



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)**

December 2006

NOTICE

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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 ABBREVIATIONS AND ACRONYMS

Ah	aryl hydrocarbon
AhR	Ah receptor
BDE-15	2,2',4,4',5,5'-hexabromodiphenyl ether
CALUX	Chemical-Activated LUCiferase gene eXpression
CAR	constitutive androstane receptor
CYP-45	cytochrome P-450
ER	estrogen receptor
EROD	ethoxyresorufin O-deethylase
FOB	functional observational battery
hexaBD	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 RNA
MUP	major urinary protein
NOAEL	no-observed-adverse-effect level
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofuran
PCR	polymerase chain reaction
PND	postnatal day
PXR	pregnane X receptor
RfC	reference concentration
RfD	reference dose
T3	triiodothyronine
T4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
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 exposure to 2,2',4,4',5,5'-hexabromodiphenyl ether. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153).

The majority of the available toxicological information relates to the congener 2,2',4,4',5,5'-hexabromodiphenyl ether or BDE-153 (CAS No. 68631-49-2). Toxicological information related to other congeners in the hexabromodiphenyl ether homolog group (CAS No. 36483-60-0) 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 Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

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

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This document and the accompanying IRIS Summary have been peer reviewed by EPA scientists and independent scientists external to EPA. Comments from all peer reviewers were evaluated carefully and considered by the Agency during the finalization of this assessment. During the finalization process, the IRIS Program Director achieved common understanding of the assessment among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and EPA's regional offices.

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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 less-than-lifetime exposure durations, and a carcinogenicity assessment.

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

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

Development of these hazard identification and dose-response assessments for 2,2',4,4',5,5'-hexabromodiphenyl ether has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and technical panel reports that were used in the development of this assessment include the following: *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity*

Risk Assessment (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2000a, 2005c), *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

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

2. CHEMICAL AND PHYSICAL INFORMATION

Hexabromodiphenyl ether (hexaBDE) is one of the possible 10 homologs of polybrominated diphenyl ethers. Figure 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 number and bromine substitution pattern of some congeners that have been investigated in various studies are given in Table 1.

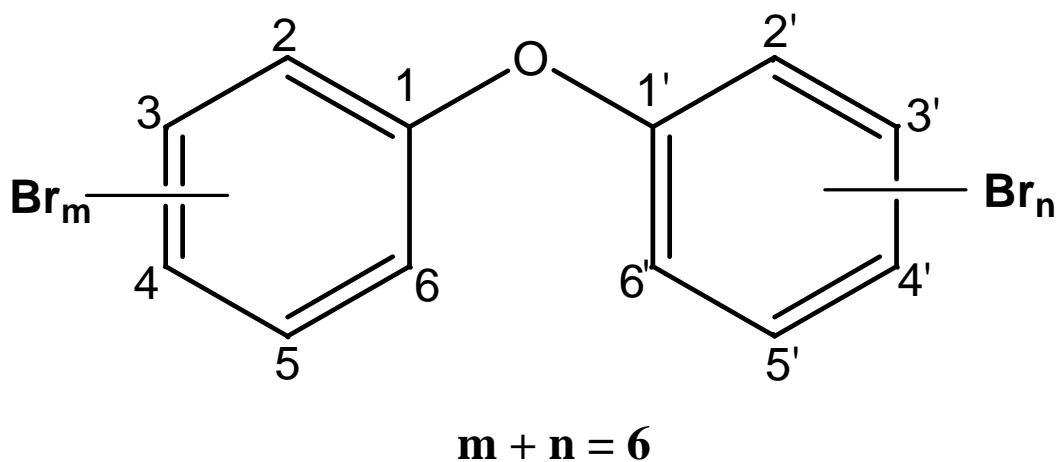


Figure 1. Chemical structure of hexabromodiphenyl ether.

Table 1. IUPAC number and bromine substitution pattern of some hexabromodiphenyl ether 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-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). Approximate composition by weight of these flame retardants is given in Table 2 (Great Lakes Chemical Corporation, 2003).

Table 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 polybrominated diphenyl ether (PBDE) congeners in the commercial penta DE-71™ and octa DE-79™ are given in Table 3 (Great Lakes Chemical Corporation, 2003).

Table 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 4.

Table 4. Physical and chemical properties of 2,2',4,4',5,5'-hexabromodiphenyl ether (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 ₄ Br ₆ O	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 (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	6.9–9.3	Braekvelt et al. (2003); ATSDR (2004)
Log octanol/air partition coefficient (K _{oa}), at 25°C	11.9	Chen et al. (2003)

3. TOXICOKINETICS

3.1. ABSORPTION

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

About 70% of a 0.6 mg/kg dose (1 μ mol/kg) was absorbed in groups of five male or female F344 rats and B6C3F1 mice after gavage dosing in corn oil (Sanders et al., 2006). The estimate of absorption was based on a comparison of 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 bioaccumulation 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 occurs. The data do not provide information on the quantitative aspects of absorption or the kinetics of tissue distribution and retention. 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 the national level.

Adipose tissue

Breast adipose samples were collected between 1996 and 1998 from 23 San Francisco Bay area women as part of a case-control study on organochlorine compounds and breast cancer (She et al., 2002). Women ranged from 28 to 62 years of age and were predominantly Caucasian and born in the United States. Pathology reports indicated 12 women had malignancies, 8 had benign tumors, and 3 had ductal carcinomas in situ, a condition considered by some as

transitional to malignancy. Breast adipose samples were collected during biopsy or breast surgery and were analyzed for tetraBDE (BDE-47), pentaBDEs (BDE-99 and BDE-100) and hexaBDEs (BDE-153 and BDE-154). Mean and median concentrations of the sum of these PBDEs were 86 and 41 ng/g lipid weight (lw), respectively, the highest human levels reported so far. Median concentrations of individual PBDE congeners are given in Table 5. 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 United States. There was an inverse relationship between the sum of the concentrations of these PBDEs in breast adipose tissue and age, with women younger than the median age of 48 years having significantly higher concentrations of PBDEs in adipose tissue than women older than 48. This may imply that different activities may expose different age groups more than others or that some PBDE congeners may accumulate differently with age. Five paired samples of breast and abdominal adipose tissues were also analyzed for tetra- to hexaBDEs. Abdominal and breast concentrations of PBDEs were highly correlated and of comparable magnitude.

In a study in New York City, adipose fat 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 5. 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 BDE-138 were not detected. Mean concentrations were, in decreasing order, 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).

Table 5. Median PBDE concentration (ng/g lipid weight) in human biological media in the United States

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

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

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

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 tri- 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 bioaccumulation in the liver with increasing degree of bromination (Guvenius et al., 2001).

Human milk

In a study conducted in 2002 of levels of PBDEs in human milk in the United States, 47 samples from Caucasian, African-American, and Hispanic nursing mothers 20–41 years of age

and living in Texas were analyzed for 13 PBDE congeners (Schechter et al., 2003). Mean and median total concentrations of tri- through decaBDEs were 74 and 34 ng/g lw, respectively. 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 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 5. There was no apparent difference in concentrations between age groups or ethnic groups. Median concentrations of PBDEs, including hexaBDEs, found in this study were substantially higher than those measured in human milk in Europe or Japan.

Breast milk was collected from 12 primiparous 24–33-year-old nursing women in Japan, at one 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 BDE-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 these samples in 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), BDE-100 and BDE-99 (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 BDE-100) and hexaBDEs (BDE-153 and BDE-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 to 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 BDE-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).

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 United States 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 United States increased to 61 ng/g lw. Median concentrations of the six individual PBDE congeners analyzed are given in Table 5. BDE-47 followed by BDE-99 and BDE-153 were the most abundant congeners (Sjodin et al., 2004).

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. 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 0–4-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- years-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 0–4-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 0–4-year-olds could be due to higher exposure from human milk and/or certain behavioral activity such as crawling or sucking on flame-retarded materials.

Concentrations of PBDE congeners BDE-47, hexaBDEs (BDE-153 and BDE-154), heptaBDE (BDE-183), and decaBDE (BDE-209) were determined in blood serum from groups of 19–20 Swedish male and female subjects in the following occupational groups: hospital workers (control), clerks working full-time at computer screens, and personnel at an electronic-dismantling plant (Sjodin et al., 1999). 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.

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 5 and in Table 6 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.

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 6). The levels of the higher brominated congeners, BDE-99 to BDE-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).

Table 6. Comparison of median PBDE congener concentrations (ng/g lipid weight) in maternal and fetal sera in Sweden and in the United States

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
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

^aMazdai et al., 2003 (United States): year of sampling 2001; number of donors 12.

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

In summary, the concentration profiles in the United States of PBDEs in maternal and fetal sera, pooled serum, and human milk are similar, although these studies were conducted in different States or regions of the United States (Table 5). The predominant congeners found in adipose tissue, human milk, and blood samples in the United States are BDE-47, BDE-99, BDE-100, and BDE-153. 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 tri-, hepta-, and decaBDE.

Current median concentrations of BDE-153 and BDE-154 found in the United States in adipose tissue, human milk, and blood are in the range of 4 to 0.7 ng/g lw, respectively. Median concentrations of the sum of PBDEs measured in human biological media are in the vicinity of 40 ng/g lw. These levels are substantially higher than the levels found in human populations in Europe or Japan.

3.2.2. Animal Data

In both F344 rats and B6C3F1 mice receiving a single 0.6 mg/kg dose (1 μ mol/kg) of BDE-153 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 for female mice were significantly higher than those for female rats and, there was a similar trend for the males, although the differences were

not significant. The levels in the kidney, lungs, and brain were considerably lower and similar within species; concentrations in these tissues for mice were 2–3 times higher than in rats, and the difference was 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 for BDE-47, BDE-99, and BDE-100 five days after the animals received a radiolabeled 1 mg/kg dose. When measured as ng/g wet weight basis, the levels of BDE-153 were higher in all tissues than the levels from the other tested materials.

In the Sanders et al. (2006) study, when the same dose was given to male rats for 1, 3, or 10 consecutive days, the tissue concentrations increased with the duration of exposure. A comparison of the results 24 hours after a 10-day exposure to 0.6 mg/kg-day were about twice as high as those following one 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. The concentration in the liver after the single dose was about twice that 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.

3.3. METABOLISM

Little information is available on the metabolism of hexaBDE in humans or experimental animals. There is one study (Sanders et al., 2005) that examined the effects of BDE-153 on several cytochrome P-450 (CYP-450) enzymes in the liver. The CYP-450s catalyze hepatic oxidation reactions for a variety of nonpolar xenobiotic compounds. Accordingly, up-regulation of their expression can signify the potential for initial oxidation as part of the metabolic profile.

Sanders et al. (2005) used a different approach for measuring the induction of the CYP-450 isozymes in the liver. 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 RNA (mRNA) was isolated from a portion of the right medial lobe of the liver and converted to its cDNA, using real-time polymerase chain reaction (PCR). Target gene-amplification was evaluated 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 20–30-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 suggest that hydroxylated metabolites may form in the liver after absorption of BDE-153.

A subsequent study of BDE-153 conducted by Sanders et al. (2006) was not able to identify metabolites of BDE-153 in feces in the 24-hour period after administration of single doses or an equimolar mixture of BDE-47, -99, and -153 to F344 rats. However, the radiolabel extracted from the liver after 1 or 3 days of BDE-153 treatment was $85 \pm 1\%$ or $94 \pm 1\%$, respectively. Unrecovered radioactivity was considered to be metabolites bound to liver proteins and recovered material 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 was identified as metabolites. Metabolites were extracted, separated using chromatography, and identified by mass spectroscopy. Three isomers 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 difference in the results of Sanders et al. (2006) and Staskal et al. (2006) are methodological in origin. Much of the fecal BDE-153 from the oral Sanders study was unabsorbed material accounting for part of the difference, but the BDE-153 in the 4-hour bile sample was also unmetabolized.

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 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 BDE-153. Only 0.5% of the dose was found in bile within 4 hours of administration and all of that was present as parent compound.

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 from mice was higher, especially for the male mice (1%); the level in the 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. 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) study regarding the presence of metabolites in the feces. The oral Sanders et al. (2006) study did not identify metabolites in the feces, whereas the intravenous Staskal et al. (2006) study 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. It may also relate to the facts that Sanders et al. (2006) used 24-hour collections from male rats while Staskal et al. (2006) used the samples from a 5-day collection period from female mice. 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).

Elimination half-lives of individual tetra, penta, and hexa components of commercial pentaBDE (Bromkal 70TM) were investigated in groups of male and female Wistar rats given a single oral dose of 300 mg/kg of Bromkal 70TM dissolved in peanut oil (von Meyerinck et al., 1990). Groups of three animals of either sex were sacrificed on days 1, 2, 3, 4, or 7 and then once a week for 10 weeks. Perirenal fat was collected and analyzed for PBDEs. The degree of bromination could be determined using gas chromatography/mass spectrometry but not the exact stereochemistry of the individual compounds. Tetra- and pentaBDEs were eliminated faster than hexaBDEs. The half-life of an unspecified hexaBDE congener was 91 days for female rats and 119 for male rats. However, the difference in half-lives between sexes was not significant. The authors indicated that the metabolic rate and therefore the elimination of PBDE may be affected by the high dose given to the animals, and half-lives of hexaBDE as determined in this study may not be representative of cases where the exposure doses are lower.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Limited or no information is available on the absorption, distribution, metabolism, and excretion of hexaBDEs in experimental animals and in humans. A model for human metabolism has not been established. Extrapolation of results from laboratory animals to humans using physiologically based pharmacokinetic models is not possible at this time.

4. HAZARD IDENTIFICATION

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

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 to 37 years old, presenting in labor at an Indiana hospital. The PBDE congeners and their concentrations measured in fetal and maternal serum samples are given in Table 5. 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. LESS-THAN-LIFETIME AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

Inhalation and oral acute, 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 spontaneous motor behavior, 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 adult mouse brain. Male neonatal NMRI mice were given single oral 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

and water. Litters had been culled to 10–12 pups within 48 hours of birth; of the remaining pups 4–7 per litter were males. Female pups were euthanized 4–5 weeks after birth.

Motor activity was measured for a 60-minute period, divided into three 20-minute periods, in mice at ages 2, 4, and 6 months. The tests were conducted in 10 mice randomly selected from the 3–5 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, a ratio was calculated between the performance period 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 between 2-, 4-, and 6-month-old mice.

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. At 4 months of age, mice exposed to 0.9 and 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 two of the variables, rearing and total activity. 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) was a slightly lower activity for the behavioral variable total activity during the first 20-minute period, compared with controls. At the other doses of 0.9 and 9.0 mg/kg, mice were significantly hypoactive and significantly hyperactive during the first and last 20-minute periods, respectively, for all three variables. The habituation capability in 2-, 4-, and 6-month-old mice concerning locomotion and total activity decreased significantly with age at 0.9 and 9.0 mg/kg BDE-153. 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.

The Morris swim maze test was performed at 6 months of age in groups of 19–24 mice from each treatment group and was used to assess spatial learning ability and memory by measuring latencies in locating a submerged platform during the acquisition period (days 1 to 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 spacial 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 four 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 through 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 ($p > 0.001$) 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 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 five was significantly ($p > 0.05$) longer than that of the controls for all hexaBDE exposed groups.

Changes in the cholinergic receptors have been proposed to affect learning and memory. For this reason, one week after completion of the swim maze test, 6 to 9 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 neurotoxic effects of BDE-153.

The NOAEL for BDE-153 (92.5% pure) 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.

4.4. OTHER STUDIES

4.4.1. *Receptor Site Interactions*

There is considerable evidence from studies of the PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs) and 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 both the aryl hydrocarbon (Ah) receptor site and the estrogen receptor (ER) site. Based on the data from the well-studied PCBs, PCDDs, and PCDFs, the activation of these receptor sites is associated with immunotoxicity, reproductive effects and carcinogenesis (Klaassen, 1996, pp. 47-49, 373-376) all endpoints of interest for PBDEs.

4.4.1.1. *Aryl Hydrocarbon Receptors*

The transcription of the genes for CYP-450, -1A1, -1A2, and -1B1 are linked to a signal transduction cascade that is initiated by activation of the Ah receptor by an appropriate ligand. The CYP-1 family of enzymes is highly conserved in mammals and is responsible for the oxidative metabolism of a variety of planar and near-planar compounds (Lewis et al., 1998). The CYP- family of enzymes metabolically activates and metabolizes polycyclic aromatic hydrocarbons and aromatic amines as well as PBDEs. Many substrates for the CYP- family enzymes are also Ah receptor (AhR) ligands. Differences in Ah receptor affinity are correlated to variations in CYP-1 inducibility. Receptor site affinity has been shown to reflect potency and the potential for a xenobiotic to cause adverse health effects.

Chen et al. (2001) studied the affinity of several PBDE congeners for rat hepatic Ah receptor 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, in liver cell lines from rainbow trout, and in rat and human tumor cell lines. HexaBDE congeners BDE-153 and -154 had Ah receptor binding affinities approximately 2×10^{-5} that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Quantitative measures of EROD induction were reported for BDE-153 and BDE-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 Ah receptor agonists or antagonists at sequential stages of the Ah receptor signal transduction pathway

leading to CYP-1A1. These issues are environmentally relevant because of the strong rank-order correlation between strength of Ah receptor binding, CYP1A induction, and toxicity for 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 to 60% that 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 BDE-154 or induction of genes that encode metabolizing enzymes have also been investigated by Peters et al. (2004). Ah receptor-mediated induction of CYP-450 enzymes 1A1 and 1B1 were 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 BDE-154 (>98% pure) did not induce EROD activity when incubated for 72 hours at concentrations that were not cytotoxic (up to 10 μ M) and, therefore, were not found to be AhR agonists in these cells. Additionally, exposure of the cells to BDE-153 or BDE-154 did not induce mRNA levels of CYP-1A1 or -1B1, indicating no effect of these hexaBDEs on AhR gene expression directly.

Peters et al. (2006) examined the interaction of BDE-153 and BDE-154 as well as other BDEs on the Ah receptor in cultured liver cells from four healthy cynomolgus monkeys (3 males and 1 female), using EROD activation as a biomarker for receptor activation. Both compounds were weak Ah agonists when co-exposures 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 localized 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 to 10 μ M) 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 Ah receptor-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 1500 picomolar TCDD standard (%-TCDD-max). A positive response was defined as any response that was greater than three standard deviations above the mean value for the control. BDE-153 failed to induce Ah receptor-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 Ah receptor 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% pure) in corn oil at 0, 1, 10, or 100 µmol/kg-day. The animals were sacrificed 24 hours after receiving the last dose. The liver was removed, and RNA from a 100 g liver sample was isolated, converted to its complementary DNA, and amplified by using PCR. The resultant DNA samples were then analyzed to determine the expression of CYP-450-1A1, a protein linked to Ah receptor activation.

BDE-100 had a significant effect on the level of CYP-1A1 (19 times the vehicle-treated controls) only at 100 µmol/kg-day (64 mg/kg-day), making it a weak activator of the Ah receptor. When the 1A1 expression from BDE-153 was compared with those for tetraBDE-47 and pentaBDE-99, the impact on the Ah receptor seemed to be correlated to the levels of polybrominated dibenzofurans in each congener, which in turn correlated with increased bromine content of the congeners.

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

4.4.1.2. Estrogen Receptors

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

The in vitro estrogenic and antiestrogenic potencies of seventeen PBDEs, including 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 ER α and ER β receptors were trypsinized and seeded in 96 well plates for the ER-CALUX (Chemical Activated LUciferase gene eXpression) assay. After allowing for cell growth, the wells were exposed to solutions containing the test compounds or estradiol and 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 ^3H -steroids from carp serum proteins that had been stripped of hormones before testing. Unlabeled estradiol and testosterone also had a limited effect on displacing the radiolabeled ligands, suggesting limited sensitivity of the assay with carp serum.

In summary, the mechanistic studies of the estrogen receptor indicate that there was no activity of BDE-153 and BDE-154 at the concentrations tested and the binding affinity of the hexaBDEs was 50,000 lower than that of estradiol.

4.4.1.3. Androgen Receptors

DE-71, a commercial pentaPBDE mixture, was found by Stoker et al. (2004) to delay puberty and suppress the growth of androgen-dependant tissues in male Wistar rats exposed to doses of 30 or 60 mg/kg during the peri-pubertal period but not to doses of 0 or 3 mg/kg. In order to examine which components of the mixture might be responsible for the observed effects, androgen receptor binding by several of the individual congeners found in DE-71 was examined in vitro (Stoker et al., 2005). The assays of the individual congeners examined competitive binding of BDE-153 (99% pure) and BDE-154 (97% pure) in the presence of a tritium-labeled androgen agonist (R1881) by using ventral prostate cytosolic extracts along with an assay in an MDA-kb2 cell line containing the human androgen receptor and a transfected luciferase reporter element.

In the assay with the ventral prostate extract, 0.001, 1.6, 3.3, 16.7, or 33 μM concentrations of BDE-153 and BDE-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 μ M.

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

4.4.1.4. Acetylcholine Receptors

As discussed in Section 4.3, changes in the cholinergic receptors have been proposed to affect movement, learning, and memory. Disturbances in the development of the cholinergic system were demonstrated to occur during the brain growth spurt in humans and laboratory animals (Viberg et al., 2003a). In mice, this period occurs in the first few weeks after birth and reaches its peak on day 10 (Viberg et al., 2003a) For this reason, one week after completion of the swim maze test, 6 to 9 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 by using tritium-labeled α -bungarotoxin. Parallel samples were treated with α -bungarotoxin to correct for nonspecific α -bungarotoxin binding. Specific binding was determined by calculating the difference in the amount of labeled toxin bound in the presence versus absence of 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 changes in the cholinergic system (decrease in density of nicotinic receptors) may be one mechanism behind the neurodevelopmental neurotoxic effects of BDE-153.

4.4.1.5. Other 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 Ah receptor. CYP-3A was slightly up-regulated but to a lesser extent than the CYP-1A1 and CYP-2B. CYP-2B and CYP-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 CAR was greater than that on PXR. The CAR and PXR receptors are classified as orphan receptors. They are both involved in the metabolism of xenobiotics and are stimulated by phenobarbital. The CAR receptor is also involved with steroid metabolism. The impact of BDE-153 on these receptors is

similar to the impact of non-coplanar PCBs on the same receptors; however, little is presently known about the effects of stimulation on the CAR and PXR receptors.

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 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 BDE-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 CYP2B-enriched rat liver microsomes resulted in the formation of metabolites that were able to displace 20 to 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 CYP1A or CYP4A3. 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. Thyroxine-binding globulin, rather than TTR is the major thyroxine-binding protein in humans.

4.4.3. Genotoxicity

Information is not available on the genotoxicity of hexaBDEs.

4.5. SYNTHESIS AND EVALUATION 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). These behavioral disturbances raise concerns about possible developmental toxicity in children.

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 United States 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 exposures 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-156 by the inhalation route of exposure.

4.5.3. Mode-of-Action Information

In the study of Viberg et al. (2003a), density of nicotinic 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. The authors hypothesized that such changes in the cholinergic system (decrease in density of nicotinic receptors) may be one mechanism behind the neurodevelopmental behavioral effects of BDE-153. Similar effects were also observed in the testing of BDE-99, a pentaBDE congener (Viberg et al., 2005, 2004a; Ankarberg, 2003).

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 TTR, one of the thyroid hormone-binding transport proteins in 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 BDE-166) did not compete with T_4 -TTR binding (Meerts et al., 2000). Lack of binding with transthyretin suggests that diminished thyroxin 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.

Studies of the interactions of hexaBDE-153, -154, and -156 with the Ah receptor indicate that these congeners are, at best, weak activators of the Ah receptor (Peters et al., 2006, 2004; Sanders et al., 2005; Chen and Bunce, 2003; Villeneuve et al., 2002; Chen et al., 2001). Accordingly, the cell signaling cascade, activated through the Ah receptor does not appear to play a role in the observed toxicity of hexaBDE.

Studies have also been conducted to evaluate the interaction between hexaBDE-138, -153, and -166 and the estrogen receptor sites. These hexaBDE congeners did not show any estrogenic activity (Villeneuve et al., 2002; Meerts et al., 2001). Androgen receptor binding by BDE-153 and -154 examined in an in vitro assay, indicate that these two congeners do not exhibit any antiandrogenic activity (Stoker et al., 2005). Lack of activity in these assays

minimizes concern that hexaBDE is an endocrine disruptor through estrogen or androgen pathways.

The data on the impact of hexaBDE on the density of nicotinic acetylcholine receptors in the hippocampal area of the brain provide the strongest link to the neurodevelopmental effects observed after hexaBDE exposure. Impaired development of the cholinergic system during the postnatal “brain growth spurt” period could change adult responses to endogenous acetylcholine and other cholinergic agents. Ankarberg (2003) determined that there appears to be a critical window of vulnerability in development of the cholinergic system during postnatal development. In mice, this period occurs in the first few weeks after birth with a peak at PND 10 (Viberg et al., 2003a). The resulting cholinergic receptor deficits are irreversible and cause a hypoactive response to exposure to cholinergic stimulants in adulthood.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

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,b).

4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.7.1. Possible Childhood Susceptibility

A population subgroup is susceptible if exposure occurs during a period of sensitivity as observed in 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 for 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 (Mazdai et al., 2003; Schechter et al., 2003; Thomsen et al., 2002) implies humans are exposed to BDE-153 during a period of rapid development of the brain, a critical window of development, 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.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

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 and learning and memory ability. 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% pure) on PND 10, a period of maximum vulnerability of the developing mouse brain (Eriksson et al., 2002). 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 protocol was unique and did not conform to health effects test guidelines for neurotoxicity screening battery or developmental neurotoxicity studies (U.S. EPA, 1998b). The dosing regimen did not include gestation and lactation exposure (U.S. EPA, 1998b); only single doses were given. In some respects the observation that effects occurred with such limited dosing argues for the importance of this study. While the study design appears to 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 non-treatment-related differences in pups born to a single dam¹. 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

¹ Eriksson et al. (2005) evaluated in 18 different litters the use of the litter (n = 9) or randomly selected individuals (n = 9, three mice randomly selected from three different litters) as a statistical unit in development toxicology in the neonate. In this study of mice neonatally exposed to BDE-99, there was no statistical difference whether the litter or the randomly selected individuals were used as the statistical unit, indicating that multiple sampling from the same litter is unlikely to affect the LOAEL.

other FOB parameters. This would be helpful in gauging the reliability of the limited parameters that were measured. As indicated in the *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), it is assumed that an agent that produces detectable adverse neurotoxic effects in experimental animal studies will pose a potential hazard to humans. For BDE-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 and long half-life chemical, such as BDE-153, will result in exposure that lasts longer than the one 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. Therefore, less than chronic exposures may lead to developmental neurotoxicity via disruption of developmental events that take place during a short critical window of development (Rice and Barone, 2000). The concept that exposure during critical periods 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 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 read 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 3000 was applied: 10 for extrapolating animal data to humans (UF_A interspecies variability), 10 for susceptible human subpopulation (UF_H interhuman variability), 3 for extrapolating from subchronic to chronic exposure (UF_S), and

10 to account for a deficient database (UF_D). The rationale for application of the UFs is described below.

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

A default UF_H of 10 was applied to account for variations in susceptibility within the human population (intraspecies or interhuman variability). This factor accounts for humans who may be more sensitive than the general population to exposure to BDE-153.

A UF_S of 3 was used for extrapolating effects seen in a single exposure neurodevelopmental study to a lifetime exposure. Exposure on PND 10 occurred during a period of rapid brain development in mice. Brain development does not continue at an equivalent rate across the lifespan and is more quiescent during adult life stages. There are a wide variety of brain structures that have very limited critical windows during development. These critical windows translate to susceptible periods of exposure that are very short in duration. However, any exposure, be it acute or chronic, that were to occur during the critical developmental window could cause equivalent effects. Accordingly, timing of exposure is a more critical factor than duration of exposure.

Days 10 through 14 appear to be a critical window for development of the brain nicotinic cholinergic receptors that serve a variety of neuromuscular and neurological functions (Ankarberg, 2003). On the basis of this rationale, it is not necessary to make a 10-fold adjustment for exposure duration. Uncertainties regarding the effects of exposures during the prenatal period, extended postnatal exposures, and latent expression of early postnatal changes in the brain are addressed as a component of the database uncertainty factor.

A UF_D of 10 was used to account for database uncertainty. The available oral database for BDE-153 lacks prenatal developmental neurotoxicity studies and reproductive toxicity studies.

Application of a total uncertainty factor of 3000 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 μ g/kg-day.

5.1.4. Previous RfD Assessment

The hexabromodiphenyl ether congener BDE-153 has not been previously assessed in IRIS. However, a health assessment of the hexabromodiphenyl ether 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 hexabromodiphenyl ether homolog group.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

No data are available for deriving a reference concentration for BDE-153.

5.3. CANCER ASSESSMENT

Data are not available to assess the carcinogenic potential of BDE-153.

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 octabromodiphenyl ether 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 exposed humans 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/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,b), there is inadequate information to assess carcinogenic potential of BDE-153.

6.2. DOSE RESPONSE

The RfD of 0.2 µg/kg-day for BDE-153 was derived from 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 3000 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 hexaBDE 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.

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