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TOXICOLOGICAL REVIEW

OF

2,2',4,4',5,5'-HEXABROMODIPHENYL ETHER (BDE-153)

(CAS No. 68631-49-2)

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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2,2',4,4',5,5'-HEXABROMODIPHENYL ETHER (CASRN 68631-49-2)

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LIST OF ACRONYMS

Ah	aryl hydrocarbon
AhR	Ah receptor
BDE-153	2,2',4,4',5,5'-hexabromodiphenyl ether
BMD	benchmark dose
BMDL	95% lower bound on the BMD
CALUX	Chemical-Activated LUciferase gene eXpression
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service Registry Number
cDNA	complementary deoxyribonucleic acid
CYP-450	cytochrome P-450
ER	estrogen receptor
EROD	ethoxyresorufin O-deethylase
FOB	functional observational battery
heptaBDE	heptabromodiphenyl ether
hexaBDE	hexabromodiphenyl ether
IRIS	Integrated Risk Information System
IUPAC	International Union of Pure and Applied Chemistry
LOAEL	lowest-observed-adverse-effect level
lw	lipid weight
mRNA	messenger ribonucleic acid
MUP	major urinary protein
NOAEL	no-observed-adverse-effect level
octaBDE	octabromodiphenyl ether
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofuran
PCR	polymerase chain reaction
pentaBDE	pentabromodiphenyl ether
PND	postnatal day
PXR	pregnane X receptor
RfC	reference concentration
RfD	reference dose
T_3	triiodothyronine
T_4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
tetraBDE	tetrabromodiphenyl ether
triBDE	tribromodiphenyl ether
TTR	transthyretin
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',5,5'-hexabromodiphenyl ether (BDE-153). It is not intended to be a comprehensive treatise on the chemical or toxicological nature of 2,2',4,4',5,5'-hexabromodiphenyl ether.

The majority of the available toxicological information on the hexabromodiphenyl ether homolog group (CASRN 36483-60-0) relates to the congener 2,2',4,4',5,5'-hexabromodiphenyl ether or BDE-153 (CASRN 68631-49-2). Toxicological information related to other congeners in the hexabromodiphenyl ether homolog group is also discussed. However, this health assessment does not deal with commercial mixtures containing hexabromodiphenyl ether congeners as one of the ingredients present in the formulation. In addition to BDE-153, IRIS health assessments have also been prepared for three other polybrominated diphenyl ether congeners: tetraBDE-47, pentaBDE-99, and decaBDE-209. These four congeners are those for which toxicological studies suitable for dose-response assessments were available and 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).

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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',5,5'-hexabromodiphenyl ether (BDE-153).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153). 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/m^3) 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 (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute ($\leq$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 are 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 $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for BDE-153 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

Hexabromodiphenyl ether (hexaBDE) is one of the possible 10 homologs of polybrominated diphenyl ethers (PBDEs). Figure 2-1 shows the chemical structure of hexaBDE. The number of possible congeners of hexaBDE is 42, with International Union of Pure and Applied Chemistry (IUPAC) numbers 128 to 169. The IUPAC numbers and bromine substitution patterns of some congeners that have been investigated in various studies are given in Table 2-1.

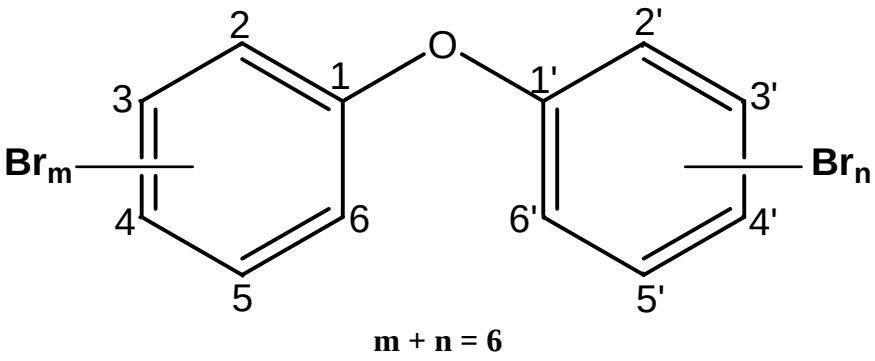

Figure 2-1. Chemical structure of hexabromodiphenyl ether.

Table 2-1. IUPAC number and bromine substitution pattern of some hexaBDE congeners

IUPAC number	Bromine substitution pattern
BDE-138	2,2',3,4,4',5'-HexaBDE
BDE-153	2,2',4,4',5,5'-HexaBDE
BDE-154	2,2',4,4',5,6'-HexaBDE
BDE-156	2,3,3',4,4',5-HexaBDE
BDE-159	2,3,3',4,5,5'-HexaBDE
BDE-166	2,3,4,4',5,6-HexaBDE

HexaBDEs are found in the commercial flame retardants penta- and octabromodiphenyl ethers (penta- and octaBDEs). The sole U.S. producer of the commercial pentaBDE and octaBDE mixtures discontinued their production in the U.S. at the end of 2004. Approximate composition by weight of these flame retardants is given in Table 2-2 (Great Lakes Chemical Corporation, 2003).

Table 2-2. Composition by weight of commercial penta- and octaBDEs

Homolog group	Commercial pentaBDE	Commercial octaBDE
Tribromodiphenyl ether	0–1%	–
Tetrabromodiphenyl ether	24–38%	–
Pentabromodiphenyl ether	50–62%	0.5%
Hexabromodiphenyl ether	4–12%	12%
Heptabromodiphenyl ether	–	45%
Octabromodiphenyl ether	–	33%
Nonabromodiphenyl ether	–	10%
Decabromodiphenyl ether	–	0.7%

The relative proportions by weight of different PBDE congeners in the commercial penta DE-71™ and octa DE-79™ are given in Table 2-3 (Great Lakes Chemical Corporation, 2003).

Table 2-3. Composition by weight of different congeners in commercial penta- and octaBDEs

Congener	DE-71™ pentaBDE	DE-79™ octaBDE
BDE-47	28%	<1%
BDE-99	43%	<1%
BDE-100	8%	Not detected
BDE-153	6%	14%
BDE-154	4%	2%
BDE-183	–	44%

Of the hexaBDE congeners, BDE-153 is present at higher levels than BDE-154 in both the penta- and octaPBDE commercial products. The predominant hexaBDE congener in environmental media, biota, and human tissues is BDE-153 (CASRN 68631-49-2), followed by BDE-154 (CASRN 207122-15-4). Physical and chemical properties of BDE-153 are listed in Table 2-4.

Table 2-4. Physical and chemical properties of BDE-153

Parameter	Value	Reference
Synonym	2,2',4,4',5,5'-Hexabromodiphenyl ether; benzene; 1,1'-oxybis-2,4,5-tribromo-; BDE-153	U.S. EPA (2004)
CASRN	68631-49-2	U.S. EPA (2004)
Chemical formula	$C_{12}H_4Br_6O$	U.S. EPA (2004)
Molecular weight	643.6	U.S. EPA (2004)
Vapor pressure (Pa) at 25°C	5.8×10^{-6}	Wong et al. (2001)
Melting point (°C)	183	Palm et al. (2002)
Solubility in water (μg/L)	0.9	Tittlemier et al. (2002); ATSDR[a] (2004)
Henry's law constant (Pa m^3 mol^{-1}) at 25°C	0.26	Cetin and Odabasi (2005)
Log octanol/water partition coefficient (K_{ow}) at 25°C	7.90	Braekevelt et al. (2003); ATSDR (2004)
Log octanol/air partition coefficient (K_{oa}) at 25°C	11.9	Chen et al. (2003)

[a]ATSDR = Agency for Toxic Substances and Disease Registry.

3. TOXICOKINETICS

Data on the toxicokinetics of the hexabrominated diphenyl ethers 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-153 in mice and rats suggest that absorption is about 70% in both species. The highest levels are found in adipose tissues, followed by muscle, liver, and skin. Metabolism appears to occur in mice to a greater extent than in rats although results differ among studies. Both species form hydroxylated metabolites via the activity of cytochrome P-450 (CYP-450) isozymes. There are no data for metabolites in rats; in mice, monohydroxylated metabolites with six, five, and four bromines have been identified. Small amounts of the hydroxylated metabolites may become conjugated with glutathione. Knowledge of the metabolic pathway and tissue distribution of the metabolites is limited. The parent compound and its metabolites are excreted with bile and feces and to a lesser extent in urine. Excretion in mice is assisted by a urinary transport protein; a similar protein has not been identified in rats.

3.1. ABSORPTION

There are no direct studies of BDE-153 absorption in humans. The data that demonstrate human absorption come from measurements of hexaBDE in human biological media after anthropogenic exposures but do not permit estimation of route-specific uptake parameters.

About 70% of a 0.6 mg/kg dose (1 μmol/kg) was absorbed in groups of five male or female F344 rats and B6C3F1 mice after gavage dosing with radiolabeled BDE-153 (96% purity; 27.8 mCi/mmol) in corn oil (Sanders et al., 2006). The estimate of absorption was based on the recovery of the radiolabel from the orally exposed animals as compared with the results from animals receiving the same dose injected intravenously.

3.2. DISTRIBUTION

The high K_{ow} of hexaBDE suggests a strong potential for accumulation in lipid-rich tissues. This property of hexaBDE is evident from the data on distribution in humans and experimental 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 quantitative information on exposure or the kinetics of absorption, tissue distribution, and 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 are available for human adipose tissue, liver, milk, and blood samples and indicate a tendency for PBDEs to distribute to these tissues. However, distribution studies have not been conducted in humans, and therefore it is not known whether hexaBDEs distribute to other tissues as well. The number of samples examined in various studies and countries is small and therefore the data should not be construed as representative at 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.

Table 3-1. Median PBDE concentration in human biological media in the U.S.

	Year[a]	N[a]	BDE-47	BDE-85	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	Σ PBDE	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	Schecter 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[b]	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)

[a]Year = year of sampling; N = number of donors.
[b]Seven serum pools with number of donors in each serum pool ranging from 40–200.

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 that 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 tetrabromodiphenyl ether (tetraBDE) (BDE-47), pentaBDEs (BDE-99 and -100), and hexaBDEs (BDE-153 and -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. Median concentrations of individual PBDE congeners are given in Table 3-1. The highest concentrations found were for tetraBDE, followed by penta- and hexaBDEs, a distribution that does not follow that of the commercial penta- or octaBDEs 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.

In a study in New York City, adipose tissue samples (n = 52) were collected in 2003–2004 from patients undergoing liposuction procedures (Johnson-Restrepo et al., 2005). Median concentrations of individual PBDE congeners are given in Table 3-1. BDE-153 and -154 were not detected. No significant difference was found in the concentrations of PBDEs between genders. 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.

Adipose tissues from persons living in the Tokyo area were collected from hospitals in 1970 (n = 10) and 2000 (n = 10). Women in their forties or fifties were selected (Choi et al., 2003). The samples were analyzed for BDE-28, -47, -99, -100, -153, -154, and -183. Median concentrations of the sum of these PBDEs were 0.03 and 1.3 ng/g lw in 1970 and 2000, respectively. In 2000, median concentrations in ng/g lw of PBDE congeners were, in decreasing order, BDE-47 (0.5), BDE-153 (0.4), BDE-100 (0.3), BDE-99 (0.1), BDE-28 (0.08), BDE-154 (0.06), and BDE-183 (0.05).

TetraBDE (BDE-47), pentaBDEs (BDE-85 and -99), and hexaBDEs (BDE-138 and -153) were analyzed in adipose tissue samples from 3 women and 10 men between the ages of 28 and 83 years and living in Spain for at least 10 years. BDE-85 and -138 were not detected. Mean concentrations, in decreasing order, were 1.8 ng/g lw BDE-153, 1.4 ng/g lw BDE-47, and 0.4 ng/g lw BDE-99. The predominant congener in both men and women in this study was BDE-153 (Meneses et al., 1999).

3.2.1.2. *Liver*

In a Swedish study, paired samples of human liver and adipose tissue obtained at autopsy from one woman (age 47) and four men (ages 66–83) were analyzed for nine tribromodiphenyl ether (triBDE) to hexaBDE congeners. PBDEs were found in all samples. TetraBDE-47,

pentaBDE-99, and hexaBDE-153 were the predominant PBDE congeners in both liver and adipose tissue. Generally, BDE-47 occurred at similar levels in adipose tissue and liver (mean approximately 2.7 ng/g lw). Mean concentrations of BDE-99 were higher in liver (3.8 ng/g lw) than in adipose tissue (1.3 ng/g lw). Average concentrations of BDE-153 were 2.1 and 1.0 ng/g lw in liver and adipose tissue, respectively. Lower concentrations were found for BDE-154, with average concentrations of 0.14 and 0.06 ng/g lw in liver and adipose tissue, respectively. Similarly to BDE-99, average concentrations of BDE-153 and -154 were therefore higher in liver than in adipose tissue, perhaps indicating a trend for selective accumulation in the liver with increasing degree of bromination (Guvenius et al., 2001).

3.2.1.3. *Human Milk*

In a study conducted in 2002 of levels of PBDEs in human milk in the U.S., 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 (Schecter et al., 2003). Mean and median total concentrations of tri- through decaBDEs were 74 and 34 ng/g lw, respectively. The maximum and mean concentrations of BDE-153 were 22 and 5 ng/g lw, respectively. Maximum and mean levels of the other hexaBDE congeners were 7 and 0.6 ng/g lw for BDE-138 and 7 and 0.8 ng/g lw for BDE-154, respectively, indicating wide variations of congener levels among nursing women. Median concentrations of individual PBDE congeners and total median concentration of PBDEs are given in Table 3-1. There was no apparent difference in concentrations between age groups or ethnic groups.

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 median concentration of 28 ng/g lw, followed by BDE-99 (5.4 ng/g lw), BDE-100 (5.3 ng/g lw), and hexaBDE-153 (4.8 ng/g lw). 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.

Breast milk was collected from 12 primiparous 24- to 33-year-old nursing women in Japan, at 1 month after delivery and analyzed for six tri- to hexaBDEs. The most abundant PBDE congener in human milk was BDE-47, followed by BDE-153, pentaBDEs (BDE-99 and -100), triBDE (BDE-28), and BDE-154. The sum of the concentrations of six tri- to hexaBDEs

ranged from 0.7 to 2.8 ng/g lw. There was a strong positive relationship between total PBDE levels in human milk and the frequency of fish consumption (Ohta et al., 2002).

In another study in Japan, 16 tri- to hepta-PBDEs were analyzed in eight pooled human milk samples collected between 1973 and 2000. PBDEs were not detected in the samples from 1973 at the limit of detection of 0.01 ng/g lw (Akutsu et al., 2003). In 2000, the sum of the concentration of these PBDEs was 1.4 ng/g lw and the predominant congeners were BDE-47 (0.5 ng/g lw), BDE-153 (0.3 ng/g lw), and BDE-99 and -100 (approximately 0.2 ng/g lw each). Concentrations of the remaining PBDE congeners, including BDE-154, were less than 0.05 ng/g lw. The relatively large concentration of BDE-153 in Japanese mothers' milk was explained by past use of a hexaBDE commercial product in Japan, consisting mostly of BDE-153 (Akutsu et al., 2003).

The breast milk concentrations of BDE-47, pentaBDEs (BDE-99 and -100), and hexaBDEs (BDE-153 and -154) were determined in samples from 93 primiparous women collected from 1996 to 1999 in Uppsala County, Sweden (Lind et al., 2003). The women ranged in age from 20–35 years. BDE-47 was the major congener (mean value 2.4 ng/g lw) and constituted 60% of the mean concentration of PBDEs of 4.0 ng/g lw, followed by BDE-99 and BDE-153 (0.6 ng/g lw each), BDE-100 (0.4 ng/g lw), and BDE-154 (0.07 ng/g lw). No significant relationship was found between breast milk concentrations of PBDEs and dietary intakes of PBDEs (through fish, meat/poultry, dairy products, and egg consumption), age, body mass index, alcohol consumption, or computer usage. After adjustments for these factors, a weak but significant association between PBDE concentrations and smoking was observed. Time-trend analysis for samples collected between 1996 and 2001 indicated a peak in total PBDE concentrations around 1998, followed by decreasing levels.

Pooled samples of breast milk collected at eight time periods between 1972 and 1997 from primiparous Swedish women were analyzed for tri- to hexaBDEs. In 1997, BDE-47 was the most abundant congener (2.3 ng/g lw), followed by BDE-99 and -153 (approximately 0.5 ng/g lw each), BDE-100 (0.4 ng/g lw), BDE-28 (0.2 ng/g lw), and BDE-154 (0.05 ng/g lw). The sum of the concentrations of these PBDE congeners in human milk increased from 0.1 to 4.0 ng/g lw during the 25-year period studied (Meironyte et al., 1999).

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. Seven tetra- to decaBDEs were analyzed in serum samples collected in the U.S. in 1988 from male blood donors (n = 12). The median serum concentration of the sum of tetra- to decaBDEs was 1.6 ng/g lw (Sjodin et al., 2001). In 2000–2002, the sum

of the median concentrations of six tetra- to hexaBDEs in serum pools collected in the U.S. increased to 61 ng/g lw. Median concentrations of the six individual PBDE congeners analyzed are given in Table 3-1. BDE-47 followed by BDE-99 and -153 were the most abundant congeners (Sjodin et al., 2004).

Serum samples from 24 immigrant Mexican pregnant women living in an agricultural community in California were collected during 1999 and 2001 (Bradman et al., 2007). Tetra-, penta-, hexa-, and heptaBDE (heptabromodiphenyl ether) 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, -99, -100, -153, and -154 of 11, 2.9, 1.8, 1.5, and 0.3 ng/g lw, respectively. There was no clear association 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 6 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). BDE-47 was the predominant congener for all ages, followed by hexaBDE-153, BDE-100, -99, and -154. 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 house dust and breast milk to contribute appreciably to the child and infant exposures. However, no firm conclusions can be drawn from this study, given the small number of subjects investigated.

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- to hexaBDEs. The total concentration of these PBDEs in men older than 60 years was 5.3 ng/g lw, with tetraBDE-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 each); and triBDE-28 (0.1 ng/g lw). The highest plasma total PBDE concentration was 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 burden. This may be explained by the fact that PBDEs are relatively new contaminants in the environment; the time period for human exposure is therefore relatively short, and different age groups (except the 0- to 4-year-old group) may thus have experienced a similar exposure period (Thomsen et al., 2002). The high level of PBDEs in the serum of the 0- to 4-year-olds could be due to higher exposure from

human milk and/or certain behavioral activity, such as crawling or sucking on flame-retardant materials.

Concentrations of PBDE congeners BDE-47, hexaBDEs (BDE-153 and -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). The sums of the median concentrations of these PBDEs were 3, 4, and 26 ng/g lw in the hospital cleaners, computer clerks, and electronic dismantlers, respectively. The median concentration of BDE-153 in serum was 0.6 and 0.9 ng/g lw in the controls and computer clerks, respectively, and 4.5 ng/g lw in the electronic-dismantling personnel. Serum concentrations of all PBDE congeners decreased in electronic-dismantling workers after vacation. The median decreases, standardized to 30 days of leave, were 14% for BDE-47, -153, and -154; 30% for BDE-183; and 66% for BDE-209. These results indicate shorter half-lives of the more highly brominated diphenyl ethers.

3.2.1.5. *Placental Transport*

Twelve paired samples of maternal and cord blood collected in 2001 from women presenting in labor in an Indiana hospital were analyzed for six tetra- to heptaBDE congeners (Mazdai et al., 2003). 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 and in Table 3-2 for comparison with a Swedish study (Guvenius et al., 2003) described below. TetraBDE-47 was the most abundant congener, followed by pentaBDE-99, pentaBDE-100, and hexaBDE-153. PBDE concentrations were highly correlated between mother and fetal sera, indicating that PBDEs cross the placenta into the fetal circulation. In addition, the results indicate that all tetra- through hepta-substituted congeners have approximately the same potential to cross the placenta. There was a decreasing trend in concentration of PBDE congeners in maternal and fetal sera with increasing degree of bromination.

Table 3-2. Comparison of median PBDE congener concentrations in maternal and fetal sera in Sweden and in the U.S.

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	0.83	25	0.98
PentaBDE-99	5.7	0.19	7.1	0.07
PentaBDE-100	4.2	0.17	4.1	0.07
HexaBDE-153	2.9	0.56	4.4	0.17
HexaBDE-154	0.3	0.04	0.7	<0.01
HeptaBDE-183	0	0.06	0	0.01
Σ PBDEs	37	2.07	39	1.69

[a]Mazdai et al. (2003) (United States): year of sampling 2001; number of donors 12.
[b]Guvenius 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 3-2). The levels of the higher brominated congeners, BDE-99 to -183, were higher in maternal blood than in cord blood, suggesting 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. There was no relationship between PBDE concentrations in the samples and frequency of fish consumption. 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).

In summary, median concentrations of PBDE congeners in the U.S. are available for human adipose tissue (Johnson-Restrepo et al., 2005; She et al., 2002), human milk (She et al., 2007; Schecter 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 adipose tissue, serum, and human milk are similar, although these studies were conducted in different regions of the U.S. (Table 3-1). The predominant congener found in adipose tissue, human milk, and blood samples in the U.S. is tetraBDE-47, followed by pentaBDE-99, pentaBDE-100, and hexaBDE-153, with current median concentrations in human biological samples of approximately 20, 7, 4, and 3 ng/g lw, respectively. HexaBDE-154 median concentration in human biological samples is usually ≤1 ng/g lw, except in adipose tissue, where BDE-153 and BDE-154 concentrations are comparable (~5 ng/g lw). Few measurements have been made of other PBDE congeners, such as triBDE and heptaBDE to decaBDE. Median concentrations of the sum of PBDEs measured in

human biological media are about 40 ng/g lw. These levels are substantially higher than the levels found in human populations in Europe or Japan.

3.2.2. Animal Data

In both F344 rats and B6C3F1 mice receiving a single 0.6 mg/kg dose (1 μmol/kg) of ^{14}C-labeled BDE-153 (96% purity; 27.8 mCi/mmol) by gavage, the highest concentrations of radiolabel were found in adipose tissue, muscle, skin, and liver when measured 24 hours after dosing (Sanders et al., 2006). The concentrations in adipose and muscle tissues of female mice were significantly higher than those of female rats, and there was a similar trend in the males, although the differences between mice and rats were not significant. The levels in the kidney, lungs, and brain were considerably lower than those in adipose tissue, muscle, skin, and liver. Concentrations in the lungs and brain of mice were two to three times higher than in rats, and the differences were statistically significant. The same pattern of tissue distribution was observed in C57BL/6 mice after intravenous dosing (1 mg/kg) in a study by Staskal et al. (2006). Tissue levels of BDE-153 were compared with those of BDE-47, BDE-99, and BDE-100 5 days after the animals received a radiolabeled 1 mg/kg dose. When measured as ng/g wet weight basis, the levels of BDE-153 in all tissues were higher than the levels of the other tested materials.

In the Sanders et al. (2006) study, when the same radiolabeled dose was given to male rats for 1, 3, or 10 consecutive days, the tissue concentrations increased with the duration of exposure. Tissue levels 24 hours after a 10-day exposure to 0.6 mg/kg-day were about twice as high in adipose tissue and skin as those following a single exposure to 6 mg/kg, demonstrating the tendency for repeat doses to accumulate in adipose deposits to a greater extent than after a single dose. On the other hand, the concentrations in the liver and other tissues (except thyroid and thymus) after the 6 mg/kg single dose were about twice those observed when the same total dose was distributed across 10 days of dosing (Sanders et al., 2006). The authors hypothesized that BDE-153 is minimally metabolized, allowing it to accumulate in the liver lipids before its gradual mobilization to adipose tissues.

Kodavanti et al. (2005) evaluated neuronal uptake by using cultures of cerebellar granule cells from 7- to 8-day-old Long-Evans rat pups. The cultures were treated with labeled PBDE-153 (0.05 μCi/mL) combined with 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, although accumulation for BDE-153 was considerably lower than that for BDE-99 and -47. When time was held constant and concentration varied, the percent cellular uptake decreased as the concentration increased from 0.67–30 μM, suggesting a limited ability to cross the cellular membrane.

3.3. METABOLISM

No information is available on the metabolism of BDE-153 in humans and little information is available in experimental animals. In F344 rats, almost all of the radiolabel in feces appeared to be parent compound (Sanders et al., 2006) in the 24-hour period after administration of BDE-153 on 1, 3, or 10 consecutive days or an equimolar mixture of BDE-47, -99, and -153 on 1 or 3 consecutive days. The total radiolabel extracted from the liver after 1 or 3 days of BDE-153 treatment was 85 ± 1% or 94 ± 1%, respectively. Unrecovered radioactivity (about 5–10%) was considered to be metabolites bound to liver proteins, and recovered material was considered to be unmetabolized BDE-153 dissolved in liver lipids and not yet transported to other tissues. These data are consistent with the hypothesis that there is minimal metabolism of BDE-153 in male rats.

The results from examination of the radiolabel in the feces and urine from mice exposed to 1 mg/kg-day BDE-153 intravenously by Staskal et al. (2006) are very different from those of Sanders et al. (2006). In mice, about two-thirds of the label in the feces and four-fifths of that in the urine were identified as metabolites. Metabolites were extracted, separated using chromatography, and identified by mass spectroscopy. Three isomers (Figure 3-1) were identified as monohydroxylated metabolites that retained all six bromines. Two isomers were identified as monohydroxylated metabolites that had lost one bromine. A single monohydroxylated isomer that had lost two bromines was identified as well as a trace amount of a sulfur-containing metabolite. Additional research is needed to determine whether there is a difference in the ability of rats and mice to metabolize BDE-153 or whether the differences in the results of Sanders et al. (2006) with rats and Staskal et al. (2006) with mice are methodological in origin. Much of the fecal BDE-153 from the oral Sanders et al. (2006) study was unabsorbed parent material, accounting for part of the difference. The BDE-153 in the 4-hour bile sample was also classified as unmetabolized.

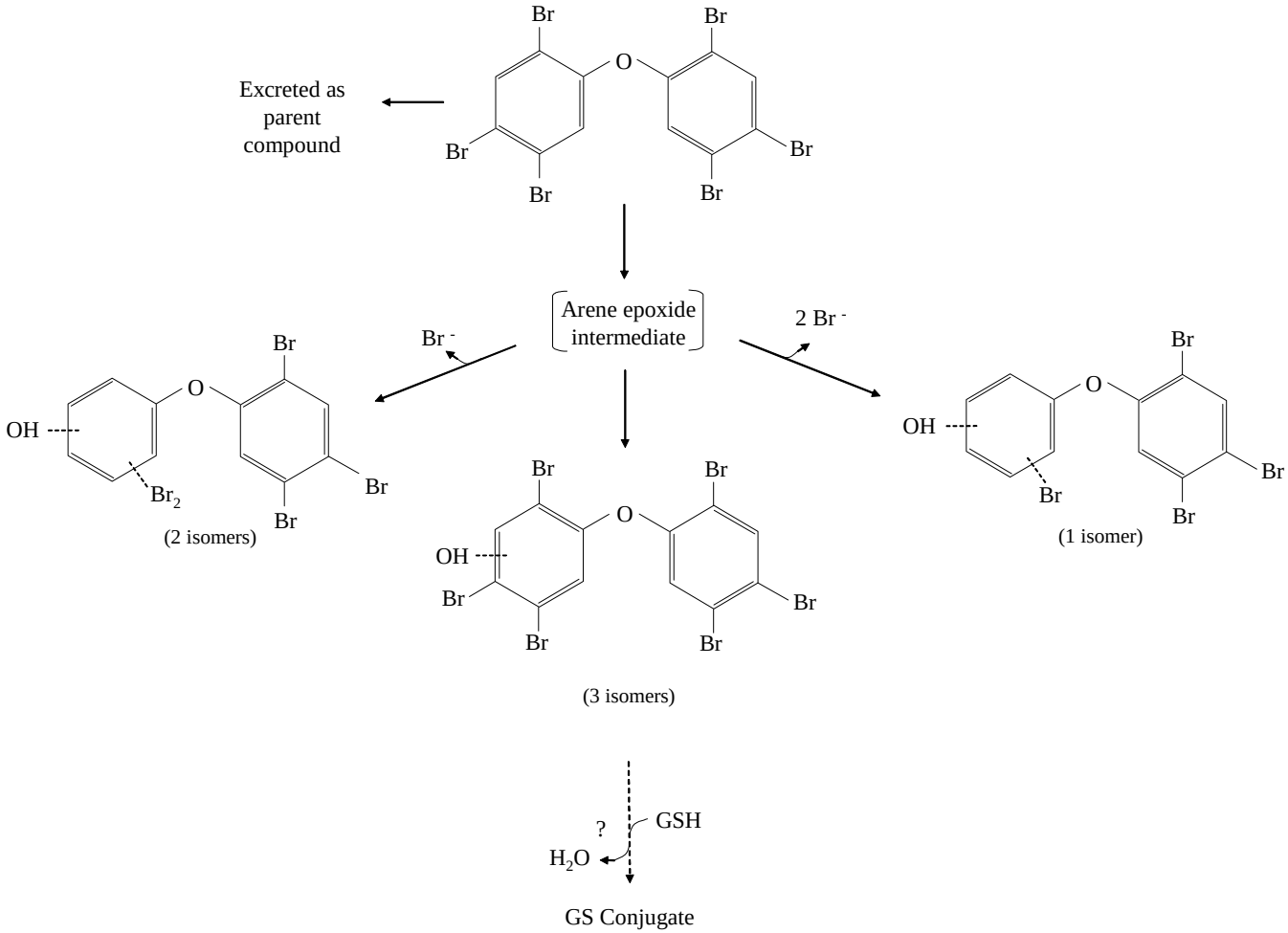

Figure 3-1. Proposed metabolic pathway for BDE-153 in B6C3F1 mice.

Source: Derived from Staskal et al. (2006).

Note: GSH = glutathione; ? = uncertainty about pathway.

Sanders et al. (2005) examined the effects of BDE-153 on several CYP-450 enzymes in the liver. The CYP-450s catalyze hepatic oxidation reactions for a variety of xenobiotic compounds. Accordingly, up-regulation of their expression or gene products can signify the potential for initial oxidation as part of the metabolic profile. Male F344 rats were treated with 0, 0.6, 6.4, or 64 mg/kg-day BDE-153 for 3 consecutive days and sacrificed 24 hours after the last dose. Messenger ribonucleic acid (mRNA) was isolated from a portion of the right medial lobe of the liver and converted to its complementary deoxyribonucleic acid (cDNA), using real-time polymerase chain reaction (PCR). Target gene amplification was evaluated by using specific probes for CYP-1A1, CYP-2B, and CYP-3A. These analyses indicated that CYP-1A1 expression was significantly up-regulated (19-fold) only with the 64 mg/kg-day dose of BDE-153. On the other hand, BDE-153 up-regulated expression of CYP-2B in a dose-related fashion for the 6.4 and 64 mg/kg-day doses. Expression of the mRNA was increased 20- to 30-fold at the highest dose. The expression of CYP-3A was up-regulated (sixfold) with the highest dose in two assays. In one of the two assays, the 6.4 mg/kg dose was associated with a fivefold increase in mRNA. The results of these assays support the concept that hydroxylated metabolites are produced by hepatic CYP-450 isozymes. The CYP-2B family isozymes appear to be favored over the CYP-1A family.

3.4. ELIMINATION

No information is available on the excretion of BDE-153 in feces or urine in humans. In F344 rats and B6C3F1 mice, about 30% of the radiolabel was found in the feces 24 hours after administration of a single radiolabeled 0.6 mg/kg dose compared to 4% in animals that received the same dose intravenously (Sanders et al., 2006). Based on the differences between the oral and intravenous routes, the authors concluded that most of the fecal material represented unabsorbed parent BDE-153. Only 0.5% of the dose was found in the bile of rats within 4 hours of administration, and all of that was present as parent compound. Staskal et al. (2006) found that cumulative fecal excretion in C57BL/6 mice was 12% 24 hours after a single 1 mg/kg intravenous dose and 18% 5 days after dosing.

Minimal radiolabel from BDE-153 was excreted in the rat urine (0.1%), and the percentage did not increase when the dose was increased from 0.6 mg/kg to 6 or 60 mg/kg. The amount in the urine of mice was higher than in rats, especially in male mice (1%); the level in female mice was 0.3%. The authors hypothesized that mouse-specific urinary carrier proteins used for chemosensory signaling may have been responsible for the higher levels of BDE-153 excreted in the urine of male mice. The excreted material appeared to be the parent compound rather than a metabolite. In the intravenous study of a slightly higher dose (1 mg/kg), Staskal et al. (2006) found that 55.1% of the urinary material in female C57BL/6 mice was bound to

protein 24 hours after administration. The protein was identified as mouse major urinary protein (MUP) isoforms MUP-2 and MUP-3.

There is a major difference between the results from the Sanders et al. (2006) and the Staskal et al. (2006) studies regarding the presence of metabolites in the feces. The oral Sanders et al. (2006) study in rats did not identify metabolites in the feces, whereas the intravenous Staskal et al. (2006) study in mice determined that more of the excreted BDE-153 was present as metabolites than as parent compound. The difference in results can partially be explained by the differences in the route of exposure (oral versus intravenous). It may also relate to the fact that Sanders et al. (2006) used 24-hour collections from male rats while Staskal et al. (2006) used female mice and the samples from a 5-day collection period. Additional research is needed to clarify the observed differences. Total urinary and fecal excretion of BDE-153 was lower than that from an equivalent 1 mg/kg dose of BDE-47, BDE-99, and BDE-100 in the study by Staskal et al. (2006).

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Little information is available on the absorption, distribution, metabolism, and excretion of hexaBDEs in humans. A model for human metabolism has not been established. The database for laboratory animals is more robust, but additional data on the effect of age on distribution and metabolism are needed. Extrapolation of results from laboratory animals to humans 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 for BDE-153 were not available.

To assess whether PBDEs may be detrimental to neurodevelopment, Mazdai et al. (2003) determined concentrations of PBDEs and total and free serum thyroxine (T_4) and triiodothyronine (T_3) 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 was no correlation between total PBDEs and T_3 or T_4 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 PBDE levels in breast adipose tissue of 23 California women, described in section 3.2.1 (She et al., 2002), there was no correlation between total concentrations of tetra- to hexaBDE in breast adipose tissues and disease status (malignancies, benign tumors, or ductal carcinomas in situ).

In summary, current evidence from these limited human studies does not support an association between exposure to PBDEs and adverse health outcomes in humans.

4.2. SHORT-TERM, SUBCHRONIC, AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

Inhalation and oral short-term, subchronic, or chronic toxicity/carcinogenicity studies of hexaBDE in experimental animals are not available.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

A study was conducted to determine whether habituation (spontaneous motor behavior in a novel environment), learning, and memory are affected in adult mice after neonatal exposure to BDE-153 (Viberg et al., 2003a). Another objective of this study was to investigate whether such neonatal exposure can affect the development of the cholinergic system by reducing the density of nicotinic receptors in the hippocampus of the mouse brain at approximately 6 months of age. Male neonatal NMRI mice were given single doses of BDE-153 (92.5% BDE-153, 7.5% heptaBDE-183) by gavage on postnatal day (PND) 10 at doses of 0, 0.45, 0.9, or 9.0 mg/kg dissolved in a 20% fat emulsion of egg lecithin-peanut oil (1:10) and water.

Motor activity was measured for a 60-minute period, divided into three 20-minute periods, in male mice at ages 2, 4, and 6 months. The tests were conducted in 10 mice randomly selected from the three to five litters that comprised each dose group at 2, 4, and 6 months of age. Motor activity 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 movements, shaking/tremors, and grooming]). From the spontaneous motor behavior test, an habituation ratio was calculated between the performance periods 40–60 minutes and 0–20 minutes for the three different variables (locomotion, rearing, total activity). This ratio was used to analyze alteration in habituation among 2-, 4-, and 6-month-old mice. Habituation is defined as the ability of the animals to adapt to a new environment and is characterized by initial investigation and exploration of their surroundings followed by a gradual acclimatization and acceptance of the new area.

There were no clinical signs of toxicity in the BDE-153-treated mice at any given time during the experimental period, nor was there any significant difference in body-weight gain or adult weight between controls and mice treated with BDE-153. In control mice, there was a distinct decrease in locomotion, rearing, and total activity at 2, 4, and 6 months of age, indicating habituation in response to the diminishing novelty of the test chamber over the 60-minute test period. Two-month-old mice exposed to 9.0 mg/kg BDE-153 displayed significantly less activity for all three test variables during the first 20-minute test period compared with controls, while during the third 20-minute period (40–60 minutes) they were significantly more active ($p \leq 0.01$). Mice receiving 0.9 mg/kg BDE-153, compared with the controls, showed significantly lower activity during the first 20-minute period for the rearing and total activity variables, but, during the third 20-minute period, they showed a significantly higher activity compared with the control animals for the two behavioral variables locomotion and total activity. No effects in activity for any of the three behavioral variables were seen in mice receiving 0.45 mg/kg BDE-153 compared with controls.

At 4 months of age, mice exposed to 9.0 mg/kg BDE-153 displayed the same pattern of activity (hypoactive during the first 20 minutes and hyperactive during the last 20 minutes) for all three behavioral variables: rearing, locomotion, and total activity. Mice receiving 0.9 mg/kg BDE-153, compared with the controls, showed significantly lower activity during the first 20-minute period for the rearing and total activity variables, but, during the third 20-minute period, they showed a significantly higher activity compared with the control animals for the locomotion and total activity variables. No effects in activity for any of the three behavioral variables were seen in mice receiving 0.45 mg/kg BDE-153 compared with controls.

Six months after neonatal exposure to BDE-153, the only effect seen in mice receiving the lowest dose of BDE-153 (0.45 mg/kg), compared with controls, was a marginal decrease in total activity during the first 20-minute period. However, since the total activity returned to

control levels during the last 20-minute period, this effect was considered of marginal significance. At 9.0 mg/kg, 6-month-old mice compared with controls were significantly hypoactive and significantly hyperactive during the first and last 20-minute periods, respectively, for all three variables. Mice receiving 0.9 mg/kg compared with controls were significantly less active and more active during the first and last 20-minute period, respectively, for the rearing variable. They were also significantly more active compared with controls for the locomotion and total activity variables during the last 20-minute period of the test but were not significantly hypoactive during the first 20-minute period.

The habituation capability in 2-, 4-, and 6-month-old mice concerning locomotion, rearing, and total activity decreased significantly with age at 0.9 and 9.0 mg/kg BDE-153 compared with controls. The lowest-observed-adverse-effect level (LOAEL) based on changes in spontaneous motor behavior, worsening with increasing age, was 0.9 mg/kg, and the no-observed-adverse-effect level (NOAEL) was 0.45 mg/kg.

Viberg et al. (2003a) also reported observations for the Morris swim maze test performed at 6 months of age in groups of 19–24 mice from each treatment group. This test was used to assess spatial learning ability and memory by measuring latencies in locating a submerged platform during the acquisition period (days1–4) and during the reversal learning period on the fifth day. For the acquisition phase of the swim maze test, the mice were first placed on the submerged platform for 30 seconds to stimulate learning. They were then tested for spatial memory by being released into the water from a set location and timed to see if they could find the submerged platform. If the mouse failed to reach the platform in the allotted time, it was once again placed on the platform to stimulate learning. Five such trials were carried out on each of the 4 acquisition days, and latencies in finding the platform were measured. On the fifth day the location of the platform was changed, and the mice were once more presented with the challenge of finding the submerged platform and given five trials.

In the swim maze test, mice exposed to BDE-153 at 0.9 and 9.0 mg/kg showed significantly longer latencies in locating the platform for the trials on days 2–4 of the acquisition period, compared with controls and mice exposed to 0.45 mg/kg. On day 5, after the platform was relocated in order to measure relearning ability in reversal trials, control mice and the 0.45 mg/kg dose groups displayed significantly ($p \leq 0.001$) longer latencies for finding the location of the platform in its new position. This is a normal behavior during relearning, since the mouse initially searches close to the previous location of the platform. Mice exposed to 0.9 and 9.0 mg/kg BDE-153 did not show any significant decrease in latency in the first trial on day 5. However, the latency observed with the fifth trial on day 5 was significantly ($p \leq 0.05$) longer than that of the controls for the 0.9 and 9.0 mg/kg BDE-153 exposed groups.

Changes in the cholinergic receptors have been proposed as affecting learning and memory. For this reason, 1 week after completion of the behavioral tests, six to nine mice in the

control, 0.9, and 9.0 mg/kg groups were sacrificed, and measurement of nicotine-binding sites in the hippocampus was performed by using tritium-labeled α-bungarotoxin, a snake neurotoxin that specifically binds to nicotinic cholinergic receptors. Density of nicotinic receptors in the hippocampus of controls and 0.9 mg/kg 6-month-old mice was not affected but was significantly decreased in mice given 9.0 mg/kg, a dose at which mice showed significant defects in learning and memory. The authors hypothesized that such changes in the cholinergic system (decrease in density of nicotinic receptors) may be one mechanism behind the neurodevelopmental effects of BDE-153. See section 4.4.1.5 for additional details concerning this component of the study.

The NOAEL for BDE-153 (92.5% purity) in this study (Viberg et al., 2003a) was 0.45 mg/kg, and the LOAEL was 0.9 mg/kg for changes in spontaneous motor behavior, worsening with increasing age, and for effects on learning and memory ability as displayed in the Morris swim maze test. Data for the three spontaneous behavior variables (horizontal movement, vertical movement, and total activity) and habituation ratio are only available in graphic form and could not be used for quantitative assessment.[1]

4.4. OTHER ENDPOINT-SPECIFIC STUDIES

4.4.1. Receptor Site Interactions

There is considerable evidence from studies of the PCBs, polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) 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 the PCBs suggest that PBDEs might activate the aryl hydrocarbon (Ah) receptor, estrogen receptor (ER), and/or androgen receptor sites. Based on the data from the well-studied PCBs, PCDDs, and PCDFs, the activation of these receptor sites is associated with immunosuppression, reproductive effects, and carcinogenesis (Klaassen, 1996; Bock, 1994), endpoints of interest for PBDEs. Table 4-1 provides a summary of the hexaBDE congeners that have been evaluated in a variety of receptor interaction studies.

[1]Attempts to obtain numerical values and other information on the data from the neurobehavioral studies were not successful.

Table 4-1. Receptor interaction studies of hexaBDE congeners

Endpoint evaluated	Congener					Findings
	138	153	154	156	166	
		X[a]				Effect levels are10^{-3} to 10^{-6} that of TCDD.[b]
Estrogen receptor		X				No estrogenic activity in the ER-CALUX[b] assay.
Androgen receptor		X				No antiandrogenic activity.
CAR[b]		X				Receptor interactions up-regulate expression of associated CYP-450 isozymes. CAR activation stronger than PXR[b].
PXR/SXR[b]		X				Receptor interactions up-regulate expression of associated CYP-450 isozymes.

[a]X indicates that the congener was tested for receptor effects; for most congeners several methodologies for evaluation of receptor interactions were employed.
[b]TCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxin; ER-CALUX = estrogen receptor-Chemical-Activated LUciferase gene eXpression; CAR = constitutive androstane receptor; PXR = pregnane X receptor; SXR = steroid X receptor.

4.4.1.1. *Aryl Hydrocarbon Receptors*

The transcription of the genes for CYP-450, -1A1, -1A2, and -1B1 is linked to a signal transduction cascade that is initiated by activation of the Ah receptor (AhR) 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). Enzymes of the CYP-450 family metabolically activate and metabolize polycyclic aromatic hydrocarbons and aromatic amines as well as PBDEs. Many substrates for CYP-450 enzymes are also AhR ligands. Differences in AhR 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 AhR through competitive binding assays and determined their ability to induce hepatic CYP-450 enzymes by means of ethoxyresorufin O-deethylase (EROD) assays (a biomarker for CYP-1A1/2 induction) in chick and rat hepatocytes, liver cell lines from rainbow trout, and rat and human tumor cell lines. HexaBDE congeners BDE-153 and -154 had AhR-binding affinities approximately 2×10^{-5} the binding affinity for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Quantitative measures of EROD induction were reported for BDE-153 and -154. BDE-153 was a very weak inducer in all cells, its relative induction potency being 10^{-5} to 10^{-6} that of TCDD. BDE-154 was not an inducer in any cell line.

Using hepatocyte cultures from Sprague-Dawley rats, Chen and Bunce (2003) investigated whether nine different PBDE congeners, including hexaBDEs, act as AhR agonists or antagonists at sequential stages of the AhR signal transduction pathway leading to CYP-1A1 induction. These issues are environmentally relevant because of the strong rank-order

correlation among strength of AhR binding, CYP-1A induction, and toxicity of many halogenated aromatic compounds. The hexaBDE congeners evaluated in this study were BDE-153 and -156.

BDE-153 and -156 were very weak activators of dioxin response element binding, showing maximum induction at about 10 and 40%, respectively, of that of TCDD but at concentrations four orders of magnitude higher than TCDD. When tested in combination with TCDD, BDE-153 and -156 tended to slightly inhibit the activity of a saturating TCDD concentration. BDE-156 induced responses of CYP-1A1 mRNA protein equivalent to the maximal response of TCDD in primary Sprague-Dawley rat hepatocytes, although at concentrations three orders of magnitude greater than TCDD. BDE-153 induced responses of CYP-1A1 mRNA protein equivalent to about 50% that of TCDD at concentrations four orders of magnitude greater than TCDD. Induction of CYP-1A1, measured through EROD assays, paralleled CYP-1A1 mRNA induction; BDE-153 and -156 gave weak to moderate responses, showing maximum CYP-1A1 levels 40–60% those of TCDD at concentrations approximately four orders of magnitude higher than TCDD. The authors concluded that PBDEs, including the hexaBDE congeners tested, contribute negligibly to dioxin-like toxicity compared with other environmental contaminants, such as PCBs and TCDD.

The possible dioxin-like effects of BDE-153 and -154 on induction of genes that encode metabolizing enzymes have also been investigated by Peters et al. (2004). AhR-mediated induction of CYP-450 enzymes CYP-1A1 and -1B1 was studied in human breast carcinoma (MCF7), human hepatocellular carcinoma (HepG2), and rat hepatoma (H4IIE) cells, using EROD activity as a marker for CYP-1A1 and -1B1 activity. BDE-153 and -154 (>98% purity) did not induce EROD activity when incubated for 72 hours at concentrations that were not cytotoxic (up to 10 μM) and, therefore, were not found to be AhR agonists in these cells. Additionally, exposure of the cells to BDE-153 or -154 did not increase mRNA levels of CYP-1A1 or -1B1, indicating no direct effect of these hexaBDEs on AhR gene expression.

Peters et al. (2006) examined the interaction of BDE-153 and -154 as well as other BDEs with the AhR in cultured liver cells from four healthy cynomolgus monkeys (three males and one female), using EROD activation as a biomarker for receptor activation. Both compounds were weak Ah agonists when coexposures of TCDD and the PBDE were tested, as evidenced by a decrease in the activation caused by TCDD alone. The impact of the PBDEs was receptor mediated rather than occurring by inhibition of the enzyme, since 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. Enzyme inhibition for BDE-153 and -154 was between 0 and 30%.

Villeneuve et al. (2002) examined the ability of BDE-153 to induce AhR-mediated gene expression in vitro, using H4IIE-luc (luciferase) recombinant rat hepatoma cells. The cells were grown in culture ViewPlates™ and then exposed to PBDE concentrations ranging from 2 to 500 ng/mL. Luminescence was measured and compared to the maximum response observed with a 1,500 picomolar TCDD standard (%-TCDD-max). A positive response was defined as any response that was greater than three standard deviations above the mean value for the control. BDE-153 failed to induce AhR-mediated gene expression in H4IIE-luc cells. These results are qualitatively consistent with those of Chen and Bunce (2003).

Sanders et al. (2005) used an in vivo approach to study AhR site activation by BDE-153 as well as several other PBDE congeners. Groups of F344 male rats (three/group), 10–12 weeks old, were dosed by gavage once daily for 3 days with BDE-153 (96% purity) in corn oil at 0, 1, 10, or 100 μmol/kg-day (0, 0.6, 6.4, or 64 mg/kg-day). 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 PCR. The resultant DNA samples were then analyzed to determine the expression of CYP-1A1, a protein linked to AhR activation.

BDE-153 had a significant effect on the level of CYP-1A1 expression (19 times that of vehicle-treated controls) only at 100 μmol/kg-day (64 mg/kg-day), making it a weak activator of the AhR. When the CYP-1A1 expression from BDE-153 was compared with those for tetraBDE-47 and pentaBDE-99, the impact on the AhR seemed to be correlated to the levels of polybrominated dibenzofuran contaminants 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 PBDEs are, at best, weak activators of the AhR. 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. *Other CYP-450 Induction Receptors*

The study of CYP-450 mRNA expression in rat liver by Sanders et al. (2005) (see section 4.4.1.1) found that expression of CYP-2B was up-regulated by BDE-153 in F344 rats to a greater extent than CYP-1A1, a biomarker for the activation of the AhR. CYP-3A was slightly up-regulated but to a lesser extent than the CYP-1A1 and -2B. CYP-2B and -3A are respective biomarkers for activation of the constitutive androstane receptor (CAR) and pregnane X receptor (PXR). In the case of BDE-153, the effect on the CAR was greater than that on the 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-153 on

these receptors is similar to the impact of noncoplanar PCBs on the same receptors; however, little is presently known about the physiological effects of stimulation of the CAR and PXR.

4.4.1.3. *Estrogen Receptors*

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

The in vitro estrogenic and antiestrogenic potencies of 17 PBDEs, including hexaBDE-138, -153, and -166, were investigated in a human T47 breast-cancer cell line based on ER-dependent luciferase reporter gene expression (Meerts et al., 2001). The modified T47D cells that contained ERα and ERβ 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 incubated. The luciferase activity was measured with a luminometer. The three hexaBDEs tested did not show any estrogenic activity in the ER-CALUX assay.

Villeneuve et al. (2002) examined the ability of 10 different PBDEs, including BDE-153 (99% purity), to initiate ER-mediated gene expression in vitro. At concentrations up to 500 ng/mL, BDE-153 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, BDE-153 as well as the other PBDEs tested did not show an appreciable capacity for displacing ^{3}H-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.

In summary, the mechanistic studies of the ER indicate that there was no activity of BDE-138, -153, or -166 at the concentrations tested and the binding affinity of the hexaBDEs was 50,000 times lower than that of estradiol.

4.4.1.4. *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, but not to doses of 0 or 3 mg/kg, during the peripubertal period. 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-153 (99% purity) and BDE-154 (97% 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 BDE-153 and -154 were incubated in the presence of 1.0 nM R1881 and 10 μM triamcinolone acetonide, an agent that blocks the progesterone and glucocorticoid receptors. BDE-154 but not BDE-153 showed very slight inhibition of R1881 binding. The maximum inhibition caused by the BDE-154 was 40% at the highest concentration tested of 33 μM.

In the assay using the MDA-kb2 cell line, BDE-153 and -154 were introduced at concentrations of 10 pM, 10 nM, 1 μM, or 5 μM in the presence of 0.1 nM of the receptor agonist dihydrotestosterone. Neither compound demonstrated any antiandrogenic activity in this assay.

4.4.1.5. *Acetylcholine Receptors*

As discussed in section 4.3, changes in the cholinergic receptors have been proposed as affecting movement, learning, and memory. Disturbances in the development of the cholinergic system have been demonstrated to occur during the brain growth spurt in humans and laboratory animals (Viberg et al., 2003a). In male mice, sensitivity of the developing cholinergic system to BDE-153 appears to peak about PND 10 (Viberg et al., 2003a). For this reason, 1 week after completion of the swim maze test, six to nine mice in the control, 0.9, and 9.0 mg/kg groups were sacrificed, hippocampal brain tissue was isolated, and measurement of nicotinic-binding sites was performed using tritium-labeled α-bungarotoxin. Parallel samples were treated with unlabeled α-bungarotoxin to correct for nonspecific binding. Specific binding was determined by calculating the difference in the amount of labeled toxin bound in the presence versus absence of unlabeled α-bungarotoxin. There were no significant differences in the density of nicotinic receptors in the hippocampus of controls and 0.9 mg/kg 6-month-old mice. However, receptor density was significantly decreased (20.6%) in mice given 9.0 mg/kg. The authors hypothesized that the decrease in the density of nicotinic receptors as a result of exposure to BDE-153 may be one mechanism contributing to the neurodevelopmental effects of BDE-153.

4.4.2. Thyroid Effects

Because PBDEs 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 transthyretin (TTR), one of the thyroid hormone-binding transport proteins in the plasma of vertebrate species. The possible interference of several hexaBDEs with T_4-TTR binding was investigated in an in vitro competitive binding assay, using human TTR and ^{125}I-labeled T_4 as the displaceable radioligand. The three hexaBDE congeners evaluated (BDE-138, -153, and -166) did not compete with T_4-TTR binding (Meerts et al., 2000).

Meerts et al. (2000) also tested these three hexaBDEs (BDE-138, -153, and -166) before and after incubation with differently induced hepatic microsomes to examine the ability of their hydroxylated metabolites to displace T_4 from TTR. The hexaBDEs 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 BDE-166 with CYP-2B-enriched rat liver microsomes resulted in the formation of metabolites that were able to displace 20–60% of the ^{125}I-T_4 from TTR. BDE-138 and -153 did not compete with T_4 for binding. No T_4-TTR displacement by hexaBDEs occurred after incubation with liver microsomes enriched with CYP-1A or -4A3. BDE-166 is therefore able to compete with T_4-TTR binding only after metabolic conversion by induced rat liver microsomes, suggesting an important role for hydroxylation. The relevance of this observation for humans has yet to be resolved. T_4-binding globulin, rather than TTR, is the major T_4-binding protein in humans.

4.4.3. Genotoxicity

Information is not available on the genotoxicity of hexaBDEs.

4.5. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.5.1. Oral

Alterations of behavioral parameters, namely impaired spontaneous motor behavior worsening with age, and effects on learning and memory capability have been shown to occur in adult male mice neonatally exposed to BDE-153 (Viberg et al., 2003a). 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-153 in the Viberg et al. (2003a) study raise concerns about possible neurobehavioral effects in children and adults. Similar neurodevelopmental effects have been observed in studies of the tetraBDE-47, pentaBDE-99, and decaBDE-209 congeners.

BDE-153 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 other countries. Fetuses and infants are exposed to BDE-153. Whether such exposures constitute a health risk for neurodevelopmental dysfunction in these population groups is not known at this time. An association between neonatal exposure to BDE-153 and neurobehavioral effects in humans has not been established.

4.5.2. Inhalation

No data are available on the toxicity of BDE-153 by the inhalation route of exposure.

4.5.3. Mode-of-Action Information

While evidence exists that demonstrates that BDE-153 (and other PBDEs) interact at the neurological level, data are inadequate to determine the mode of action for BDE-153. Researchers from the laboratory of Eriksson/Viberg 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). In the study by Viberg et al. (2003a), the density of nicotinic acetylcholine receptors in the hippocampus of 6-month-old mice was significantly decreased in mice given BDE-153 at 9.0 mg/kg, a dose at which mice showed significant defects in learning and memory, as reflected in the swim-maze performance test. The authors hypothesized that such a decrease in density of nicotinic receptors during development may play a role in the behavioral effects of BDE-153. Similar effects on cholinergic neurons were also observed in the testing of BDE-99 and -209 **(**Viberg et al., 2007, 2005, 2004a; Ankarberg, 2003).

The Eriksson/Viberg group 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). Impaired development of the cholinergic system during the postnatal "brain growth spurt" period could change adult responses to endogenous acetylcholine and other cholinergic agents. The resulting cholinergic receptor deficits persisted across the duration of testing, apparently causing a hyperactive response to exposure to cholinergic stimulants in adulthood.

Evidence that BDE-153 can enter the neurological system was reported by Staskal et al. (2006). BDE-153 was found in the brain tissue of C57BL/6 10-week-old mice when measured 5 days following intravenous administration of a single radiolabeled dose of 1 mg/kg BDE-153. Interestingly, the concentrations of BDE-153 distributed to the mouse brain tissue were significantly greater than those for the other measured PBDEs, BDE-47, -99, and -100 (Staskal et al., 2006). Additional evidence that BDE-153 reaches the brain and potentially interacts with

the neuronal growth and cognitive function was observed by Kodavanti et al. (2005) in which a dose-related increase in accumulation of BDE-153 was seen within 1 hour in cultures of cerebellar neurons and mixed cerebellar neurons and glia from neonatal Long-Evans rats (Kodavanti et al., 2005). Furthermore, uptake of BDE-153 by the neurons was coincident with translocation of protein kinase C. Kodavanti et al. (2005) note that concurrent uptake of BDE-153 and protein kinase C, which is associated with neuronal growth, memory, and learning, suggests a potential for interaction at the neuronal level; however, data showing this interaction for BDE-153 are unavailable.

The data on the impact of hexaBDE on the density of nicotinic acetylcholine receptors in the hippocampal area of the brain in combination with that on neuronal uptake of BDE-153 provide the strongest link to the neurodevelopmental effects observed after BDE-153 exposure. However, brain development is complex, and many neurochemical changes that occur during the postnatal growth spurt and that could impact motor activity have not yet been investigated. While evidence exists that demonstrates that BDE-153 may have an impact on neurochemical events during early postnatal development, data are inadequate to determine the mode of action for BDE-153.

Because PBDEs have some structural similarity with the thyroid hormone T_4, it has been suggested that interference with thyroid hormone transport by competitively binding to TTR, one of the thyroid hormone-binding transport proteins in the plasma of vertebrate species, may explain the neurobehavioral effects. The possible interference of several hexaBDEs with T_4-TTR binding was investigated in an in vitro competitive binding assay, using human TTR and ^{125}I-labeled T_4 as the displaceable radioligand. The three hexaBDE congeners evaluated (BDE-138, -153, and -166) did not compete with T_4-TTR binding (Meerts et al., 2000). Lack of binding with TTR suggests that diminished T_4 transport due to competitive binding of BDE-153 to the transport protein does not provide an explanation for the observed neurodevelopmental impairment following early life exposures. Thyroid hormone levels and behavioral activity were not co-measured in the study in mice of Viberg et al. (2003a), and, despite the possibility of interactions between BDE-153 and thyroid hormone levels, there are no mode-of-action data that link thyroid hormones to the neurobehavioral observations reported by Viberg et al. (2003a).

HexaBDE-153, -154, and -156 are, at best, weak activators of the AhR (Peters et al., 2006, 2004; Sanders et al., 2005; Chen and Bunce, 2003; Villeneuve et al., 2002; Chen et al., 2001). Congeners BDE-138, -153, and -166 did not show any estrogenic activity in tissue culture studies (Villeneuve et al., 2002; Meerts et al., 2001). Congeners BDE-153 and -154 do not exhibit antiandrogenic activity in an MDA-kb2 cell line containing the human androgen receptor and a luciferase reporter (Stoker et al., 2005). Lack of activity in these assays minimizes concern that hexaBDE is an endocrine disruptor through estrogen or androgen pathways. However, the implications of these interactions are unknown.

4.6. EVALUATION OF CARCINOGENICITY

Animal chronic toxicity/carcinogenicity studies have not been conducted for BDE-153. There is inadequate information to assess the carcinogenic potential of BDE-153 (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 Viberg et al. (2003a) with adult mice exhibiting neurobehavioral effects following neonatal exposure to BDE-153. 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 concern for early lifetime exposure (i.e., fetal or infant exposure) to the chemical. The identification of BDE-153 in human maternal and cord blood, milk, and plasma (Bradman et al., 2007; She et al., 2007; Mazdai et al., 2003; Schecter et al., 2003; Thomsen et al., 2002) implies humans are exposed to BDE-153 during a period of rapid development of the brain, indicating a potential for susceptibility. Whether exposure to BDE-153 constitutes a health risk for adverse neurodevelopmental effects in children is not known at this time because of the limited toxicological database for BDE-153. An association between prenatal or neonatal exposures to BDE-153 and neurobehavioral dysfunction in humans has not been established.

4.7.2. Possible Gender Differences

It is unknown whether susceptibility to BDE-153 differs in male and female humans or experimental animals. Two major urinary proteins promote excretion of BDE-153 in male mice once the pathways for synthesis become operational. This decreases retention of BDE-153 in juvenile and mature males compared to females but the pathway is not available during the early postnatal period of development (Staskal et al., 2006).

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

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

There is only one study available for dose-response assessment and derivation of an RfD for BDE-153. The study of Viberg et al. (2003a) identified a NOAEL and a LOAEL in mice for effects on spontaneous motor behavior, learning, and memory. In this study, male mice were administered single oral doses (0, 0.45, 0.9, or 9.0 mg/kg) of BDE-153 (92.5% purity) on PND 10, which is, according to Eriksson et al. (2002), a period of maximum vulnerability of the developing mouse brain. Adverse effects noted in adult mice at 0.9 and 9.0 mg/kg included hypoactive spontaneous motor behavior in the beginning of the test period, hyperactive behavior at the end of the test period (with these disturbances becoming more pronounced with increasing age), and effects on learning and memory capability.

There are several concerns regarding the design of the Viberg et al. (2003a) study. The study was conducted in male mice only. The protocol was unique and did not conform to health effects test guidelines for a neurotoxicity screening battery or developmental neurotoxicity studies (U.S. EPA, 1998b). The dosing regimen did not include gestation and lactation exposure (U.S. EPA, 1998a); only single doses were given. In some respects the observation that effects occurred with such limited dosing argues for the importance of this study. While the study design appears to identify a developmental window of susceptibility, it is not adequate to determine the effect of longer dosing. Translating the implications of these data to more traditional dosing regimens is problematic, particularly with regard to evaluating the implications of in utero and postnatal exposure. Another concern is that, based on the data provided in the published report, more than one pup per litter was used for the behavioral testing (10 mice were randomly selected from three to five different litters in each treatment group). Increasing the number of samples from each litter may bias the analyses towards false positives, and the observed neurobehavioral effects may be attributable to differences that are not treatment related in pups born to a single dam. Another concern regarding the study design is the limited number of neurobehavioral parameters (related to motor activity and cognitive function) that were assessed. The absence of a full functional observational battery (FOB) limits the ability to correlate the reported effects with other FOB parameters. This would be helpful in gauging the reliability of the parameters that were measured in the study. As indicated in the *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), it is assumed that an agent that produces detectable adverse neurotoxic effects in experimental animal studies will pose a potential hazard to humans. For BDE-153, in the absence of human evidence, data from experimental animal studies are used as the basis for the RfD.

While study design limitations cloud the utility of this study, several additional considerations support the use of these data. Acute exposure to a highly lipophilic chemical such as BDE-153 will result in exposure that lasts longer than the 1 day of dosing. In addition, there are a wide variety of brain structures that have very limited critical windows during development. These short critical windows translate to susceptible periods of exposure that can be very short. The concept that exposure during critical periods can induce functional neurological effects later in development has been demonstrated with structurally related PBDE congeners, including
tetra-, penta-, and decaBDEs (Kuriyama et al., 2005; Viberg et al., 2005, 2004a,b, 2003b, 2002; Ankarberg, 2003; Branchi et al., 2002; Eriksson et al., 2002, 2001). Therefore, the observed neurobehavioral effects in the Viberg et al. (2003a) study are biologically plausible, and exposure to BDE-153 may pose a potential hazard to humans (U.S. EPA, 1998a).

Taken together, these considerations support the use of the Viberg et al. (2003a) study for deriving the RfD for BDE-153.

5.1.2. Methods of Analysis

The study of Viberg et al. (2003a) in mice was not amenable to a benchmark dose (BMD) approach because the data needed for the use of a BMD approach are not available in the published study. Experimental data points for locomotion, rearing, and total activity and their standard deviations are displayed graphically, and such values cannot be determined with any accuracy from the graphs. Therefore, the NOAEL of 0.45 mg/kg is used as a point of departure for estimating the RfD for BDE-153.

5.1.3. RfD Derivation

A NOAEL of 0.45 mg/kg for BDE-153 was identified in the Viberg et al. (2003a) study. To calculate the RfD, a total uncertainty factor (UF) of 3,000 was applied: 10 for extrapolating animal data to humans (UF_A interspecies variability), 10 for susceptible human subpopulation (UF_H interhuman variability), 3 for extrapolating from a single dose to a lifetime exposure duration (UF_S), and 10 to account for a deficient database (UF_D). The rationale for application of the 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-153 in animals have been evaluated, no adequate description of toxicokinetics of BDE-153 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. 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-153. A default value is warranted because insufficient information is currently available to assess human-to-human variability in BDE-153 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 lifespan and is more quiescent during adult life stages. Many brain structures have a very limited critical window during development in early life. Following BDE-153 exposure, toxicokinetic data suggest that a mouse urinary protein becomes functional some time between PNDs 28 and 40, which leads to an increase in BDE-153 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-153 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 applied because a NOAEL was used as the point of departure.

A UF_D of 10 was used to account for database uncertainty. The available oral database for BDE-153 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.

Application of a total UF of 3,000 to the NOAEL of 0.45 mg/kg results in a reference dose for BDE-153 of 1.5×10^{-4} mg/kg-day or 0.2 μg/kg-day.

5.1.4. Previous RfD Assessment

The hexaBDE congener BDE-153 has not been previously assessed in IRIS. However, a health assessment of the hexaBDE homolog group (CASRN 36483-60-0) was previously entered

in the IRIS database in 1990 (U.S. EPA, 1990). Information was not available to derive an RfD or RfC or to assess the carcinogenic potential of the hexaBDE homolog group.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

No data are available for deriving a chronic RfC for BDE-153.

5.3. CANCER ASSESSMENT

There is inadequate information to assess the carcinogenic potential of BDE-153 (U.S. EPA, 2005a).

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

6.1. HUMAN HAZARD POTENTIAL

BDE-153 (CASRN 68631-49-2) is a component of the commercial penta- and octaBDE flame retardants. BDE-153 has been found in human milk, adipose tissue, and blood. As a result, fetuses and infants are exposed to BDE-153.

No data are available regarding the potential toxicity of BDE-153 in humans exposed via the oral route. However, the available animal data indicate that the nervous system is a sensitive target organ. Neurobehavioral developmental toxicity has been identified as the critical endpoint of concern in adult male mice following neonatal oral exposure to BDE-153 (Viberg et al., 2003a). Since fetuses and infants are exposed to BDE-153 via maternal and cord blood and human milk, such exposure may constitute a health risk for adverse neurodevelopmental effects in these population groups.

There are no studies of the potential carcinogenicity of BDE-153 in humans or experimental animals. Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess carcinogenic potential" of BDE-153.

6.2. DOSE RESPONSE

The RfD of 0.2 μg/kg-day (rounded from 1.5×10^{-4} mg/kg-day) for BDE-153 was derived from the study of Viberg et al. (2003a), which identified a NOAEL of 0.45 mg/kg and a LOAEL of 0.9 mg/kg for effects on spontaneous motor behavior, learning, and memory capability in mice. A total UF of 3,000 was applied to the NOAEL: 10 for interspecies variability, 10 for interindividual variability, 3 for extrapolation from single to lifetime exposure, and 10 for database deficiencies.

No data are available regarding the potential toxicity of BDE-153 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-153 among humans is unknown, representing another important area of uncertainty in the RfD. However, subpopulations expected to be more susceptible to BDE-153 toxicity are fetuses and children. Chronic studies relevant to BDE-153 toxicity have not been performed in experimental animals.

The principal study for the RfD (Viberg et al., 2003a) examined a number of behavioral parameters in adult male NMRI mice that had been neonatally exposed to BDE-153 (three doses, administered in a single day, using a limited number of animals). Aside from this study, the oral database is sparse. No information is available for the testing of BDE-153 in assays of

reproductive toxicity or chronic toxicity. The overall confidence in the RfD assessment for BDE-153 is low.

7. REFERENCES

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

The "Toxicological Review" for hexabromodiphenyl Ether (BDE-153) 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-153 was conducted in concert with the external peer review of other PBDE congeners (i.e., BDE-47, BDE-99, and BDE-209), and some external peer review charge questions were specific to congeners other than BDE-153. 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 responses 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 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-153 was coupled with the review of the documents for BDE-47, -99, 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-153. The charge to the external peer reviewers and final external peer review report (February 2007) pertaining to the toxicological reviews of the four PBDE 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, -99, -153, or -209?*

Comment 1: Three reviewers stated that they were unaware of any other relevant studies that would contribute to the BDE-153 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
Schecter, 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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Kodavanti, PRS; Derr-Yellin, EC. (2002) Toxicol. Sci. 68:451-457
Kodavanti, PRS; et al. (2005) Toxicol. Sci. 88:181-192
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-153. 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 that recently published, relevant studies are included in the IRIS assessment. The only study added to the "Toxicological Review" from the literature search was that by She et al. (2007).

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, -99, -153, and -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-153 based on the neurobehavioral toxicity study by Viberg et al. (2003a) was clearly and transparently described. Two reviewers commented that the neurobehavioral study by Viberg et al. (2003a) is the only study that provides data for deriving the BDE-153 RfD. The peer reviewers acknowledged the limitations and concerns with the study; however, they felt that its limitations were transparently discussed in the "Toxicological Review." 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, -99, -153, and -209 to a large extent on the Eriksson/Viberg neurobehavioral studies?*

Comment 1: All peer reviewers supported the use of the Viberg et al. (2003a) neurobehavioral study as the basis for the derivation of the BDE-153 RfD, given the limited body of toxicological information available. Two reviewers noted that the neurobehavioral studies for 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 selected more than one pup per litter, ignoring the "litter" effect. Treating littermates as independent experimental units could confuse dose effects with litter effects. 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 RfD for BDE-153 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). None of the reviewers stated that the Viberg et al. (2003a) study should not be used as the basis for the derivation of the RfD for BDE-153.

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 Viberg et al. (2003a) has been added to the discussion in section 5.1.1 of the "Toxicological Review." Additionally, the neurobehavioral effects reported in Viberg et al. (2003a) are supported by an expanding body of literature (Rice et al., 2007; Viberg et al., 2007, 2005, 2004a, b, 2003b, 2002; Kuriyama et al., 2005; Eriksson et al., 2002, 2001; Branchi et al., 2002) that details 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 methodologies that have generated toxic effects similar to the Eriksson/Viberg group. These studies, although conducted with BDE-99 or -209, support the findings of Viberg et al. (2003a) that exposure to these PBDE congeners in early developmental stages 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. (2004b) (BDE-99), Viberg et al. (2003a) (BDE-153), and Viberg et al. (2003b) (BDE-209) studies appropriate for determining the point of departure? Have the strengths and weaknesses of the Viberg and Eriksson studies been appropriately characterized and considered?*

Comment 1: All four reviewers believed that the Viberg et al. (2003a) study is appropriate for determining the point of departure for BDE-153. One reviewer felt that the data were appropriate as long as the document emphasizes that the neurochemical data also show alterations in normal developmental patterns. Another reviewer noted that Viberg et al. (2003a) was the only study with dose-response data for determining the point of departure for BDE-153. None of the reviewers suggested alternative studies for determining the point of departure for BDE-153. Two reviewers explicitly stated that the strengths and weaknesses were identified and clearly presented.

Response**:** The neurochemistry data for BDE-153 are discussed in section 4.4.1.5 of the "Toxicological Review" because they are studies of the acetylcholine receptor in the brain. At the suggestion of the reviewers, text has been added to the discussion of the toxicological endpoints evaluated in the study (section 4.3) referring the reader to section 4.4.1.5 for a more detailed discussion of the neurochemical data. The neurochemical data for BDE-153 as well as that from BDE-99 and -209 are also discussed in section 4.5.3 of the "Toxicological Review" on mode of action.

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

Comment 1**:** All four reviewers agreed with the selection of the neurobehavioral effects of BDE-153 as the critical effect for identifying a point of departure. One of the reviewers felt that the neurochemical data 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." Another reviewer was confused as to why the actual data could not be recovered from the study authors to allow for dose-response modeling and BMD estimation, given that the studies were published fairly recently (2003). This reviewer recommended that the Agency attempt to recover the neurobehavioral toxicity data from the study authors.

Response**:** Descriptions of the neurochemical data are fully summarized in section 4.4.1.5 and are referenced in the discussions of the neurodevelopmental data from the principal study (section 4.3). The evidence of neurochemical interactions and the potential relationship with the neurobehavioral effects are highlighted in the mode-of-action section of the "Toxicological Review" (section 4.5.3). The "Toxicological Review" presents the hypothesis proposed by the Eriksson/Viberg group in which the observed effects on locomotion and habituation are related 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 that 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. In the case of BDE-153, there are no data on levels in the brains of mice to illustrate if timing of exposure influenced the amount in the brain.

The actual motor activity components (locomotion, rearing, and total activity) and habituation ratios for BDE-153 were reported in graphical form only 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. The NOAEL for the motor behavior data (activities and ratios) served as the point of departure for the assessment.

Comment 2: Two reviewers thought that the rationale for the point of departure had been transparently and objectively described. Another reviewer felt the document provided clear rationalization for the selection of the point of departure. None of the reviewers stated that the rationale for the point of departure was not appropriately described.

Response**:** No response needed.

Comment 3**:** One reviewer noted that a 95% lower bound on the BMD (BMDL) would be better than a NOAEL for deriving an RfD.

Response**:** The Agency recognizes that a NOAEL can be limiting since it is highly dependent on the doses selected and the sample size and does not account for the dose-response curve. Alternatively, a BMDL is more independent of study design, takes into account the dose-response curve and the statistical significance of the observed effects, and therefore is preferable to a NOAEL as a point of departure. However, the data from the principal study do not provide quantitative dose response and can only be used to establish a NOAEL. The graphic reporting of observational measures of effects in the critical study is insufficient to provide the necessary information to perform BMD modeling, and the Agency's attempts to obtain the raw data from the authors were unsuccessful.

Charge Question 6. *Have the rationale and justification for each uncertainty factor (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: 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-153 in animals and humans. However, additional explanation for applying the default interspecies UF_A was added to section 5.1.3.

Comment 3: Two reviewers suggested lowering the intrahuman UF_H. One of these reviewers felt 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: The 10-fold UF_H for intraspecies uncertainty is retained based on the lack of information concerning the toxicokinetics and mode of action of BDE-153 in humans. In the absence of human data, the effects in potentially susceptible populations exposed to BDE-153 cannot be determined. Additional explanation for applying the default intraspecies UF_H 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 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 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-153, 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-153 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-153. 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-153 from exposures during pregnancy and lactation are not available. Accordingly, a UF_S as described above was applied. Furthermore, there are no data for BDE-153 from any conventional studies of chemical toxicity that can be used to determine whether or not long-term exposures might identify effects that were co-critical with the sensitive neurodevelomental toxicity identified in mice following a single dose exposure during its critical window.

Comment 5: 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_D "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 UFD, 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-153 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 UFD. The "IRIS Summary" and "Toxicological Review" contain sufficient information on the rationale for the database UFD to allow a risk manager to consider the impact of this UFD during the risk management process.

Comment 6: Two reviewers believed that 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 on BDE-47 and -99 to determine if a body burden approach could be used for these congeners during the development of the toxicological reviews. It was determined that existing half-life, exposure, metabolite, and mode-of-action data could not support a body burden calculation for these congeners. There are no data for BDE-153 that could support a body burden approach.

Charge Question 8. *Do you agree with the rationale described in the "Toxicological Review" of BDE-99 that the data on the window of susceptibility of the cholinergic receptors to BDE-99 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-99 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-99 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-153 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). There are neurochemical data for BDE-153, but there was no testing to determine the impact of varying the time of dosing. 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-153. The following statement has been added to the mode-of-action summary (section 4.5.3): "While evidence exists that demonstrates that BDE-153 may have an impact on neurochemical events during early postnatal development, data are inadequate to determine the mode of action for BDE-153."

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; tetrabromodiphenyl ether (BDE-47), pentabromodiphenyl ether (BDE-99),

hexabromodiphenyl ether (BDE-153), and decabromodiphenyl ether (BDE-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 assessments. 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 on BDE-153 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 proposed metabolic pathway that integrates the available data on the metabolites that have been identified.

Response: A diagram of a proposed metabolic pathway for BDE-153 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. In cases where the data from one study are discussed in more than one section of the "Toxicological Review," a reference to the location of prior mention of the study is provided.

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, and in the case of BDE-153 there is only one study that provides data on metabolites. That study used an intraveneous rather than oral route of administration.

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 has been added to the toxicokinetics section that provides an integration of the BDE-153 toxicokinetic data for mice and rats and identifies any differences seen between species. There are no metabolite data from rats and there are inconsistencies between the intraveneous data on mice from the Staskal et al. (2006) study and the oral Sanders et al. (2006) study in rats.

Comment 7: One reviewer commented that there appears to be no correlation between PND exposure, concentration in the brain, and increased adverse responses.

Response: There are no data on the levels of BDE-153 or its metabolites in the brain after oral dosing during either the postnatal or adult periods.

Comment 8: 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 9: 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-153 document.

Response: Considering the lack of other studies to compare to the principal study, a table is not warranted for only one study.

Comment 10: One reviewer recommended adding data for BDE-209 found in human biological media to Table 3-1, "Median PBDE concentrations in human biological media in the U.S."

Response: Data for BDE-209 were not included in Table 3-1 for BDE-153 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 11: 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 12: 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 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-153 is acknowledged in the "Toxicological Review" as part of section 3, 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-excretion can be determined. The data are currently inadequate to determine the impact of the oily vehicle on the distribution and uptake of BDE-153.

Comment 13: One reviewer noted that considering the antithyroid effects observed with DE-71 (a formulation that might be reasonably linked to neurodevelopmental effects observed with BDE-47 and -99) in Zhou et al. (2002) occurred at doses that are higher than those that produce the neurodevelopmental effects of BDE-47 and -99. The reviewer suggested that it could 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: There are minimal data on the thyroid effects of BDE-153. In human sera there was no correlation between total PBDEs and T_3 or T_4 concentrations (total or free), but the sample sizes were small (Mazdai et al., 2003). None of the animal studies of BDE-153 examined the serum levels of thyroid hormones. Neither BDE-153 nor its hydroxylated metabolites BDE-138 and -153 were found to compete with T_4 for binding to TTR (Meerts et al., 2000). In addition, DE-71 is a commercial mixture and cannot be considered to be fully equivalent to BDE-153. DE-71 contains only 6% BDE-153.

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 Viberg et al. (2003a) 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-153 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-153 and that its limitations were transparently discussed in the "Toxicological Review." Additionally, the neurobehavioral effects reported in Viberg et al. (2003a) are supported by an expanding body of literature (Viberg et al., 2007, 2005, 2004a, b, 2003b, 2002; Kuriyama et al., 2005; Eriksson et al., 2002, 2001; Branchi et al., 2002) that details changes in motor and cognitive activity in rodents following administration of single or repeated perinatal doses of PBDEs.