPAC) numbers 40 to 81 (Agency for Toxic Substances and Disease Registry [ATSDR], 2004). The IUPAC number and bromine substitution pattern of some congeners that have been investigated in various studies are given in Table 2-1.

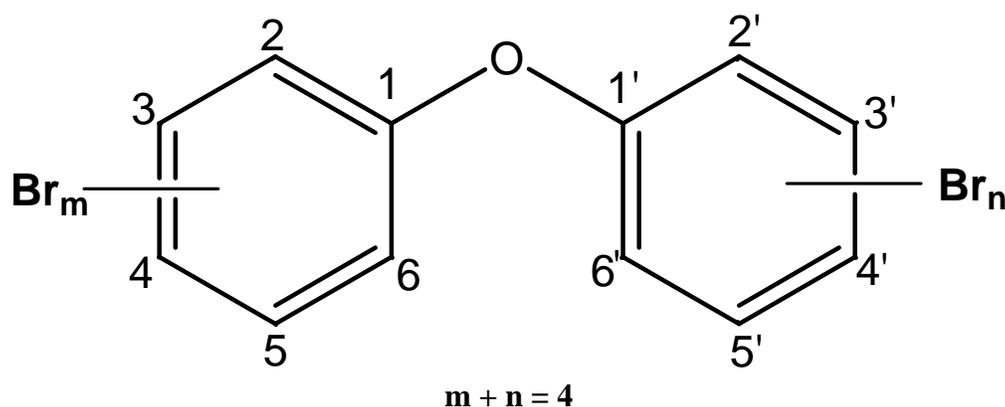


Figure 2-1. Chemical structure of tetraBDE.

Table 2-1. IUPAC number and bromine substitution pattern of some tetraBDE 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 (pentaBDE), which is usually composed of a mixture of tribromodiphenyl ether (triBDE) to hexabromodiphenyl ether (hexaBDE) congeners. The relative proportions by weight of various PBDE homologs and 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 sole U.S. producer of the commercial pentaBDE mixture discontinued its production in the U.S. at the end of 2004.

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 2-2.

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

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	6.81	Braekevelt et al. (2003); ATSDR (2004)
Log octanol/air partition coefficient (K _{oa}) at 25°C	10.5	Chen et al. (2003)

3. TOXICOKINETICS

Data on the toxicokinetics of the tetraBDEs in humans are limited to findings on levels in adipose tissues, blood, liver, and maternal milk that demonstrate that they are absorbed from the environment and distributed to tissues. Studies of BDE-47 in several strains of mice and rats suggest that absorption is $\geq 80\%$ in both species and indicate that mice tend to absorb more of an equivalent dose than rats. As with humans, the highest levels found in mice and rats are in adipose tissues, followed by lung and liver. Metabolism appears to differ between mice and rats. Much of the absorbed dose in mice is excreted unchanged, particularly in urine where excretion is assisted by a urinary transport protein. Both species form hydroxylated metabolites via the activity of cytochrome P-450 (CYP-450) isozymes. The metabolites are excreted with bile and feces and to a lesser extent in urine. Small amounts of the hydroxylated metabolites may become conjugated with glutathione. In rats, there may also be hydrolysis of the ether bond forming brominated phenols excreted in the urine as glucuronate or sulfate conjugates. Knowledge of the metabolic pathway and tissue distribution of the metabolites is limited.

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 uniformly ^{14}C -radiolabeled BDE-47 (12.2 Ci/mol) 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 labeled BDE-47 diluted with unlabeled compound (purity 98%; 1 Ci/mol) 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, possibly with chylomicrons.

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 $>97\%$; 26.7 mCi/mmol) diluted with unlabeled compound in corn oil at 0, 0.1, 1.0, 10, or 100 mg/kg ($<2\mu\text{Ci/mouse}$). 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 among 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 four or six C57BL-6J 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 suggests 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 (four to five animals/sex) were given a single dose of 1 $\mu\text{mol/kg}$ (approximately 0.5 mg/kg) of ^{14}C -BDE-47 (purity 99%; 3.6 $\mu\text{Ci/kg}$) by gavage in corn oil. BDE-47 was absorbed from the gastrointestinal tract 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 ^{14}C -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 accumulation 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 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 occur. The data do not provide information on the quantitative aspects of absorption or the kinetics of tissue retention. The PBDE congener profiles in human biological media differ from the congener profiles of the commercial PBDE mixtures. The reasons for this difference in congener distribution are not known with any certainty. 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, thorough distribution studies have not been conducted in humans; 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 being representative at a national level.

Biomonitoring data with emphasis on levels of PBDE congeners found in the U.S. are summarized in Tables 3-1 and 3-2.

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 U.S. 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. Concentrations of BDE-47 ranged from 7–196 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 the composition of the commercial pentaBDE used in the U.S. 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). Concentrations of BDE-47 ranged from 5–196 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 -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) and analyzed for tri- to hexaBDEs (Güvenius 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 (Choi et al., 2003), 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.

3.2.1.2. *Liver*

In the study by Güvenius et al. (2001) of PBDEs in adipose tissue, liver tissue samples were also obtained in 1994 at autopsy from the same five Swedish subjects discussed above. 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 of levels of PBDEs in human milk in the U.S. conducted in 2002 (Schechter et al., 2003), 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. 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.

Milk samples were collected in 2003 from 40 first-time mothers with 2- to 8-week-old infants and residing in urban areas in the Pacific Northwest of the U.S. (Montana, Oregon, and Washington State) and Canada (British Columbia) (She et al., 2007). Mean and median total concentrations of 12 tri- through decaBDE congeners were 96 and 50 ng/g lw, respectively. These values are substantially higher than the values reported in the study of Schecter et al. (2003) and could be due to the fact that the mothers in the later study had been nursing for longer periods of time. BDE-47 was found at the highest level with maximum, mean, and median concentrations of 201, 50, and 28 ng/g lw, respectively. HexaBDE-153 and pentaBDE-99 and -100 were the next highest congeners with median concentrations of approximately 5 ng/g lw each. Except for triBDE-28 with a median concentration of about 2 ng/g lw, all other concentrations of PBDE congeners were <1 ng/g lw. In 7% of the samples, hexaBDE-153 was the dominant congener. DecaBDE-209 with median concentration of 0.4 ng/g lw was a minor congener in breast milk, contributing 1.2% to the total PBDE concentration.

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. Levels of PBDEs found in breast milk in Japan and Sweden in comparable sampling years were substantially lower than those found in the U.S. or Canada.

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 U.S. 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 U.S. was 34 ng/g lw. The number of donors in the serum pools ranged from 40–200. When the BDE-47 concentrations in archived serum samples were arrayed by collection periods, there was a progressive increase from 1985–1999 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).

Serum samples from 24 pregnant Mexican immigrant women living in an agricultural community in California were collected during 1999 and 2001 (Bradman et al., 2007). Tetra-, penta-, hexa-, and hepta-BDE congeners were measured in the serum samples. The median concentration of the sum of tetra-, penta-, hexa-, and heptaBDE congeners was 21 ng/g lw with a median concentration for BDE-47 of 11 ng/g lw, comparable to the level found in a Laotian immigrant population (Petreas et al., 2003). There were no clear associations among blood levels of PBDEs and demographic characteristics, including age, lactation, and parity. There was a slight correlation between number of years living in the U.S. and PBDE blood levels.

Concentrations of tetra-, penta-, hexa-, and decaBDE congeners were measured in serum samples collected during 2004 from a family residing in Berkeley, California (35- and 36-year-old father and mother, respectively, 5-year-old daughter, and 18-month-old son) (Fischer et al., 2006). The 18-month-old was exclusively breast-fed for six months and was breast-feeding during the study period. PBDE levels for the sum of the five lower brominated congeners BDE-47, -99, -100, -153, and -154 were much higher in the infant (418 ng/g lw) and child (247 ng/g lw) than in their parents (mother 106 ng/g lw, father 64 ng/g lw), with BDE-47 being the predominant congener for all ages. Levels of BDE-209 in the infant (233 ng/g lw) and child (143 ng/g lw) were unusually high compared with those in the parents (mother 14 ng/g lw, father 23 ng/g lw). The authors suspected that house dust and breast milk contributed appreciably to the child and infant exposures. However, no firm conclusions can be drawn from this study because of the small number of subjects investigated.

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 were no correlations between plasma levels of BDE-47 and 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 and 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 among levels of the thyroid hormones triiodothyronine (T_3), thyroxine (T_4), and thyroid-stimulating hormone (TSH) and PBDE levels in the plasma of 11 subjects, aged 20–55 years, working at an electronic

recycling facility (Julander et al., 2005). PBDEs studied were triBDE-28, tetraBDE-47, pentaBDE-99 and -100, hexaBDE-153 and -154, and heptabromodiphenyl ether (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-183 (<0.19), BDE-154 (0.14), and BDE-28 (0.11). 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 (in pmol/g lw) 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 concentrations was highest for the 0- to 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 seemed to experience elevated exposure, there was a lack of an age-related trend of PBDE body burdens. 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- to 4-year-old 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 3-1. BDE-47 was the most abundant congener, followed by pentaBDE-99 and pentaBDE-100. PBDE concentrations were highly correlated between maternal 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).

Table 3-1. Median PBDE congener concentrations in maternal and fetal sera in the U.S. and Sweden

PBDE congener	Maternal serum		Fetal serum	
	Mazdai et al. (2003) ^a	Guvenius et al. (2003) ^b	Mazdai et al. (2003) ^a	Guvenius et al. (2003) ^b
	ng/g lw			
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. (2003) (U.S.): year of sampling, 2001; number of donors, 12.

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

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 3-1). 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).

A summary of the data described above on concentrations of PBDEs in various human biological media in the U.S. is given in Table 3-2. Median concentrations of PBDE congeners are available for human adipose tissue (Johnson-Restrepo et al., 2005; She et al., 2002), breast milk (She et al., 2007; Schechter et al., 2003), and serum (Bradman et al., 2007; Sjodin et al., 2004; Mazdai et al., 2003). In the U.S., the concentration profiles of PBDEs in maternal and fetal sera and human milk are similar, although these studies were conducted in different regions.

The chief congeners found in human biological samples in the U.S. 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 U.S. 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 3-2. Median PBDE congener concentrations in human biological media in the U.S.

	Year ^a	N ^b	BDE-47	BDE-85	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	Σ	Reference
			ng/g lw								
Adipose tissue	1996–1998	23	18	–	7	3	4	6	–	41	She et al. (2002)
Adipose tissue	2003–2004	52	29	<1	10	12	<1	<1	–	75	Johnson-Restrepo et al. (2005)
Breast milk	2002	47	18	0.4	6	3	2	0.2	0.1	34	Schechter et al. (2003)
Breast milk	2003	40	28	0.6	5	5	5	0.4	0.2	50	She et al. (2007)
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 ^c	34	0.7	11	6	7	1	–	61	Sjodin et al. (2004)
Serum, pregnant women	1999–2001	24	11	0.3	2.9	1.8	1.5	0.3	<0.1	21	Bradman et al. (2007)

^aYear = year of sampling.

^bN = number of donors.

^cSeven serum pools with number of donors in each serum pool ranging from 40–200.

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, apparently stored as the parent compound in adipose tissue, which had the highest concentration of ¹⁴C on an lw basis. The lung had the second highest ¹⁴C concentration on an lw basis, amounting to twice the concentrations found in kidney and liver and 10 times 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 an lw basis, concentrations of ¹⁴C in mice were highest in adipose and liver tissues and were of similar magnitude. Kidney and lung had about half that concentration, while levels in the brain were about one-tenth of the concentrations

in adipose and liver tissues. As in rats, ^{14}C levels in mouse plasma were low. The low levels in the 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 exposure, the concentration in adipose tissue (ng/g tissue) was highest. Levels in skin, liver, muscle, and lung were intermediate 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. 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 3 and 2 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 approximately 15% of the applied compound remained at the site of administration five days after dosing.

Staskal et al. (2006a) examined the distribution of BDE-47 in female C57BL/6 mice after repeat exposures to 10 consecutive 1.0 mg/kg-day doses (total dose 10 mg/kg). The 10th dose was radiolabeled (>97% purity; ~5 $\mu\text{Ci/mL}$) 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 or 10 mg/kg dose. As reported by the authors, the mean percent of the radiolabel dose in the adipose tissue, blood, brain, skin, and kidney was significantly higher ($p \geq 0.05$) when a 10th, radiolabeled 1 mg/kg dose was administered after 9 days of 1 mg/kg pretreatment, compared with a single 1 mg/kg dose given to naive mice. The mean percent of the radiolabel dose in adipose tissue was higher (although not statistically significantly higher) after repeated doses (10×1 mg/kg) than after the single 10 mg/kg dose (20 versus 15%). However, when expressed as percent dose/g tissue the difference was significant (14 versus 8%). The mean percent of the radiolabel dose in the other tissues was roughly comparable; muscle tissue was an exception and also showed a significant difference between the percent of the dose/g tissue from repeated dosing as compared to the 10 mg/kg single dose.

The overall distribution of ^{14}C -labeled BDE-47 was studied in mice, using a qualitative whole-body autoradiography technique (Darnerud and Risberg, 2006). ^{14}C -BDE-47 (>95% purity; 12.2 mCi/mmol) was administered to male and female C57BL mice by intravenous injection at 20 $\mu\text{mol/kg}$ 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 2–3 months old). Groups of four to five animals were given a single dose of 1 $\mu\text{mol/kg}$ (approximately 0.5 mg/kg) of ^{14}C -BDE-47 (purity 99%; 36.5 $\mu\text{Ci/kg}$) 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 1,000 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 mg/kg; 3.65 Ci/kg) of ^{14}C -BDE-47 were administered for 1, 5, or 10 consecutive days. Accumulation of radioactivity

showed a linear response with time and did not appear to reach saturation in adipose and other major tissues, excluding lung, muscle, and thyroid over the course of the 10 daily doses. BDE-47-derived radioactivity appeared to be at or near steady state in the lung, muscle, and thyroid by the fifth dose. Male rats and mice were evaluated in a similar fashion but with a higher dose (1 $\mu\text{mol/kg}$, approximately 0.5 mg/kg) in the rats and the elimination of the 5-day sacrifice. 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 exposure (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 C57BL mice sacrificed 24 hours after intravenous administration of 10 mg/kg of ^{14}C -BDE-47 (purity >95%; 12.2 mCi/mmol) on gestational days 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 tissue 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 (>96% purity; $\sim 5 \mu\text{Ci/mL}$) 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 in adult rats from the Staskal et al. (2005) study discussed above. The percent of dose/g brain tissue in 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 but lower than those at 24 hours; the 10-day concentration in pups was about twice the 5-day value and significantly higher than in adults. 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, with the difference 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 (from the first phase) were included in the comparison, the blood concentrations

declined across all five time points and the 10-day levels in pups were significantly higher than those in adults. Adipose tissue levels for the 10-day and 22-day animals were approximately equivalent and more than two times higher than for the other time points. The authors hypothesized that the younger animals were less able to excrete 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- 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 0.01–3.0 μM BDE-47 and incubated at 37°C for 60 minutes. The concentration of the BDE-47 in the cultured 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 hydrophobic interactions with nonpolar areas within folded serum proteins. The uptake in the presence of serum proteins is likely to be more representative of *in vivo* uptake by neurons from serum than from buffered, protein-free 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.

Kodavanti et al. (2005) carried out a similar study by using cultures of cerebellar granule cells from 7- to 8-day-old Long-Evans rat pups. The cultures were treated with labeled PBDE-47 (0.05 $\mu\text{Ci/mL}$) combined with different concentrations of unlabeled compound (0–30 μM) for 15 minutes to 1 hour. For each concentration tested there was a linear increase in percent accumulation over the 1-hour exposure period. When time was held constant and concentration varied, the percent accumulation increased linearly with concentration for the 0.67–30.67 μM range at 15, 30, and 60 minutes. *In vitro* uptake of BDE-47 by the neurons exceeded that of BDE-99 and -153.

3.3. METABOLISM

No human data on the metabolism of BDE-47 are available. The database on the metabolism of BDE-47 in animals is more complete but still lacks information on some of the sequential steps in 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 and quantitative data are lacking in most cases. Figure 3-1 presents the general features of metabolism as suggested from the data summarized below.

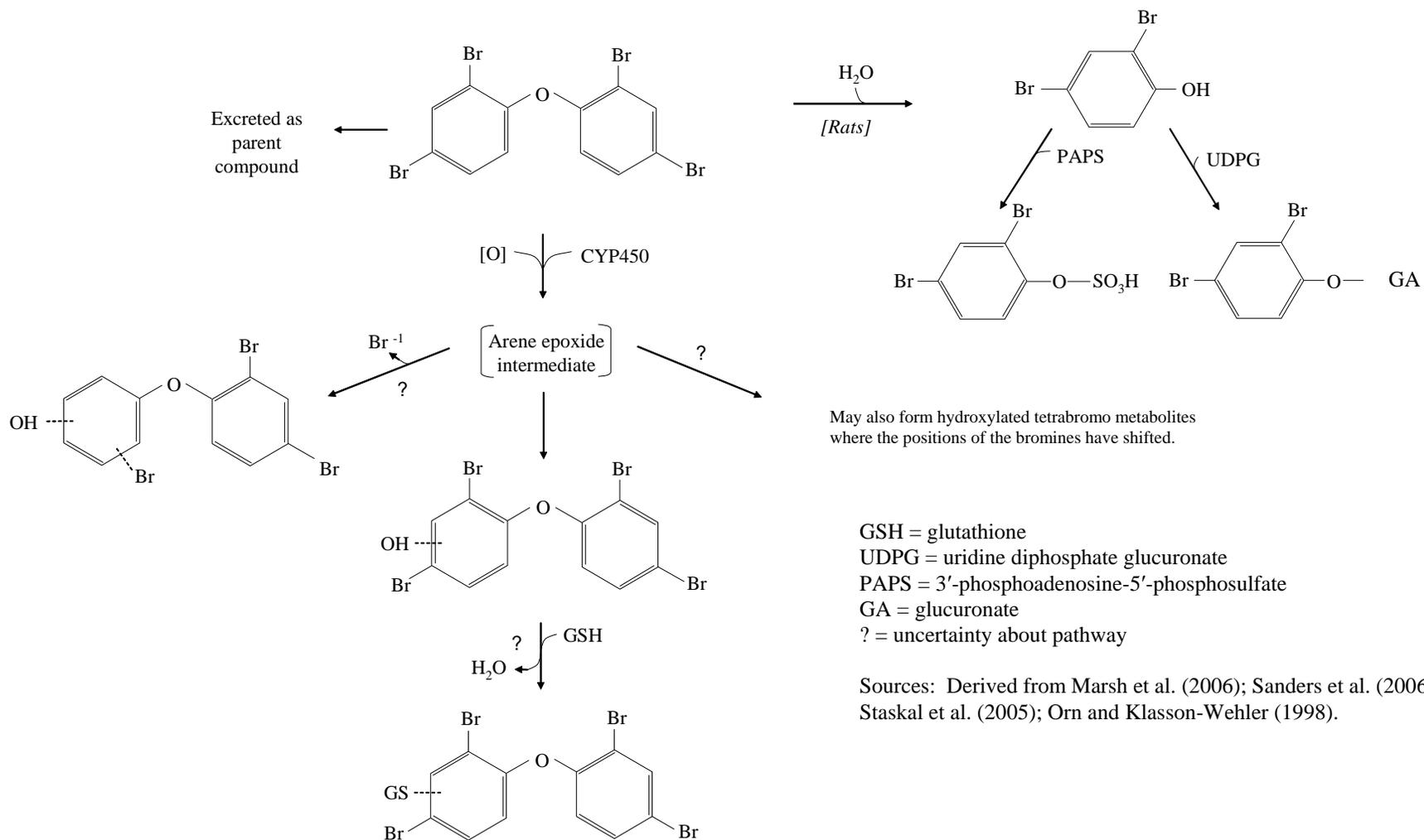


Figure 3-1. Proposed metabolic pathway for BDE-47.

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 mice 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.

Orn and Klasson-Wehler (1998) reported that the mouse formed and excreted a highly water-soluble metabolite that accounted for 1% of the urinary label. This metabolite was unstable and apparently at least partially reverted to the parent compound during attempts to isolate it by using silica gel. Retrospectively, Sanders et al. (2006) proposed that the putative metabolite was the parent compound bound to a urinary transport protein and had become dissociated from the protein in the postextraction processing.

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. According to the authors, metabolites covalently bound to 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 column, were detected in small amounts in the feces and tissues (liver, lung, kidney, and brain) 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. However, in mouse urine the radiolabel was primarily parent BDE-47 after both 1 dose and 10 consecutive doses. Metabolites were present in the feces of both species. The metabolites present in feces were increased after administration of the 10 consecutive doses as compared with the single dose. In rats the fecal metabolites increased from 0% of the radiolabel in fecal matter collected at 24 hours from animals receiving a single dose of BDE-47 to $39 \pm 4\%$ of the radiolabel in the

fecal matter collected at 24 hours from animals 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 the bile collected from rats receiving $1 \mu\text{mol/kg}$ (approximately 0.5 mg/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 by using positive ion electron spray ionization-mass spectrometry (ESI-MS) were glutathione conjugates formed through an arene oxide intermediate. The metabolites were tentatively identified as 5-(glutathion-S-yl)-2,2',4,4'-tetraBDE and 6-(glutathion-S-yl)-2,2',4,4'-tetraBDE. Two urinary metabolites from male rats treated with $1,000 \mu\text{g/kg}$ were also isolated by 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) and Marsh et al. (2006), 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 the parent compound. However, 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. PBDE metabolites identified 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 CYP-450 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 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 CYP-1A1 and -1A2 enzyme activities, respectively, while PROD is a marker of CYP-2B 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 T_4 , the fact that there was no induction of this enzyme suggests that the tetraBDE hydroxylated metabolites do not require up-regulated expression of the enzyme if they are conjugated with glucuronic acid. 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% purity) 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 suggest greater involvement of CYP-2B enzymes in rats than in mice and a possibility for glucuronidation (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 influenced the induction of enzyme activity than a single dose of 10 mg/kg or less.

Sanders et al. (2005) dosed groups of three male F344 rats with 0.49, 4.9, or 49 mg/kg-day BDE-47. Twenty-four hours after the last dose, the animals were sacrificed. Ribonucleic acid (RNA) was extracted from the right lobe of the liver, converted to its complementary deoxyribonucleic acid (cDNA), and amplified by using polymerase chain reaction. Levels of CYP-1A1, -2B1, and -3A1 were measured by using the appropriate primer probes. CYP-2B1 showed the greatest response to the increase in dose, with the levels increasing 12- to 21-fold as the concentration increased from 0.49–49 mg/kg-day. Concentrations of CYP-3A1 and CYP-1A1 increased about threefold over the same concentration range.

In a similar study (Pacyniak et al., 2007), C57BL/6 mice (10 weeks of age), were injected with a dose of 10 or 100 µmol/kg in corn oil for 4 days. The livers were removed 24 hours after the last dose and the levels of messenger RNA (mRNA) measured by Northern blot and branched DNA (bDNA) analyses. The bDNA was considered to be the more accurate of the two assay systems. Northern blot analysis indicated that the levels of CYP-2B10 were induced 11- and 42-fold, respectively, at the two doses tested, while the bDNA results indicated twofold and 22-fold inductions. CYP-3A11 did not show a difference with respect to the dose administered but was induced fivefold by the Northern blot analysis and 1.8-fold by the bDNA analysis.

Richardson et al. (2007) administered a single oral gavage (in corn oil) dose of 0, 3, 10, or 100 mg/kg-day BDE-47 (purity >98%) to female C57BL/6 mice for 4 days. Hepatic EROD activity and CYP-1A1 mRNA expression were measured. The authors found that hepatic EROD activity was significantly increased (1.4-fold) at 100 mg/kg-day, although the mRNA expression

for hepatic CYP-1A1 was not altered by BDE-47 exposure at any dose. Additionally, Richardson et al. (2007) examined hepatic PROD activity and CYP-2B10 mRNA expression. There were significant increases in PROD activity; 1.2-, 1.8-, and 4.8-fold at 3, 10, and 100 mg/kg-day doses, respectively. Hepatic CYP-2B10 mRNA expression was increased 2.5- and 19.9-fold at 10 and 100 mg/kg-day, respectively. No changes were observed in CYP-3A11 mRNA expression.

To date there has been no clear identification of a favored receptor-linked CYP isozyme responsible for the oxidation of BDE-47. Slight to moderate increases in the activities of CYP-1A1/2, -2B1, -2B10, -3A1, and -3A11 have been noted, but the results are not completely consistent across studies. The data support involvement of the 2B family of isozymes to a greater extent than the A family isozymes that have been evaluated.

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 a 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 30.4% of the BDE. 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 is 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 CYP-1A1/2, -2B, and/or -3A isozymes. Conjugations of the hydroxylated metabolites with glutathione are possible as indicated by the detection of a thiol metabolite in feces. The metabolites in urine were not identified; however, Marsh et al. (2006) and Staskal et al. (2006c) identified hydroxylated metabolites in the feces of rats and mice. Sanders et al. (2006) also identified dibromphenol metabolites in rat urine. Methylated metabolites were 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 ^{14}C than rats. A total of 53% of the administered dose was excreted by male mice, 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 was supported by the autoradiography data of Darnerud and Risberg (2006), which showed radiolabeling of the bile and intestinal contents following intravenous injection of 10 mg/kg of ^{14}C -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 $\mu\text{mol/kg}$ BDE-47 (approximately 0.5 mg/kg). Radiolabel in the bile accounted for $2.8 \pm 0.6\%$ of the injected dose over the 6-hour collection period evaluated.

Species 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 $\mu\text{mol/kg}$ dose of ^{14}C -BDE-47 was present in the urine of F344 rats. B6C3F1 mice excreted more of the dose in 24-hour cumulative urine than did rats (~2% in females 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 $\mu\text{mol/kg}$ ^{14}C -BDE-47, $0.9 \pm 0.2\%$ was collected in urine over the 24-hour period after the last dose in rats and $57 \pm 3\%$ 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 (five males and five females 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 facilitated 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. The amount excreted following the intratracheal, intraperitoneal, and oral administration was 30%. The amount excreted following intravenous administration was about 42%. 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.

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

Epidemiological studies of BDE-47 are unavailable.

In the study of PBDE levels in breast adipose tissue of 23 California women, described in section 3.2.1.1 (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, ages 23–79 years and consuming varying amounts of fish (0–32 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, luteinizing hormone (LH), prolactin, TSH, free T₃ and T₄, 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 were no correlations among levels in plasma of BDE-47 and plasma levels of LH, prolactin, free T₃ or T₄, 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 18–37 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 3-1. 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 were no

correlations among 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 the 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). 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 et al. (2003) 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. SHORT-TERM, SUBCHRONIC, AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Studies

4.2.1.1. *Short-term and Subchronic Studies*

4.2.1.1.1. *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 (n = 8) mg/kg-day 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 over control values, but no statistically significant differences were found for thymus or spleen somatic indices. BDE-47 significantly decreased plasma free and total T₄ levels. The effects were 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

activities were highest 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.

Richardson et al. (2007) administered a single oral gavage (in corn oil) dose of 0, 3, 10, or 100 mg/kg-day BDE-47 (purity >98%) to female C57BL/6 mice for 4 days. This study was designed to examine the changes in circulating thyroid hormone concentrations and correlating factors related to the impact of BDE-47 on thyroid hormone homeostasis, including T₄ glucuronidation, membrane transport of glucuronidated T₄, and hepatic transthyretin synthesis. Mice were sacrificed 24 hours after the last dose. There were no changes in body weight in the treated animals. Increases of 14 and 10% were observed for absolute and relative liver weights in animals treated with 100 mg/kg-day BDE-47. Serum total T₄ was decreased approximately 43% in the 100 mg/kg-day dose group compared with the controls, a dose higher than the 18 mg/kg-day tested in the Hallgren et al. (2001) study. There were no effects on liver weight and serum T₄ at the two lower doses of BDE-47. In examining UDPGT induction, Richardson et al. (2007) showed 1.2-, 1.3-, and 1.7-fold increases in UDPGT1A1, UDPGT2B5, and UDPGT1A7 isozyme expression, respectively, at 100 mg/kg-day. UDPGT1A7 expression was increased 1.3-fold at 10 mg/kg-day. There was no change in UDPGT1A6. The changes in UDPGT isoform expression correlated with the observed T₄ decreases. However, in contrast to the observed changes in UDPGT mRNA expression, BDE-47 treatment did not change hepatic T₄-UDPGT enzyme activity. The authors suggested that the T₄-UDPGT enzyme assay was not adequately sensitive to measure changes in activity of individual UDPGT isoforms.

There was a significant increase (47%) in the expression of a major glucuronide transporter, hepatic multidrug resistance-associated protein 3 (MRP3) mRNA, at 100 mg/kg-day BDE-47. The hepatic MRPs are a family of export pumps that play an important role in hepatobiliary excretion. The mRNA for this transporter correlated significantly with T₄ decreases ($R^2 = 0.46$, $p < 0.001$). Significant, dose-dependent decreases in hepatic MDR 1A (but not MDR 1B), a transporter for glucuronides and thyroid hormones, were observed at all doses of BDE-47; however, these decreases did not correlate with the decreases in T₄ ($R^2 = 0.17$, $p = 0.08$). Monocarboxylate transporter 8 (MCT8), a thyroid hormone uptake transporter, was significantly decreased at 100 mg/kg-day to a level 80% of that of the controls. The authors suggested that MCT8 may play a role in thyroid hormone changes, although the decrease in MCT8 mRNA expression did not correlate with T₄ decreases ($R^2 = 0.02$, $p = 0.56$). The hepatic mRNA for transthyretin, a major rodent serum T₄ transport protein, was significantly decreased at 100 mg/kg-day and correlated with the decrease in T₄ ($R^2 = 0.61$, $p < 0.0001$).

4.2.1.1.2. 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

gavage doses of 0, 1, 6, or 18 mg/kg-day BDE-47 (>98% purity) 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-T₄ to the plasma thyroid hormone transporter transthyretin (TTR) were studied. In addition, microsomal phase I enzyme activities (EROD, MROD, and PROD) were also assayed. Induction of these enzymes might suggest metabolic transformation of BDE-47. 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 or thyroid somatic indices and body-weight gains were unaffected. No effects were seen on thyroid morphology at any dose. Plasma levels of free T₄ 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 activities 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 UDPGT activity, significant only at 18 mg/kg-day, but the increase in UDPGT activity did not correlate well with the decrease in T₄ level.

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 ¹²⁵I-T₄ 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 T₄ 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 T₄ 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 T₄ was found to be the most sensitive indicator of imbalance in thyroid hormone status in this study.

4.2.1.2. Chronic Studies

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

4.2.2. Inhalation Studies

Inhalation toxicological studies of BDE-47 in experimental animals 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/peanut oil in water) were administered by gavage to male NMRI mice on PND 10, a period of rapid brain growth and increased susceptibility in neonatal mice. 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. The mice were tested only once. Spontaneous motor behavior was measured for a 60-minute period, divided into three 20-minute periods, at both doses. Spontaneous motor behavior tests 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 versus 4-month-old mice), data from the spontaneous motor behavior tests were used. Habituation is defined as the ability of the animals to adapt to a new environment and is characterized as initial investigation and exploration of their surroundings followed by gradual acclimatization and acceptance of the new area. A habituation ratio was calculated by dividing the motor behavior measures from the 40- to 60-minute observation period by those from the 0- to 20-minute period and multiplying by 100 for each of the three different variables: locomotion, rearing, and total activity. The 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 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. Data for the three spontaneous behavior variables (horizontal movement, vertical movement, and total activity) are only available in graphic form and could not be used for quantitative assessment.¹ Numerical values suitable for dose-response assessment are only available for the habituation ratio. 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)

¹ Attempts to obtain numerical values and other information on the data were not successful.

but were significantly more active than controls during the third 20-minute period (hyperactivity). The habituation ratios based on total activity (i.e., ratio between the total activity counts at 40–60 minutes versus 0–20 minutes) in 2-month-old mice were 11.7, 15.9, and 50.6 in the control, low-dose, and high-dose groups, respectively. These same ratios in 4-month-old mice were 13.1, 16.2, and 79.7 in the control, 0.7, and 10.5 mg/kg dose groups, respectively. 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 animals 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, presented in graphic form only, 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 DURATION- OR ENPOINT-SPECIFIC 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 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 the aryl hydrocarbon (Ah) receptor, estrogen receptor, and/or androgen receptor. Based on the data from the well-studied PCBs, CDDs, and CDFs, the activation of these receptor sites is associated with immunosuppression, reproductive effects, and carcinogenesis (Klaassen, 1996; Bock, 1994), all endpoints of interest for PBDEs. Table 4-1 provides a summary of the tetraBDE congeners that have been evaluated in a variety of receptor interaction studies.

Table 4-1. Receptor interaction studies of tetraBDE congeners

Congener evaluated	47	49	51	66	71	75	77	80	Findings
Ah receptor	X ^a	X		X	X	X	X	X	Effect levels are 10^{-3} to 10^{-5} that of TCDD ^b ; planar BDEs (e.g., 77) appear to be slightly more potent than nonplanar (e.g., BDE-47).
Estrogen receptor	X		X		X	X	X		Weak estrogenic activity compared to estradiol.
Androgen receptor	X								Antiandrogenic activity.
Constitutive androstane receptor	X								Receptor interactions up-regulate expression of associated CYP-450 isozymes. Constitutive androstane receptor activation appears to be stronger than pregnane X receptor.
Pregnane X / steroid X receptor	X								Receptor interactions up-regulate expression of associated CYP-450 isozymes.

^aX indicates that the congener was tested for receptor effects; for most congeners several methodologies for evaluation of receptor interactions were employed.

^bTCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxin.

4.4.1.1. Aryl Hydrocarbon Receptors

The transcription of the genes for CYP-1A1, -1A2, and -1B1 is linked to a signal transduction cascade that is initiated by activation of the Ah receptor by an appropriate ligand. The CYP-1 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 CYP-1 family of enzymes metabolically activates and metabolizes polycyclic aromatic hydrocarbons and aromatic amines as well as PBDEs. Many substrates for the CYP-1 family enzymes are also Ah receptor ligands. Differences in Ah receptor affinity are correlated to variations in CYP-1 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 in competitive binding assays and determined their ability to induce hepatic CYP-450 enzymes by means of EROD assays (a biomarker for CYP-1A1/2 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} times that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and none of the receptor/tetraBDE complexes were found to bind with the nuclear dioxin response element (the segment of DNA that is activated by dioxin-stimulated transcription factors). The Ah receptor binding of the tetraBDE congeners did not seem to be strongly influenced by whether or not the phenyl rings were coplanar. The authors hypothesized that the large atomic

volume of bromine substituents 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. BDE-47 did not act as an EROD inducer in any cell line. EROD induction in all cell lines was strongest with planar BDE-77, although its relative induction potency in the different cell cultures was approximately 10^{-3} to 10^{-4} times that of TCDD. Induction of EROD in the rodent cell lines was slightly lower than in the human cell line. BDE-66 was a very weak inducer in rat hepatocytes and inactive in the other cell lines. The tetraBDE congeners evaluated were 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 with the Ah receptor in cultured liver cells from four healthy cynomolgus monkeys (three males, one female), using EROD activation as the indicator of receptor activation. Both compounds were weak Ah agonists when coexposures of TCDD and PBDEs were tested, as evidenced by a decrease in the activation caused by TCDD alone. The action of the PBDEs was receptor mediated rather than through inhibition of the enzyme because no EROD inhibition occurred if TCDD exposure preceded the PBDE exposure. Environmentally relevant concentrations of PBDEs (1–10 μM) 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 CYP-1A1 induction. These issues are environmentally relevant because of the strong rank-order correlation among strength of Ah receptor binding, CYP-1A induction, and toxicity for many halogenated aromatic compounds.

There were four components to the Chen and Bunce (2003) study: (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 CYP-1A mRNA and CYP-1A 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 CYP-1A1 mRNA and CYP-1A1 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 BDE-66 (another tetraBDE congener) 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 2–500 ng/mL BDE-47 or -77. Luminescence was measured and compared to the maximum response observed with a 1,500 pM 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 with 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. Male F344 rats (three/group), 10–12 weeks old, were dosed by gavage once daily for 3 days with BDE-47 (99% purity) 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% purity). The animals were sacrificed 24 hours after receiving the last dose. The liver was removed, and RNA from a 100 mg liver sample was isolated, converted to its cDNA, and amplified by using the polymerase chain reaction. The resultant DNA samples were then analyzed to determine the expression of the CYP-4501A1, a protein linked to Ah receptor activation.

BDE-47 had a significant effect on the level of CYP-1A1 (2.4 times the vehicle-treated controls) only at 100 $\mu\text{mol/kg-day}$ (approximately 50 mg/kg-day), making it a weak activator of the Ah receptor. BDE-80 had only a weak effect on CYP-1A1 expression, despite its more planar conformation. When the CYP-1A1 expression by BDE-47 was compared with those by pentaBDE-99 and hexaBDE-153, the impact on the Ah receptor seemed to be correlated to the

levels of polybrominated dibenzofuran impurities 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 estrogen receptors (ERs). Activation of estrogen receptors induces cell division in female reproductive organs, mammary glands, and the liver. Receptor-induced mitogenic activity has been linked to tumor formation in the affected organs (Klaassen, 1996).

The *in vitro* estrogenic and antiestrogenic potencies of 17 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 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 μ M, respectively, in comparison with the EC₅₀ value of 1.0×10^{-5} μ M for estradiol. These tetraBDEs were thus 300,000–700,000 times 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, and -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 -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 the 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, they examined the impact of BDE-47 on induction of the enzymes responsible for the oxidation of estradiol at the 2- and 4-positions of the steroid backbone. It has been suggested that the carcinogenic properties of estradiol are related to its hydroxylation at these sites, resulting in the formation of a catecholestragen that 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-ring 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 CYP-450 form that catalyzes 4-hydroxylation has not been identified; aromatase (CYP-19) 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 increase bioavailability and receptor stimulation. In this study, the human sulfotransferase that is active in liver, endometrium, mammary gland, and testes was incubated with varying concentrations of 4-hydroxy metabolites of selected PBDEs. Tri-, tetra-, and pentaBDE hydroxy metabolites were evaluated using concentrations of up to 1,000 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 1,000 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 suggested that the inhibition was noncompetitive (i.e., the interaction with the enzyme did not involve the active site).

In summary, the mechanistic studies of the estrogen receptor indicate that the potencies of the tetraBDEs are much lower than those of dioxin and PCBs. Estrogen receptor mediated activity 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-dependent 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% purity) 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 that blocks 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 used at concentrations of 10 pM, 10 nM, 1 μM , or 5 μM in the presence of 0.1 nM of the receptor agonist dihydrotestosterone. BDE-47 elicited a concentration-dependent antiandrogenic activity in this assay with an IC_{50} of 5 μM .

4.4.1.4. Other CYP-Inducing Receptors

The study of CYP-450 mRNA expression in rat liver by Sanders et al. (2005) (section 4.4.1.1) found that expression of CYP-2B and -3A was up-regulated by BDE-47 in F344 rats to a greater extent than that of CYP-1A1. CYP-2B and -3A are biomarkers for activation of the constitutive androstane receptor (CAR) and pregnane X receptor (PXR), respectively. The authors concluded 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 are both involved in the metabolism of xenobiotics and are stimulated by phenobarbital. The CAR is also involved in steroid metabolism. The impact of BDE-47 on these receptors is similar to the impact of noncoplanar PCBs on the same receptors; however, the implications of CAR or PXR activation are not well understood.

Pacyniak et al. (2007) carried out additional work with the PXR receptor and its human counterpart, the steroid X receptor (SXR), using HepG2 cells transvested with the appropriate cDNA, the receptor response elements, and a luciferase reporter vector. The cultured cells were exposed to 0, 0.1, 1, 10, or 100 μM concentrations of BDE-47. With the PXR there was a dose-related increase in relative luciferase activity that showed a significant increase above the control for all but the lowest dose. With the SXR, there was a linear significant response to dose for both the 10 and 100 μM concentrations but not for the 0.1 and 1 μM concentrations. The authors also compared the response in PXR-knock-out mice (10–12 weeks old) with the wild type and found that CYP-3A11 was induced to a greater extent in the wild-type animals, indicating that BDE-47 seems to be a ligand for the PXR.

Richardson et al. (2007) suggested that the significant dose-dependent increases in PROD activity and CYP-2B10 mRNA expression suggest that CAR may be one of the major nuclear receptors activated by BDE-47 or its metabolites. In the Richardson et al. (2007) study, the lack of an increase in CYP3A11 mRNA expression in female mice was in contrast to the PXR activation in male mice reported by Pacyniak et al. (2007) and may be a result of gender or exposure routes.

4.4.2. Thyroid Effects

Because PBDEs, 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 the 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. None of the five tetraBDE congeners evaluated (BDE-47, -51, -71, -75, and -77) competed with T_4 -TTR binding (Meerts et al., 2000).

Meerts et al. (2000) also tested the same five tetraBDEs before and after incubation with hepatic microsomes to examine the ability of hydroxylated metabolites to displace T_4 from TTR. The tetraBDEs were individually incubated with liver microsomes prepared in the presence of phenobarbital (a CYP-2B inducer), β -naphthoflavone (a CYP-1A inducer), or clofibrate (a CYP-4A3 inducer). Incubation of the tetraBDEs with CYP-2B-enriched rat liver microsomes resulted in the formation of metabolites that were able to displace ^{125}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}I - T_4 from TTR. Only tetraBDE-71 showed a lower ability to displace ^{125}I - T_4 from TTR (20–60%). T_4 -TTR displacement by tetraBDEs after incubation with liver microsomes enriched with CYP-1A or CYP-4A3 was much lower than that with CYP-2B. BDE-47 was inactive after treatment with both the CYP-1A and CYP-4A enriched microsomes, and tetraBDE-51 was inactive after the treatment with the CYP-1A enriched microsomes. TetraBDEs are therefore

able to compete with T₄-TTR binding only after metabolic conversion by the appropriate CYP-450 isozyme, suggesting an important role for hydroxylation. The relevance of this observation for humans has yet to be resolved. T₄-binding globulin, rather than TTR, is the major T₄-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 numbers 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, suggesting 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 significantly 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 those in controls, and no alterations in the proportion of the CD4 and CD8 lymphocyte subpopulations were found in the thymuses of mice exposed to BDE-47. No effect was seen on the *in vitro* production of immunoglobulin G (IgG) in supernatants of 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 (≥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 IgG by using an antihuman 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 for intragenic recombination at an endogenous locus in mammalian cells at concentrations of 0–40 µg/mL in two *in vitro* V79 Chinese hamster cell-line assays, Sp5 and SDP8. 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 spontaneously revert 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 among 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 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. Similar neurodevelopmental effects have been observed in studies of the pentaBDE-99, hexaBDE-153, and decaBDE-209 congeners.

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 U.S., 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

There are minimal mode-of-action data that apply to the developmental neurotoxicity of BDE-47. Researchers from the laboratory of Eriksson have hypothesized that the effects on locomotion and habituation observed with decaBDE-209 (Viberg et al., 2007), hexaBDE-153 (Viberg et al., 2003a), and pentaBDE-99 (Viberg et al., 2004a) are related to impaired development of the cholinergic system during the postnatal “brain growth spurt” period resulting

in a reduced number of cholinergic receptors. They have further hypothesized that the sensitivity of the cholinergic system occurs in the vicinity of PND 10 and have tested this hypothesis by varying the time of dosing and observing differences in the habituation effect for BDE-99 (Eriksson et al., 2002) and BDE-209 (Viberg et al., 2007). The resulting deficit in cholinergic receptors persisted across the duration of testing and could cause an abnormal response to exposure to cholinergic stimulants in adulthood. Testing of this hypothesis has not been conducted in studies of BDE-47.

Evidence that BDE-47 can enter the neurological system and possibly interact with the growth of neurons, memory, and learning was reported by Kodavanti et al. (2005) and Mundy et al. (2004). A dose-related increase in accumulation of BDE-47 was observed within 1 hour in cultures of cerebellar neurons and mixed cerebellar neurons and glia from neonatal Long-Evans rat pups (Kodavanti et al., 2005; Mundy et al., 2004). This uptake by the neurons was coincident with translocation of protein kinase C, which is involved in neuronal growth, memory, and learning. NMR mice pups exposed to BDE-99 on PND 10 showed differential expression (up- or down-regulated) of several proteins in brain tissues that are involved with the protein kinase C signaling complex (Alm et al., 2006). Coincident uptake of BDE-47 with protein kinase C translocation would suggest a potential for interaction at the neuronal level; however, a study, comparable to Kodavanti et al. (2005) in BDE-99 has not been conducted with BDE-47. While some evidence exists that PBDEs may have an impact on neurochemical events during early postnatal development, the data are inadequate to determine the mode of action for BDE-47.

Staskal et al. (2006b) studied the distribution of BDE-47 in neonatal mice (10 days old) as compared with juvenile (22–40 days old) and adult mice. They found the body burdens as reflected in adipose tissue levels, blood concentrations, and total residuals 24 hours after dosing were higher in the 10- and 22-day-old mice than in the other age groupings. The blood and tissue levels of the radiolabel 24 hours after BDE-47 administration were inversely related to urinary excretion. Label in the urine increased dramatically and significantly in the 40-day-old and adult mice compared with that in the younger mice. The authors hypothesized that the differences in body burden could be related to a late developing mouse urinary protein, which, when produced, increased urinary excretion of BDE-47 and its metabolites (Staskal et al., 2006c). A comparable protein has not been identified in rats. However, developmental changes in renal excretion may contribute to the age-specific effects of BDE-47.

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, Richardson et al., 2007). 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). Thyroid hormone levels and behavioral activity were not comeasured in the study in mice of Eriksson et al. (2001).

Decrease in T₄ 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 TTR (Hallgren and Darnerud, 2002). Hydroxylated tetraBDE metabolites have been shown in vitro to compete with T₄ 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.

Despite the possibility of 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 neurobehavioral observations reported by Eriksson et al. (2001).

Studies of tetraBDE interactions with the Ah 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). The tetracongeners appear to have an antiandrogenic impact on the androgen receptor (Stoker et al., 2005). Some data (Pacyniak et al., 2007; Richardson et al., 2007; Sanders et al., 2005) suggest that the CAR and PXR may be impacted to a greater extent in vivo and in vitro than the Ah, estrogen, and androgen receptors. However, the implications of these interactions are unknown.

4.6. EVALUATION OF CARCINOGENICITY

Epidemiological studies of exposure to BDE-47 and cancer occurrence in humans are not available. Animal chronic toxicity/carcinogenicity studies have not been conducted with BDE-47. BDE-47 demonstrated low or no recombinogenic potential in two in vitro Chinese hamster cell 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).

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 cerebellar and neocortical neuron BDE-47 accumulation in newborn rats (Kodavanti et al., 2005; 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; Schechter 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. This is a critical window of development and indicates 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. A major urinary protein has been identified in male mice, which facilitates excretion of BDE-47 once the pathway for its synthesis becomes operational. This decreases retention of BDE-47 in juvenile and mature males compared to females but is not functional during the early postnatal period of development.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

The only study suitable for use in dose-response assessment is the neurobehavioral study of Eriksson et al. (2001). As previously discussed in section 4.2.1, subchronic animal studies in mice (Richardson et al., 2007; 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. However, changes in receptor-linked enzyme induction were not consistently dose related, and the observed changes in thyroid hormone levels occurred at a dose greater than that seen in the Eriksson et al. (2001) study. Therefore, these changes were not considered as the basis for the dose-response assessment. The study used as the basis for the dose-response assessment of BDE-47 was Eriksson et al. (2001).

In Eriksson et al. (2001), three groups of eight male NMRI mice were administered single oral doses of 0, 0.7, or 10.5 mg/kg of BDE-47 on PND 10. Following this single exposure, the effects of BDE-47 on spontaneous behavior were evaluated in these mice at both 2 and 4 months of age. Comparison of mice in the high-dose group versus controls showed significant changes in the habituation ratios derived from three different measures of spontaneous behavior (i.e., locomotion, rearing, and total activity) at both 2 and 4 months of age. Alterations in spontaneous behaviors, manifested as a decreased habituation capability, were also observed to worsen with age. The habituation ratios based on total activity (i.e., ratio between the total activity counts at 40–60 minutes versus 0–20 minutes) in 2-month-old mice were 11.7, 15.9, and 50.6 in the control, low-dose, and high-dose groups, respectively. These same ratios in 4-month-old mice were 13.1, 16.2, and 79.7 in the control, 0.7, and 10.5 mg/kg dose groups, respectively. These increases in habituation ratios with increasing dose of BDE-47 suggest that the capability of these animals to habituate to a new environment was adversely affected by exposure to BDE-47. Based on these results, the LOAEL identified in this study was 10.5 mg/kg and the NOAEL was 0.7 mg/kg.

Although the Eriksson et al. (2001) study was deemed suitable for dose-response assessment, several concerns exist regarding the design of this study. First, the study was conducted in male mice only. Second, the protocol was unique and did not conform to existing health effects test guidelines for neurotoxicity screening batteries or developmental neurotoxicity studies (U.S. EPA, 1998b). Also, the dosing regimen did not include exposure during gestation and lactation (U.S. EPA, 1998a), and only single doses were given. However, supporting data that exposure occurred during the period of maximum vulnerability of the developing mouse brain come from a study of pentaBDE-99, which demonstrated that vulnerability of adult mice to neurodevelopmental effects occurs during a narrow phase of neonatal brain development

(Eriksson et al., 2002). In some respects, the fact that effects were observed with such limited dosing argues for the importance of this study, and the potential potency of BDE-47. While the dosing appears to have been administered during an appropriate developmental window of susceptibility, it is inadequate for determining the effects of longer-term dosing. Whether these data would be similar under more traditional dosing regimens is problematic, particularly with regard to evaluating the potential impact of in utero and postnatal exposures. Another concern with Eriksson et al. (2001) is that, based on the data provided in the published report, more than one pup per litter was used for the behavioral testing (i.e., eight mice were randomly selected from three to four different litters in each treatment group). An increased number of samples from a particular litter may bias the results of the analyses towards false positives. Although the dams were not treated and the pups were not exposed during gestation, the observed neurobehavioral effects may be attributable to non-treatment-related differences in pups born to a single dam. Finally, the study design was such that only a limited number of neurobehavioral parameters were assessed. The absence of a full functional observational battery (FOB) limits the ability of this study to correlate the reported effects with other FOB parameters. This type of analysis would be helpful in gauging the reliability of the 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 animals will pose a potential hazard to humans. For BDE-47, in the absence of human evidence, data from experimental animal studies were 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 of short duration.

The concept that exposure during critical periods of development can induce functional neurological effects later in development has been demonstrated with BDE-47 (Eriksson et al., 2001) and 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 using the benchmark dose (BMD) approach employing data on habituation ratios reported by Eriksson et al. (2001). In the case of habituation ratios, no specific change exists that is generally regarded as indicative of an adverse

response. In the absence of consensus regarding the magnitude of response that should be considered adverse, the benchmark response (BMR) selected was a change in the mean equal to one standard deviation (SD) of the control mean, which is consistent with the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c). In addition to employing the standard BMR corresponding to a change of 1 SD from the control mean, BMRs associated with a change in mean response equivalent to 0.5 and 1.5 times the control SD were also employed in order to evaluate the impact of BMR selection on model-derived BMDs and their 95% lower bounds (BMDLs).

Habituation ratios (based on the spontaneous behaviors of locomotion, rearing, and total activity) were modeled as continuous variables by fitting the linear, polynomial, and power models available in EPA's benchmark dose software (BMDS). Based on this modeling, habituation ratios based on locomotion and total activity in 2- and 4-month-old male mice were deemed the most suitable endpoints for identifying a point of departure (POD).

Of the BMDs and BMDLs estimated from the continuous models in BMDS that provided an adequate fit, the lowest BMD and BMDL were obtained by fitting a linear model to habituation ratios based on total activity in 4-month-old mice. In this case, the estimated BMD_{1SD} is 0.47 mg/kg and the $BMDL_{1SD}$ is 0.35 mg/kg. 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 the 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. Further details regarding the BMD modeling results are presented in Appendix B.

5.1.3. RfD Derivation

Using the BMD approach, a $BMDL_{1SD}$ of 0.35 mg/kg was selected as the POD for the RfD. This POD is based on a decrease in the habituation ratio (based on total activity) in 4-month-old male mice exposed to BDE-47 on PND10 by Eriksson et al. (2001). To calculate the RfD, a total uncertainty factor (UF) of 3,000 was applied to this POD. This total UF consists of the following: (1) a factor of 10 for extrapolating from animals to humans (UF_A interspecies variability), (2) a factor of 10 to account for susceptible human subpopulations (UF_H intrahuman variability), (3) a factor of 3 for extrapolating from a single dose to a lifetime exposure duration (UF_S), and (4) a factor of 10 to account for database deficiencies (UF_D). The rationale for applying each of these UFs is described below.

A 10-fold UF_A was used to account for laboratory animal to human interspecies differences. Although the toxicokinetics of BDE-47 in animals have been evaluated, no adequate description of toxicokinetics of BDE-47 in humans exists. The critical effect for

deriving the RfD, altered behavior due to exposure during development, is expected to be relevant to humans. No quantitative data were identified to compare relative human and rodent sensitivity to these changes. However, given the longer period of brain development in humans as compared to rodents and the higher importance of cognitive function, it is appropriate to consider that humans may be more sensitive than rodents in the absence of specific data. Based on these considerations the default UF_A value of 10 was applied.

A default intraspecies UF_H of 10 was applied to account for variations in susceptibility within the human population (intrahuman variability). This factor accounts for the segment of the human population that may be more sensitive than the general population to exposure to BDE-47. A default value is warranted because insufficient information is currently available to assess human-to-human variability in BDE-47 toxicokinetics or toxicodynamics.

A UF_S of 3 was used to account for uncertainties in extrapolating from 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 over a mouse's lifetime and is more quiescent during adult life stages. Many brain structures have a very limited critical window during development in early life. Following BDE-47 exposure, toxicokinetic data suggest that a mouse urinary protein becomes functional some time between PNDs 28 and 40, which leads to a dramatic increase in BDE-47 urinary excretion, especially in males. This increased excretion reduces the total body burden of BDE, including the levels of radiolabel reaching the brain 24 hours after dosing in older mice compared with that in younger mice. These data thus suggest that the risk of neurodevelopmental effects in neonatal mice may be greater than in older mice because of rapid postnatal brain growth and coincident increased retention of BDE-47 and/or its metabolites. Therefore, chronic exposure is not expected to result in more serious effects. However because the mice received only a single dose rather than repeated doses over multiple days within the hypothesized critical window, a threefold UF was applied.

A UF_L for LOAEL-to-NOAEL extrapolation was not used because the Agency's current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a change in the mean equal to 1 SD of the control mean was assumed to represent a minimal biologically significant change.

A UF_D of 10 was used to account for database uncertainty. The available oral database for BDE-47 lacks prenatal developmental neurotoxicity studies, reproductive toxicity studies, and standard chronic or subchronic studies of systemic toxicity. 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 UF.

Therefore, application of a total UF of 3,000 to the POD of 0.35 mg/kg results in an RfD for BDE-47 of 1.17×10^{-4} mg/kg-day or 0.1 µg/kg-day. For comparison purposes, a NOAEL/LOAEL approach to the derivation of the RfD, with a total UF of 3,000 applied to the NOAEL of 0.7 mg/kg for neurodevelopmental effects identified in Eriksson et al. (2001), would result in a reference dose for BDE-47 of 2.3×10^{-4} mg/kg-day or 0.2 µg/kg-day.

5.1.4. Previous RfD Assessment

The tetraBDE congener, BDE-47, has not been previously assessed in IRIS. However, a health assessment of the tetraBDE 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 a RfD or RfC or to assess the carcinogenic potential of the tetraBDE homolog group.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

No data were available for deriving an RfC for BDE-47.

5.3. CANCER ASSESSMENT

Inadequate information currently exists to assess the carcinogenic potential of BDE-47 (U.S. EPA, 2005a).

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 commercial pentaBDE flame retardants. BDE-47 has been found in human milk, adipose tissue, and blood. As a result, fetuses and infants can be 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 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 exposures may constitute a health risk for adverse neurodevelopmental effects in these population groups.

No studies of the potential carcinogenicity of BDE-47 in humans or experimental animals were found. Therefore, under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is “inadequate information to assess carcinogenic potential” of BDE-47.

6.2. DOSE RESPONSE

The principal study from which the RfD was derived (Eriksson et al., 2001) examined a number of behavioral parameters in two groups of adult male NMRI mice that had been neonatally exposed to BDE-47 in a single acute dose, using a limited number of animals. Aside from this study, the oral database is sparse. No information is available on the testing of BDE-47 in assays of reproductive toxicity.

The RfD for BDE-47 of 1.17×10^{-4} mg/kg-day (0.1 µg/kg-day) was derived from a POD of 0.35 mg/kg by using a BMD approach based on changes in habituation ratios (based on total activity) in adult mice observed by Eriksson et al. (2001). In deriving the RfD, a total UF of 3,000 was applied to the POD: 10 for interspecies variability, 10 for interindividual human variability, 3 for extrapolation from acute to chronic exposure, and 10 for database 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 yet been performed in experimental animals.

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

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APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The “Toxicological Review” for tetraBDE (BDE-47) has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a, 2000a). The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. The external peer review for BDE-47 was conducted in concert with the external peer review of other PBDE congeners (i.e., BDE-99, BDE-153, and BDE-209), and some external peer review charge questions were specific to congeners other than BDE-47. External peer reviewer comments on all of the PBDEs and the Agency response are included below for completeness. A summary of significant comments made by the external reviewers and EPA’s response to these comments arranged by charge question follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. Synthesis of comments from individual peer reviewers resulted in summaries that combine similar statements from peer reviewers that were mentioned in conjunction with more than one charge question. In such cases, the comment and its response have been placed under the most relevant charge question. Some of the peer review comments were not directly related to charge questions. Those comments are categorized as miscellaneous and placed after those related to the charge questions. EPA also received scientific comments from the public. These comments and EPA’s responses are included in a separate section of this appendix.

The peer review of the “Toxicological Review” of BDE-47 was coupled with the review of the documents for BDE-99, -153, and -209. Accordingly, most of the charge questions address all four congeners. The responses to the charge questions in this appendix apply primarily to comments related to BDE-47. The charges to the external peer reviewers and final external peer review report (February 2007) pertaining to the toxicological reviews of the four polybrominated diphenyl ether congeners are available at <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=161970>. The public comments received can be found at <http://www.regulations.gov/fdmspublic/component/main> under the docket EPA-HQ-ORD-2006-0838.

EXTERNAL PEER REVIEWER COMMENTS

The reviewers made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

A. General Comments

Charge Question 1. *Are you aware of other published peer-reviewed toxicological studies not included in these toxicological reviews that could be of relevance to the health assessment of BDE-47, BDE-99, BDE-153, or BDE-209?*

Comment 1: Three reviewers stated that they were unaware of any other relevant studies that would contribute to the BDE-47 IRIS assessment. One reviewer identified the following potentially relevant additional literature:

Jones-Ortazo, HA; et al. (2005) Environ. Sci. Technol. 39:5121-5130
Wilford, BH; et al. (2005) Environ. Sci. Technol. 39:7027-7035
Schechter, A; et al. (2005) J. Toxicol. Environ. Health Part A 68:501-513
Hites, RA; et al. (2004) Environ. Sci. Technol. 38:4945-4949
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Fischer, D; et al. (2006) Environ. Health Perspect. 114:1581-1584
Bradman, A; et al. (2007) Environ. Health Perspect. 115:71-74
Kodavanti, PRS; Derr-Yellin, EC. (2002) Toxicol. Sci. 68:451-457
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Reistad, T; Mariussen, E. (2005) Toxicol. Sci. 87:57-65
Reistad, T; et al. (2006) Arch. Toxicol. 80:785-796

Response: The Agency reviewed and evaluated the studies recommended by the reviewer and has included the relevant studies for BDE-47. Fischer et al. (2006), Bradman et al. (2007), and Kodavanti et al. (2005) were found to be relevant and were added to the document. The remaining studies suggested by the reviewer fell outside the scope of the IRIS assessment (i.e., exposure data, commercial mixtures). Additionally, a new literature search was conducted to ensure recently published, relevant studies are included in the IRIS assessment. Two new studies (Pacyniak et al., 2007 and She et al., 2007) were thus identified and have been included in the “Toxicological Review” of BDE-47.

B. Oral Reference Dose (RfD) Values

Charge Question 2. *Have the rationale and justification for deriving RfDs on the basis of the neurobehavioral toxicity studies been transparently and objectively described in the draft toxicological reviews of BDE-47, BDE-99, BDE-153, and BDE-209? Are there additional studies that should be considered for deriving the RfDs for any of the four PBDE congeners?*

Comment 1: Three reviewers stated that the rationale for deriving the RfD for BDE-47 based on the Eriksson et al. (2001) neurobehavioral study was clearly and transparently described. The peer reviewers acknowledged the limitations and concerns with the study; however, they felt that its limitations were transparently discussed in the “Toxicological Review.” Two reviewers commented that the Eriksson et al. (2001) study provides the most appropriate dose-response data on which to base the health assessment for BDE-47. None of the reviewers suggested additional studies that should be considered for deriving the RfD.

Response: No response needed.

Charge Question 3. *Do you agree or disagree with EPA basing the health assessment of BDE-47, BDE-99, BDE-153, and BDE-209 to a large extent on the Eriksson/Viberg neurobehavioral studies?*

Comment 1: All reviewers supported the use of the Eriksson/Viberg neurobehavioral study as the basis for the derivation of the BDE-47 RfD, given the limited body of toxicological information available. Two reviewers noted that the studies for the four congeners (BDE-47, -99, -153, and -209) are limited by the fact that they originated from the same laboratory. One reviewer was concerned that the experimental design of the principal study ignored the “litter” effect and suggested the study authors should have used one pup per litter rather than more than one pup per litter. Another reviewer was concerned with the specificity of the neurobehavioral data for developmental neurotoxicity and suggested that independent confirmation of the endpoints is essential. One peer reviewer identified the use of a single sex (male mice) as a limitation of the critical study that had not been identified in the “Toxicological Review” discussion of study limitations. One of these reviewers stated that these limitations do not hinder the derivation of the BDE-47 RfD but make the confidence low. Another reviewer noted that the neurobehavioral findings of the Eriksson/Viberg laboratory have been corroborated in a study examining BDE-99 (Kuriyama et al., 2005). No reviewer suggested that the Eriksson et al. (2001) study should not be used as the basis for the derivation of the RfD.

Response: The “Toxicological Review” contains a detailed summary of the concerns with the study design and methods utilized in the principal study (see section 5.1.1). A discussion of the use of only male mice in the study by Eriksson et al. (2001) has been added to the discussion in section 5.1.1 of the “Toxicological Review.” Additionally, the neurobehavioral effects reported in Eriksson et al. (2001) are supported by an expanding body of literature on other congeners

(Rice et al., 2007; Viberg et al., 2007, 2005, 2004a, b, 2003a, b, 2002; Kuriyama et al., 2005; Eriksson et al., 2002; Branchi et al., 2002) that detail changes in motor and cognitive activity in rodents following administration of single or repeated perinatal doses of PBDEs. Some of the concerns associated with the methodology of the Eriksson/Viberg neurobehavioral studies are alleviated by other studies (Rice et al., 2007; Kuriyama et al., 2005; Branchi et al., 2002) using more traditional neurodevelopmental methodologies than the Eriksson/Viberg group and have noted similar neurodevelopmental effects.

For BDE-47, the principal study by Eriksson et al. (2001) is supported by results from a study recently completed, but not yet published, by the EPA National Health and Environmental Effects Research Laboratory (e-mail from Ginger Moser, National Health and Environmental Effects Research Laboratory, Research Triangle Park, North Carolina, to Samantha Jones, U.S. EPA, dated August 14, 2007). Since this study has not been published, it is not reported in the "Toxicological Review." The study was designed to determine if effects analogous to those reported by Eriksson et al. (2001) could be observed in another strain of mice exposed to BDE-47. There were some differences in the endpoints monitored and the monitoring conditions used by Moser, but, as was the case for the Eriksson et al. (2001) study, the mice were exposed to a single dose on PND 10 and then observed at two time periods several months later. The researchers observed hyperactivity in BDE-47-treated animals, and this effect tended to increase (worsen) with age. These results are similar to Eriksson et al. (2001) for BDE-47 as well as Viberg et al. (2004a) for BDE-99, Viberg et al. (2003a) for BDE-153, and Rice et al. (2007) for BDE-209. Altogether, these studies support the findings of Eriksson et al. (2001) that exposure to these PBDE congeners in early development can result in lasting changes in the neurobehavioral activity of mice.

Charge Question 4. *Are the Eriksson et al. (2001) (BDE-47), Viberg et al. (2004a) (BDE-99), Viberg et al. (2003a) (BDE-153), and Viberg et al. (2003b) (BDE-209) studies appropriate for determining the POD? Have the strengths and weaknesses of the Viberg and Eriksson studies been appropriately characterized and considered?*

Comment 1: All four reviewers believed that the critical study for BDE-47 (Eriksson et al., 2001) along with those for the other congeners (Viberg et al., 2004a [BDE-99], Viberg et al., 2003a [BDE-153], and Viberg et al., 2003b [BDE-209]) were appropriate for determining the PODs. One reviewer felt that these data were appropriate as long as the document emphasizes that the neurochemical data for other congeners show alterations in neurotransmitter levels and brain chemistry that are consistent with the neuromotor changes observed. Another reviewer noted that Eriksson et al. (2001) appeared to be the only study available for determining the

BDE-47 POD. None of the reviewers suggested alternative studies for determining the POD. Two reviewers explicitly stated that the strengths and weaknesses were identified and clearly presented.

Response: No response needed.

Charge Question 5. *Have the most appropriate critical effect and POD been selected? And has the rationale for the POD been transparently and objectively described?*

Comment 1: All four reviewers agreed with the selection of the neurobehavioral effects as the critical effects for BDE-47 and that these effects were appropriate for identifying a point of departure. One of the reviewers felt that the neurochemical data for some of the other congeners also provided critical information and should be presented centrally rather than as supporting data. One reviewer stated that there was no correlation between PND 10 of exposure and the concentration of the chemical in the brain. One reviewer added that the individual motor activity data on decreased habituation might be as appropriate as or more appropriate than the habituation ratio as an indicator of toxicity, while another believed that the actual behavioral data, rather than the habituation ratio, should have been presented in the “Toxicological Review.” This reviewer recommended that the Agency attempt to recover the neurobehavioral toxicity data from the study authors.

Response: The document presents the hypothesis proposed by the Eriksson/Viberg group based on data for BDE-99, -153, and -209 that attributes the observed effects on locomotion and habituation to impaired development of the cholinergic system during the postnatal brain growth spurt. However, brain development is complex, and many neurochemical changes that occur during the postnatal growth spurt and could impact motor activity have not yet been investigated. Therefore the available data, although supportive of the critical effects, are insufficient to establish a mode of action. For BDE-47 this discussion is presented in section 4.5.3 of the “Toxicological Review.”

Staskal et al. (2006b) examined tissue disposition in neonatal (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 (>96% purity; ~5 µCi/mL) 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 in adult rats from the Staskal et al. (2005) study discussed above. The percent of dose/g brain tissue in 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 but lower than those at 24 hours; the 10-day concentration in pups was about twice the 5-day value and significantly higher than in adults. These data are presented in section 3.2.2 of the “Toxicological Review.”

There are no measurements of levels of BDE-47 in the brain or other tissues at the time of neurobehavioral testing at 2 or 4 months to show if any differences exist in the brain or other tissues at those time points.

The actual motor activity components (locomotion, rearing, and total activity) that gave rise to the habituation ratios were reported in graphical form in the published paper and could not be reasonably estimated as presented. The Agency’s attempts to obtain the raw data from the authors were unsuccessful. Thus, the habituation ratios, rather than the actual motor activity data, served as the basis for determining the POD. Increased discussion of the habituation ratio and its derivation was added to section 4.3.

Comment 2: Two reviewers thought that the rationale for the POD had been transparently and objectively described. Another reviewer felt the document provided clear rationalization for the selection of the POD. None of the reviewers made a comment suggesting that the rationale for the POD was not appropriately described.

Response: No response needed.

Comment 3: One reviewer questioned the decision to not consider the changes in thyroid hormone levels reported by Hallgren and Darnerud (2002) to derive the RfD.

Response: Hallgren and Darnerud (2002) reported a decreasing trend for plasma levels of free T₄ that was significant only at 18 mg/kg-day. No effects in thyroid morphology were seen at any dose. 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. Thus, the LOAEL (18mg/kg-day) was greater than that observed in the Eriksson et al. (2001) study (10.5 mg/kg-day). No effects on T₄ levels were observed over the same dose range in the not yet published study by the Moser research group (e-mail from Ginger Moser, National Health and Environmental Effects Research Laboratory, Research Triangle Park, North Carolina, to Samantha Jones, U.S. EPA, dated August 14, 2007). The neurobehavioral effects were shown to be more sensitive than the thyroid effects.

Charge Question 6. *Have the rationale and justification for each UF selected in the draft toxicological reviews of BDE-47, -99, -153, and -209 been transparently described? If the selected UFs are not appropriate, what alternative UFs would you suggest and what are the scientific rationales for those suggested? Does the database support the determinations of the RfDs for BDE-47, -99, -153, and -209?*

Comment 1: Two reviewers agreed that the document described the rationale and justification for each UF, and another reviewer noted that the selection of the UFs was described in detail.

Response: No response needed.

Note: The peer reviewers provided fairly extensive comments about the individual components of the combined UF. For that reason the following reviewer comments and EPA responses have been grouped by the area of uncertainty to which they apply.

Comment 2: Two reviewers suggested lowering the intrahuman UF_H. One of these reviewers felt that the relatively specific and sensitive nature of the neurobehavioral and neurochemical measures compared with conventional endpoints warranted a decrease in the UF_H. The other reviewer recommended decreasing the 10-fold UF_H to threefold based on the sensitivity of the test species population (neonates).

Response: There is little information that applies to either toxicokinetics or adverse effects of exposure to BDE-47 in humans, and, in the absence of data, there is no scientific rationale for moving away from the default value for the intraspecies UF. However, additional explanation for default intraspecies UF was added to section 5.1.3.

Comment 3: One reviewer suggested decreasing the interspecies UF_A considering the relatively specific and sensitive nature of the neurobehavioral and neurochemical measures compared with conventional endpoints. However, another reviewer felt that the 10-fold UF_A was justifiable based on the lack of data on the mode of action in animals and humans.

Response: The 10-fold UF_A for interspecies uncertainty is retained based on the lack of mode-of-action, toxicokinetic, and human data that would sufficiently illustrate the similarities and differences for the effects of BDE-47 in animals and humans. However, additional explanation for applying the default interspecies UF_A was added to section 5.1.3.

Comment 4: One reviewer disagreed with the treatment of a single-dose experiment as equivalent to a subchronic exposure when applying a UF to account for differences in exposure duration. This reviewer stated that the principal study needs to be treated as a single-dose study and not a subchronic study. The reviewer also felt that the threefold UF_S was inappropriate and suggested raising the UF_S from 3 to 10 to consider the extent to which the mother's pre-pregnancy accumulated body burden would influence the developmental outcome, especially since these data are unavailable. One reviewer felt that the accumulation of the chemical should be considered in the calculation of the RfD. One reviewer agreed with the application of a threefold UF_S, recognizing that for the observed neurobehavioral effects the timing of exposure is more critical than the duration of exposure. This reviewer regards the UF_S as accounting for uncertainty from lack of prenatal exposure rather than uncertainty regarding potential effects of chronic exposure. One reviewer suggested that the threefold UF_S may not be necessary considering that exposure during a window of susceptibility indicates that chronic exposure may not necessarily result in greater adverse effects.

Response: For BDE-47, the principal study identified endpoints that, for the most part, reflect an impact during a critical period of postnatal brain development in mice. The hypothesized window of susceptibility, proposed by the study authors, is based on the observation that the developmental neurotoxic effects observed following exposure to BDE-47 on PND 10 will not occur once the toxicokinetics of intestinal uptake and excretion have matured and the animal brain is developmentally less active (outside the window of susceptibility). The Eriksson/Viberg group has suggested that the period of maximum vulnerability for the developing cholinergic system that coincides with the most pronounced neurodevelopmental effects from BDE-99 exposure is from PNDs 10–14. The UF_S was viewed as a dosing duration adjustment rather than simply a comparison of the effects of a subchronic to a chronic exposure, data that are lacking for BDE-47. A threefold UF_S was applied because the critical study dosed the animals only once within the hypothesized critical window, not because the chronic exposures would have exacerbated the impact on habituation.

In response to the comment concerning accumulation of the chemical, the Agency acknowledges that this is an important uncertainty and that it was considered as part of the database UF_D. As discussed in the response to Charge Question 7, many of the variables needed to determine body burden for BDE-47 from exposures during pregnancy and lactation are not available. Accordingly a UF as described above was applied. There are no data for BDE-47 from any conventional studies of chemical toxicity that can be used to determine whether or not long-term exposures might identify effects that are co-critical with the sensitive neurodevelopmental toxicity identified in mice following a single dose exposure during its critical window.

Comment 5: One reviewer recommended applying a threefold UF to the BMDL POD to account for uncertainty in extrapolating from a dose of non-negligible toxicity (BMDL₁₀) to a dose of negligible toxicity.

Response: A UF_L for LOAEL-to-NOAEL extrapolation was not used because the Agency's current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a change in the mean equal to 1 SD of the control mean was assumed to represent a minimal biologically significant change. The rationale for not applying UF_L has been added to section 5.1.3.

Comment 6: One reviewer disagreed with the use of a 10-fold database UF_D stating that "if the database is so uncertain as to require a UF_D of 10, then the database is too limited to allow the derivation of meaningful RfDs." This reviewer recommended a value of 1 based on the relatively sensitive nature of the neurobehavioral endpoint, the consistent observation of the neurobehavioral effects across the four PBDE congeners, and the availability of the dose-response data for deriving a BMDL. Another reviewer commented on the specificity and sensitivity of the neurobehavioral and neurochemical measures and stated that it is inappropriate to apply the database UF_D. This reviewer observed that this UF "addresses questions that go beyond this endpoint and focus on risks that might occur, but there are no relevant data." The reviewer felt that this UF_D is more appropriately applied at the point of risk management. The other two reviewers did not comment specifically on the database UF_D but noted generally that the database was poor and the overall confidence in the assessment is low (see next comment).

Response: EPA's practice is to apply a database UF_D, generally ranging from 1–10, in the health assessment to account for the potential for deriving an underprotective RfD as a result of an incomplete characterization of a chemical's toxicity because of missing studies. In deciding to apply this factor to account for deficiencies in the available data set and in identifying its magnitude, EPA considers both the data lacking and the data available for particular organ systems as well as life stages. EPA acknowledges that the principal study (involving postnatal exposure) has identified what appears to be a relatively sensitive effect; however, the database for BDE-47 lacks a prenatal toxicity study and a two-generation reproduction study, as well as subchronic and chronic toxicity studies. In light of the inadequate nature of the database, the Agency retains a 10-fold UF_D. The "IRIS Summary" and "Toxicological Review" contain sufficient information on the rationale for the database UF_D to allow a risk manager to consider the impact of this UF during the risk management process.

Comment 7: Two reviewers believed the database supports the determination of the RfD but stated that the overall confidence in the RfD assessment is low. Another reviewer believed the database is very poor and suggested that the RfDs be acknowledged as temporary while waiting for additional studies that increase confidence.

Response: The statement that the overall confidence in the RfD is low is included in the “Toxicological Review” in section 6.2. The Agency does not develop temporary RfDs for IRIS assessments. However, the availability of new information is one of the factors considered in selecting a chemical for reassessment

C. Body Burden Approach

Charge Question 7. *Are there adequate data for considering body burden as an alternative dose metric to administered doses in any of the RfD derivations?*

Comment 1: All four reviewers agreed that the data were inadequate to consider body burden as an alternative dose metric for the derivation of the RfD. Two of the reviewers stated that body burden is a possible alternative but the data are too limited.

Response: EPA examined the data to determine if a body burden approach could be used for BDE-47 during the development of the “Toxicological Review.” It was determined that existing half-life, exposure, metabolite, and mode-of-action data could not support a body burden calculation.

Charge Question 8. *Do you agree with the rationale described in the “Toxicological Review” of BDE-47 that the data on the window of susceptibility of the cholinergic receptors to BDE-47 tend to minimize body burden concerns?*

Comment 1: Three reviewers stated that the question was unclear. One reviewer accepted the concept as a basis for the experimental design, given the available information. A second reviewer stated that there was no direct evidence that BDE-47 directly affects cholinergic receptors and suggested that the mechanism of the interaction must be complex and indirect. A third reviewer stated that, although there are no definitive data on mode of action, this hypothesis is plausible. This reviewer acknowledges that the data on the window of susceptibility of the cholinergic receptors to BDE-47 are suggestive but believes there are too many other possibilities for mode of action for this rationale to minimize body burden concerns.

Response: Mode of-action data available that describe the developmental neurotoxicity of BDE-47 are limited. The Eriksson/Viberg group, the principal study authors, have hypothesized that the observed effects on locomotion and habituation are related to impaired development of the cholinergic system during the postnatal “brain growth spurt” period (Viberg et al., 2005, 2004a, 2003a). They have further hypothesized that the sensitivity of the cholinergic system occurs in the vicinity of PND 10 and have tested this hypothesis by varying the time of dosing and observing differences in the habituation effect for BDE-99 and -209 (Viberg et al., 2007; Eriksson et al., 2002). The resulting deficit in cholinergic receptors persisted across the duration of testing and could cause a hyperactive response to exposure to cholinergic stimulants in adulthood. Testing of this hypothesis has not been conducted in studies of BDE-47. The following statement has been added to the mode-of-action summary (section 4.5.3): “While some evidence exists that demonstrates PBDEs may have an impact on neurochemical events during early postnatal development, data are inadequate to determine the mode of action for BDE-47.”

Miscellaneous Comments

Comment 1: Three reviewers felt that the assessment would benefit from the combination of the individual documents for the four congeners into one comprehensive document to compare and cohesively present the similarities and differences among the congeners.

Response: The Agency has recently completed IRIS assessments for four individual PBDE congeners: BDE-47, -99, -153, and -209 (see Foreword). These congeners were selected based on frequent detection in human tissues and the environment, the availability of animal toxicological studies suitable for human health assessment, and their common occurrence in commercial PBDE mixtures. Although there is some repetition in the four documents, and similarities in the design of many studies, the outcomes are sufficiently different from one congener to another to support the separation of the four IRIS toxicological reviews. This improves reader comprehension and keeps the information on each congener separate from that of the others. Keeping the congeners separate improves the ability of the user to navigate the toxicological reviews and find the appropriate congener-specific data. However, in response to the comments from the peer reviewers, the Agency has increased the text that compares the data derived from comparable methodological approaches of BDE-47 to that for the other congeners evaluated.

Comment 2: One reviewer noted that the document failed to cite the purities of the radioactive chemicals in most of the studies, position of the label, location of radioactivity in the brain, and

specific activities of the ^{14}C compounds. Another reviewer felt that it was important to be careful when comparing the data on levels of radiolabel in tissues with data from direct chemical measurements. The reviewer felt that some of the radiolabel data, especially when the label was given after a period of dosing with unlabeled compound might have been misinterpreted.

Response: The requested data were added to the descriptions of the pertinent studies (in section 3.) when they were provided by the authors of the paper. Frequently, the position of the radiolabel was not specified. In a few cases the radiolabel was described as “uniform,” suggesting that all carbons carried the radiolabel. If the authors of the paper used the term “uniform,” it has been added to the discussion of the study. No change was made if the authors of the paper did not comment on the position of the radiolabel. In presenting the results of the studies where the radiolabel was given after a period of dosing with unlabeled compound, the conclusions were checked against those expressed by the authors of the studies and made consistent if necessary.

Comment 3: One reviewer was concerned that the doses and concentrations of the compound and the metabolites in biological tissues were presented in differing units (i.e., μmol , μg , and percent of dose).

Response: Doses and concentrations are reported as given by the authors. If a dose was given in molar or mole units per unit body weight, the doses have been provided parenthetically as mg/kg body-weight values. Otherwise the units are those provided in the published papers.

Comment 4: One reviewer suggested including a metabolic pathway that integrates the available data on the metabolites that have been identified.

Response: A diagram of a proposed metabolic pathway for BDE-47 has been included as Figure 3-1 in section 3.3. The diagram is based on the metabolites that have been identified in several studies. The metabolic pathway is described as “proposed” and uncertainties are indicated. The individual publications that provided data on metabolites are cited in Figure 3-1.

Comment 5: One reviewer acknowledged that developmental neurotoxicity is consistently observed following exposure to the PBDEs despite very different patterns of metabolism, distribution, and persistence within the body. This reviewer recommended rationalizing the relative potency of the PBDEs considering the differences in the extent of metabolism.

Response: Information is currently insufficient to adequately identify differences in the potency of the four congeners based on the metabolism data. The metabolite data are primarily

qualitative rather than quantitative. There are no data that indicate whether it is the parent compound and/or metabolites that are responsible for the adverse effects.

Comment 6: One reviewer suggested that the Agency provide conclusions on the extent of metabolism and the presence of metabolites in excreta for the PBDEs or provide a statement if conclusions cannot be drawn. One reviewer suggested the addition of a summary at the beginning of the toxicokinetics section to reduce potential confusion.

Response: An overview to the toxicokinetics section has been added to section 3 that provides an integration of the toxicokinetic data for mice and rats and identifies any differences seen between species. Most of the metabolite data are qualitative rather than quantitative.

Comment 7: One reviewer recommended presenting the receptor site interaction information in a summary table.

Response: A summary table for the receptor studies has been added to section 4.4 of the “Toxicological Review.”

Comment 8: One reviewer recommended the use of a table summarizing the developmental and reproductive effects of the BDE-99 and suggested adding a similar table to the BDE-47 document.

Response: Considering the lack of other studies to compare to the principal study a table is not warranted for BDE-47.

Comment 9: One reviewer recommended adding data for BDE-209 found in human biological media to Table 3-2, Median PBDE congener concentrations in human biological media in the U.S.

Response: Data for BDE-209 were not included in Table 3-2 for BDE-47 because, except for the She et al. (2007) study, data on median concentrations of BDE-209 were not provided in the references used to develop the table.

Comment 10: One reviewer felt that the text that compares levels of congeners in human biological media for the U.S. to that of other countries and the relative differences in the congeners identified are important issues and the studies need to be presented to support or refute the discussions of differences observed.

Response: The Agency has provided information on levels in biological media that reflect exposure in the U.S. and other countries for comparison purposes. Although the data reflect exposure, they are not direct measurements of exposure, given that there is some metabolism of PBDEs generating metabolites and, in the case of BDE-209, less highly brominated PBDEs. Therefore the data presented are not data on total exposures to any of the congeners. While the Agency agrees that exposure analysis is a critical component of risk assessment, a comprehensive presentation and analysis of exposure data is outside the scope of the IRIS health assessment.

Comment 11: One reviewer stated that the large number of bromine atoms of the PBDEs can impart electrophilic and lipophilic properties to the aromatic ring of the chemical and also noted that oily vehicles (e.g., corn oil) were used in most of the in vitro and in vivo animal studies. This reviewer was concerned that the vehicle could significantly alter the distribution and tissue uptake of the PBDEs between the oily vehicle and the biological system. These conditions could lead to decreased absorption and distribution with subsequent alteration in metabolism and excretion.

Response: The lipophilicity of the BDE-47 is acknowledged in the “Toxicological Review” as part of section 3 on toxicokinetics. It will be necessary to determine if absorption occurs via the chylomicrons along with the body lipids or via direct membrane transport before the full impact of the vehicle on absorption, distribution, metabolism, or excretion can be determined. The data are currently inadequate to determine the impact of the oily vehicle on the distribution and uptake of BDE-47.

Comment 12: One reviewer noted that the antithyroid effects observed with DE-71 (a formulation that might be reasonably linked to neurodevelopmental effects observed with BDE-47) in Zhou et al. (2002) occurred at doses that are higher than those that produce the neurodevelopmental effects of BDE-47. The reviewer suggested that it must be concluded that the neurodevelopmental effects cannot be linked to the antithyroid effects of these compounds. Additionally, the antithyroid effects have not been substantiated.

Response: Although the Zhou et al. (2002) study in rats observed a significant decrease in T₄ in offspring after exposure to 10 mg/kg-day, DE-71 is a commercial mixture and cannot be considered to be fully equivalent to BDE-47. BDE-47 is 28% of the DE-71 commercial mixture.

Comment 13: One reviewer noted that the linear model (utilized in modeling decreases in the total activity habituation ratio in 4-month-old male mice [Eriksson et al., 2001]) fits best in terms of its having the highest p value; however, it also has the highest Akaike Information Criterion (AIC). This reviewer stated that this is not consistent with the approach utilized in the BDE-209 assessment, in which the lowest AIC is one of the criteria for model selection, and suggested that AIC be used consistently.

Response: The Agency reevaluated the approach utilized in modeling the data for BDE-47 and determined that, among the models that adequately fit the data, the linear model yielded the lowest AIC, indicating that it is the “best-fit” model for the total activity habituation data in 4-month-old male mice (Eriksson et al., 2001). The rationale for this decision is explained further in section 5.1.2 and Appendix B.

Comment 14: One reviewer felt that the use of a BMDL rather than a BMD as the POD should be explicitly stated.

Response: The following statement can be found in section 5.1.3: “[A] BMDL_{1SD} of 0.35 mg/kg was selected as the POD for the RfD.”

PUBLIC COMMENTS

The public commenters made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

Comment 1: One public commenter suggested that the Agency consider a body burden approach.

Response: The Agency presented this issue to the peer reviewers in the form of a charge question. In response to the charge question about use of a body burden approach for dose evaluation, the peer reviewers agreed that, whereas the body burden approach might be appropriate for some of the congeners given their lipophilicity and distribution to adipose tissue, data to support such an approach are not presently available.

Comment 2: One public commenter questioned the selection of Eriksson et al. (2001) as the principal study for the derivation of the RfD and questioned the methods utilized by the principal study authors.

Response: The Agency has included a detailed summary of the concerns with the study design and methods utilized in the principal study (see section 5.1.1). These issues were raised during the external peer review of the BDE-47 IRIS assessment. The peer reviewers acknowledged the limitations and concerns with the study; however, all of the reviewers agreed that this study was appropriate for derivation of the RfD for BDE-47 and that its limitations were transparently discussed in the “Toxicological Review.” Additionally, the neurobehavioral effects reported in Eriksson et al. (2001) are supported by an expanding body of literature on other PBDEs (Viberg et al., 2007, 2005, 2004a, b, 2003a, b, 2002; Kuriyama et al., 2005; Eriksson et al., 2002; Branchi et al., 2002) that details changes in motor and cognitive activity in rodents following administration of single or repeated perinatal doses of PBDEs.

APPENDIX B. BENCHMARK DOSE MODELING FOR BDE-47

The data from Eriksson et al. (2001) on BDE-47 exposure and its effect on habituation ratios were modeled using EPA's BMDS. A BMR corresponding to a change of 1 SD from the control mean was selected for use in determining the POD. The habituation ratio data were modeled as continuous variables by using the linear, polynomial, and power models available in BMDS. The Hill model was not able to be fit because the number of parameters that need to be estimated in this model exceeds the number of dose groups in the study. Version 1.3.2 of BMDS was used to fit the power model, while BMDS version 1.4 (beta) of BMDS was used to fit the linear and polynomial models. The reason different versions of the software were employed is that the continuous models in BMDS version 1.3.2 calculate incorrect degrees of freedom for p value determinations in some situations, but this problem has been corrected in BMDS version 1.4 (beta), which was therefore used for fitting the available linear and polynomial models. However, the power model in BMDS version 1.4 (beta) occasionally fails to converge, whereas version 1.3.2 is more successful in this regard; therefore, the power model was fit by using BMDS version 1.3.2, even though this version of the power model is known to sometimes provide incorrect degrees of freedom for goodness-of-fit tests. To prevent this problem, the degrees of freedom of the power model were checked, and, if errors were found, they were corrected manually.

Habituation ratios based on the spontaneous behaviors, locomotion, rearing, and total activity in male mice (Table B-1) were modeled as continuous variables. The test for equality of variances across dose groups failed for all data sets modeled, and so, for each model fit, the standard deviation was modeled as a power function of the mean. This variance model adequately (i.e., goodness-of-fit p value >0.10) predicted the observed variances for all endpoints.

Table B-1. Habituation ratio data for total activity in four-month-old male mice exposed to BDE-47

Dose (mg/kg-day)	Observed mean ^a	Observed standard deviation
0	13.1	2.86
0.7	16.2	4.25
10.5	79.7	11.7

^aEight animals per dose group were analyzed.

Source: Eriksson et al. (2001).

Results

The BMD_{1SD} and BMDL_{1SD} estimates from the continuous models in BMDS that provided an adequate fit to the mean habituation ratios based on locomotion and total activity are summarized in Table B-2. Although the power model was also fit to these data, there are more parameters in this model than dose groups, so a goodness-of-fit test could not be performed. All of the continuous models in BMDS fit to the habituation ratios based on rearing activity exhibited significant lack of fit, and thus the results from these models are not shown in Table B-1.

Table B-2. BMD_{1SD} and BMDL_{1SD} estimates based on BMDS continuous dose-response models that did not show significant lack of fit for habituation ratios based on locomotion and total activity in male mice exposed to BDE-47

Endpoint	Model	Age (months)	BMD _{1SD} (mg/kg)	BMDL _{1SD} (mg/kg)
Habituation ratio (locomotion)	Linear	2	0.54	0.39
	Polynomial (2 ^o)	4	1.6	1.4
Habituation ratio (total activity)	Polynomial (1 ^o)	2	0.54	0.39
	Linear	4	0.47	0.35

Based on the results shown in Table B-2, the BMDL_{1SD} estimates ranged from 0.35 mg/kg to 1.4 mg/kg, with most of the estimates nearer to 0.35 mg/kg than 1.4 mg/kg. Therefore, the BMDL_{1SD} of 0.35 mg/kg was selected as the POD. Figure B-1 shows the dose-response curve corresponding to the fitted linear model for mean habituation ratios based on total activity. The detailed BMDS model output is also presented below.

Habituation Ratio for Total Activity in Four-Month-Old Male Mice

Linear Model
BMR = 1.0 SD

```

=====
      Linear 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
=====
  
```

BMDS MODEL RUN

The form of the response function is:

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

Dependent variable = MEAN
 Independent variable = mg/kg
 Signs of the polynomial coefficients are not restricted
 The variance is to be modeled as $\text{Var}(i) = \alpha \cdot \text{mean}(i)^\rho$

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

Model	Likelihoods of Interest		
	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*log(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

Figure B-1. Linear model fit to habituation ratio data for total activity in four-month-old male mice exposed to BDE-47.

