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TOXICOLOGICAL REVIEW

OF

2,2',4,4',5-PENTABROMODIPHENYL ETHER (BDE-99)

(CAS No. 60348-60-9)

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-PENTABROMODIPHENYL ETHER (CAS No. 60348-60-9)

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LIST OF ACRONYMS

Ah	aryl hydrocarbon
AIC	Akaike Information Criterion
BDE-99	2,2',4,4',5-pentabromodiphenyl ether
bDNA	branched DNA
BMD	benchmark dose
BMDL	95% lower bound on the BMD
BMDS	benchmark dose software
BMR	benchmark response
CALUX	Chemical-Activated LUciferase eXpression
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service Registry Number
CB3	Coxsackie virus B3
CDD	chlorinated dibenzo-p-dioxin
CDF	chlorinated dibenzofuran
cDNA	complementary DNA
CYP-450	cytochrome P-450
decaBDE	decabromodiphenyl ether
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
EC_{50}	median effective concentration
ER	estrogen receptor
EROD	ethoxyresorufin O-dealkylase
FOB	functional observational battery
GD	gestational day
GSH	glutathione
hexaBDE	hexabromodiphenyl ether
i.v.	intravenous
IC_{50}	median inhibitory concentration
IGF	insulin-like growth factor
IgG	immunoglobulin G
IRIS	Integrated Risk Information System
IUPAC	International Union of Pure and Applied Chemistry
LBI	light beam interruption
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
lw	lipid weight
mRNA	messenger RNA
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MUP	major urinary protein
NOAEL	no-observed-adverse-effect level
octaBDE	octabromodiphenyl ether
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl

PCR	polymerase chain reaction
pentaBDE	pentabromodiphenyl ether
PKC	protein kinase C
PND	postnatal day
PR	progesterone receptor
PROD	pentoxyresorufin O-dealkylase
PTU	6-n-propyl-2-thiouracil
PXR	pregnane X receptor
QNB	quinuclidinyl benzilate
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
s.c.	subcutaneous
SD	standard deviation
SXR	steroid X receptor
T_3	triiodothyronine
T_4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
tetraBDE	tetrabromodiphenyl ether
triBDE	tribromodiphenyl ether
TTR	transthyretin
UDPGT	uridine diphosphoglucuronosyl transferase
UF	uncertainty factor

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to 2,2',4,4',5-pentabromodiphenyl ether. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of 2,2',4,4',5-pentabromodiphenyl ether (BDE-99).

The majority of the available toxicological information on the pentabromodiphenyl ether homolog group (CAS No. 32534-81-9) relates to the pentabromodiphenyl congener BDE-99 (CASRN 60348-60-9). Toxicological information related to other congeners in the pentabromodiphenyl ether homolog group is also discussed. However, this health assessment does not deal with commercial mixtures of brominated diphenyl ether homologs containing pentabromodiphenyl ether as one of the constituents of commercial formulations. In addition to BDE-99, IRIS health assessments have also been prepared for three other polybrominated diphenyl ether congeners: tetraBDE-47, hexaBDE-153, 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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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-pentabromodiphenyl ether (BDE-99). 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 (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for BDE-99 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

Pentabromodiphenyl ether (CASRN 32534-81-9) is one of the possible 10 homologs of polybrominated diphenyl ethers (PBDEs). Figure 2-1 shows the chemical structure of pentabromodiphenyl ether (pentaBDE). The number of possible congeners of pentaBDE is 46, with International Union of Pure and Applied Chemistry (IUPAC) numbers 82 to 127. The IUPAC number and bromine substitution pattern of some pentaBDE congeners that have been investigated in various studies are given in Table 2-1.

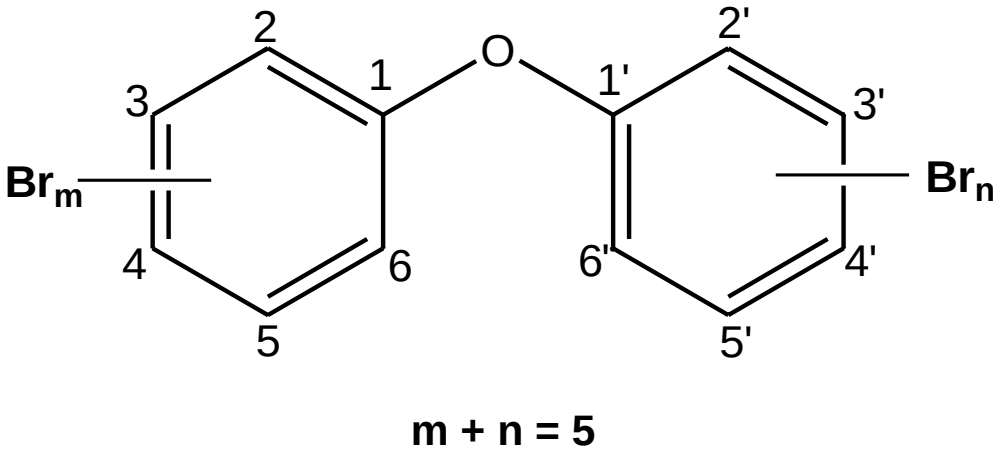

Figure 2-1. Chemical structure of pentaBDE.

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

IUPAC number	Bromine substitution pattern
BDE-85	2,2',3,4,4'-PentaBDE
BDE- 99	2,2',4,4',5-PentaBDE
BDE-100	2,2',4,4',6-PentaBDE
BDE-105	2,3,3',4,4'-PentaBDE
BDE-119	2,3',4,4',6-PentaBDE
BDE-126	3,3',4,4',5-PentaBDE

PentaBDE is found in commercial pentaBDE, which is usually composed of a mixture of pentaBDE (50–62%), tetraBDE (24–38%), and hexaBDE (4–12%) (U.S. EPA, 2005c). The relative proportions by weight of various PBDE congeners in the commercial pentaBDE DE-71™ are approximately 43% (pentaBDE-99), 28% (tetraBDE-47), 8% (pentaBDE-100), 6% (hexaBDE-153), and 4% (hexaBDE-154). Tribromodiphenyl ether (triBDE)-28 and -33 and tetrabromodiphenyl ether (tetraBDE)-49 and -66 are present at about 1% or less in the

formulation (Great Lakes Chemical Corporation, 2003). Manufacture of the commercial pentaBDE mixture was phased out in the U.S. at the end of 2004.

The predominant pentaBDE congener in environmental media, biota, and human tissues is usually BDE-99 (CASRN 60348-60-9), followed by BDE-100 (CASRN 189084-64-8). Physical and chemical properties of BDE-99 are listed in Table 2-2.

Table 2-2. Physical and chemical properties of BDE-99

Parameter	Value	Reference
Synonym	Benzene, 1,2,4,-tribromo-5-(2,4-dibromophenoxy)-; 2,2',4,4',5-pentabromodiphenyl ether; BDE-99	U.S. EPA (2004)
CASRN	60348-60-9	U.S. EPA (2004)
Chemical formula	$C_{12}H_5Br_5O$	U.S. EPA (2004)
Physical form	Amber solid	Flemming et al. (2000)
Molecular weight	564.7	U.S. EPA (2004)
Vapor pressure (Pa) at 25°C	5×10^{-5}	Wong et al. (2001)
Melting point (°C)	93	Palm et al. (2002)
Solubility in water (µg/L)	2.4	Stenzel and Markley (1997)
Henry's law constant ($Pa \times m^3 \times mol^{-1}$) at 25°C	0.60	Cetin and Odabasi (2005)
Log octanol/water partition coefficient (K_{ow}) at 25°C	6.5–8.4	Braekevelt et al. (2003); ATSDR[a] (2004)
Log octanol/air partition coefficient (K_{oa}) at 25°C	11.3	Chen et al. (2003)
Relative density (at 25°C)	2.28	Flemming et al. (2000)

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

3. TOXICOKINETICS

Data on the toxicokinetics of the pentaBDEs in humans are limited to findings on levels in adipose tissues, blood, liver, and maternal milk, which demonstrate that they are absorbed from the environment and distributed to tissues. Studies of BDE-99 in several strains of rats suggest that absorption varies between 60 and 90%. Quantitative data on absorption in mice are more limited; however, there is uptake from the gastrointestinal track based on radiolabel found in tissues and excreta. The highest levels of radiolabel following oral exposure are found in adipose tissues, muscle, skin, and liver. Much of the metabolism data have been derived from studies in rats rather than mice. Both species form a variety of hydroxylated metabolites via the activity of cytochrome P-450 (CYP-450) isozymes. In rats there appears to be some hydrolysis of the ether bond resulting in production of brominated phenols. There is evidence that conjugation with glutathione (GSH), sulfate, and/or glucuronic acid can occur. There are no data on whether the radiolabel in tissues is present as metabolites or parent, although it is presumed that the material in adipose tissue is parent compound based on its lipophilicity. The metabolites are excreted with bile and feces and to a lesser extent in urine. Some of the urinary metabolites in rats are excreted bound to albumin, while in mice metabolites are found bound to one (BDE-99) or two (BDE-100) major urinary proteins.

3.1. ABSORPTION

There are no direct studies of BDE-99 absorption in humans. The data that demonstrate human absorption come from measurements of BDE-99 in human biological media after anthropogenic exposures but do not permit estimation of route-specific uptake parameters.

Data on absorption of BDE-99 in several strains of rats and mice are available. There are also some data for BDE-85 and BDE-100. In a study by Hakk et al. (2002a), ^{14}C-BDE-99 (>98% purity) in corn oil was given as a single oral dose of 8 mg/kg (1.0 μCi/rat) to groups of conventional (three/group) and bile-duct-cannulated (five/group) male Sprague-Dawley rats. Urine, feces, and bile were collected at daily intervals over 3 days. In the first 24 hours after administration, 22 ± 16% of the dose was present in the feces in the conventional rats. This suggests that absorption was very variable among the rats, and estimates of absorption range from about 60–90%. The fecal excretion on day 1 was much higher in the bile-duct-cannulated rats (53 ± 27%), indicating that gastrointestinal emulsion by bile was a major factor governing absorption. The estimated amounts absorbed by the bile-duct-cannulated rats were also quite variable and ranged from 21–74%. There was excretion of BDE-99 in bile on day 1 after exposure (0.7 ± 0.8% of the dose). However, since it was low, it had little effect on the estimates of absorption.

Chen et al. (2006) estimated the absorption of ^{14}C-BDE-99 (95.6% purity; 34–37 μCi/kg) to be 85% in male F344 rats administered a 0.6 mg/kg (1 μmol/kg) dose in corn oil by gavage. This estimate was derived from a comparison of the excretion data from male rats receiving the same dose intravenously compared with those exposed orally. The percent of the dose in the feces 24 hours after oral exposure was 43.1 ± 4.7% compared to 27.3 ± 5.1% for the intravenous (i.v.) exposure. The dose used by Chen et al. (2006) was lower than that used by Hakk et al. (2002a) and, thus, is consistent with the higher average estimate of absorption.

The toxicokinetics of BDE-100 (2,2',4,4',6-pentaBDE) were studied in male Sprague-Dawley rats (Hakk et al., 2006). Doses of 4.1 mg/rat of ^{14}C-BDE-100 (purity >95%; 0.9 μCi/rat, equivalent to about 17 mg/kg) were administered to groups of nine conventional or bile-duct-cannulated rats. Urine, bile, and feces were collected at 24-hour intervals for 72 hours. About 11.5 ± 10.1% of the radiolabel was found in the feces of conventional rats 24 hours after administration, suggesting that absorption of BDE-100 varies among animals and is greater than 80%. As was the case for the Hakk et al. (2002a) study discussed above, the level in the collected 24-hour fecal matter from the bile-duct-cannulated rats was higher than in the conventional rats (16.8 ± 25.7%).

Chen et al. (2006) found that the absorption of ^{14}C-BDE-99 (95.6% purity; 34–37 μCi/kg) in male B6C3F1 mice administered 0.6 mg/kg (1 μmol/kg) was comparable to that in F344 rats (85%), based on the comparison of fecal loss after oral (27.1 ± 5.3%) versus i.v. (12.9 ± 2.2%) exposures.

Darnerud and Risberg (2006) examined the tissue distribution of uniformly radiolabeled BDE-85 or BDE-99 in adult female C57BL mice after i.v. or gavage exposure to 20 μmol/kg of the congener in dimethyl sulfoxide (25 μCi/g body weight; about 11 mg/kg), using a qualitative whole-body autoradiography technique. The presence of radiolabel in tissues 24 hours and 96 hours after oral dosing demonstrated absorption via the gastrointestinal tract. The levels of radiolabel in the gastrointestinal tract were higher after oral exposures than after i.v. exposures, demonstrating some excretion of unabsorbed compound with the fecal matter.

Eriksson et al. (2002) demonstrated that radiolabeled BDE-99 can be taken up and retained in the neonatal mouse brain. Neonatal NMRI male mice (five/group) were administered 0 or 8 mg/kg of ^{14}C-BDE-99 (purity >98%; 40.5μCi/kg) in a fat emulsion on postnatal day (PND) 3, 10, or 19, and the animals were sacrificed 24 hours or 7 days after administration. The amount of radioactivity in the brain was between 0.4 and 0.5% of the administered dose in the three different age categories 24 hours after administration. Seven days after administration, ^{14}C-BDE-99 or its metabolites could still be detected in the brain. These data cannot be used to quantify absorption but do demonstrate uptake from the gastrointestinal tract and transport across the blood-brain barrier in mice.

3.2. DISTRIBUTION

The high K_{ow} of BDE-99 suggests a strong potential for accumulation in lipid-rich tissues. This property of BDE-99 is quite evident from the data on distribution in humans and animals.

3.2.1. Human Data

The human data described below come from monitoring of PBDEs in human populations rather than from measured dosing studies. The data demonstrate that humans are exposed to PBDEs and that absorption and distribution to some tissues occur. The data do not provide information on the quantitative aspects of absorption or the kinetics of 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 the differences in congener distributions 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 media. However, distribution studies have not been conducted in humans, and therefore it is not known whether BDE-99 distributes to other tissues as well. The number of samples examined in various studies and countries is small, and therefore the data should not be construed as representative at the national level.

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

3.2.1.1. *Adipose Tissue*

Breast adipose samples were collected between 1996 and 1998 from 23 San Francisco Bay area women as part of a case-control study on organochlorine compounds and breast cancer (She et al., 2002). Women ranged from 28–62 years of age and were predominantly Caucasian and born in the U.S. Pathology reports indicated 12 women had malignancies, 8 had benign tumors, and 3 had ductal carcinomas in situ, a condition considered by some as transitional to malignancy. Breast adipose samples were collected during biopsy or breast surgery and were analyzed for tetraBDE (BDE-47), pentaBDEs (BDE-99 and -100), and hexabromodiphenyl ethers (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. Median concentrations of individual PBDE congeners are given in Table 3-1. The highest concentrations found were for tetraBDE, followed by hexaBDEs and pentaBDEs, a distribution that does not follow that of the commercial pentaBDE used in the U.S. There was an inverse relationship between the sum of the concentrations of these PBDEs in breast adipose tissue and age, with women younger than the median age of 48 years having significantly higher concentrations of PBDEs in adipose tissue than women older than 48. This may imply that different activities may expose different age

groups more than others or that some PBDE congeners may accumulate differently with age. However, only 23 women were tested and firm conclusions cannot be made. 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.

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

	Year[a]	N[b]	BDE-47	BDE-99	BDE-100	BDE-85	BDE-153	BDE-154	BDE-183	Σ PBDEs	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	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	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[c]	34	11	6	0.7	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.
[b]N = number of donors.
[c]Seven serum pools with number of donors in each serum pool ranging from 40–200.

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). BDE-47 was the major congener detected, followed by BDE-100 and -99. Median concentrations of individual PBDE congeners and the sum of these PBDEs are given in Table 3-1 and are the highest human levels reported so far. 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 and/or time course of exposure and disposition.

In a study in Japan, 10 human adipose samples taken from the general Tokyo population in 1970 and in 2000 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, tetraBDE-47 (0.5), hexaBDE-153 (0.4), pentaBDE-100 (0.3), pentaBDE-99 (0.1), hexaBDE-154 (0.06), and heptaBDE-183 (0.05) (Choi et al., 2003).

Tetra-, penta-, and hexaBDEs were analyzed in the adipose tissues from 3 women and 10 men between the ages of 28 and 83 and living in Spain for at least 10 years. The average concentrations of tetraBDE-47, pentaBDE-99, and hexaBDE-153 were 1.4, 0.4, and 1.8 ng/g lw, respectively. An unidentified pentaBDE congener was found at an average concentration of 0.5 ng/g lw. The predominant congener in both men and women in this study was hexaBDE-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 tri- to hexaBDE congeners. PBDEs were found in all samples. BDE-47, -99, and -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). For the pentaBDEs, BDE-99 was the predominant congener in both liver and adipose tissue, followed by BDE-100 and -85. The mean total concentrations of these three pentaBDEs were higher in liver than in adipose tissue and amounted to 4.3 and 1.7 ng/g lw, respectively (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-99 were 111 and 14 ng/g lw, respectively. Median concentrations of individual PBDE congeners are given in Table 3-1. There was no correlation between age and level of PBDEs in human milk.

Milk samples were collected in 2003 from 40 first-time mothers with 2- to 8-week-old infants and residing in urban areas in the Pacific Northwest of the U.S. (Montana, Oregon, and Washington State) and Canada (British Columbia) (She et al., 2007). Mean and median total concentrations of 12 triBDE through decabromodiphenyl ether (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 one month after delivery. The most abundant PBDE congeners in human milk were BDE-47 and the hexaBDE congener BDE-153, followed by pentaBDEs (BDE-99 and -100) and triBDEs. The sum of the concentrations of 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. The average total PBDE concentration (1.7 ng/g lw) in five women representing the highest frequency of fish consumption (every day) was double that found in three women (0.8 ng/g lw) representing relatively low fish consumption (1–2 days/week) (Ohta et al., 2002).

In another study in Japan, PBDEs were not detected in eight pooled human milk samples collected in 1973 (Akutsu et al., 2003). In 2000, BDE-47 was the predominant congener (0.5 ng/g lw), followed by hexaBDEs (0.4 ng/g lw), pentaBDEs (0.3 ng/g lw), triBDEs (0.1 ng/g lw), and heptaBDEs (0.04 ng/g lw). Of the pentaBDEs, BDE-100 was the most abundant (0.17 ng/g lw), followed by BDE-99 (0.15 ng/g lw) and BDE-85 (0.01 ng/g lw) (Akutsu et al., 2003). The relatively large concentration of hexaBDEs in mothers' milk seen in Japan was explained by the authors to be due to past use in Japan of a hexaBDE commercial product consisting mostly of BDE-153.

The breast milk concentrations of BDE-47, two pentaBDEs (BDE-99 and -100), and two hexaBDEs (BDE-153 and -154) were determined in samples from 93 primiparous women, collected from 1996–1999 in Uppsala County, Sweden. 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 -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 among 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 suggested a peak in BDE-47 and total PBDE concentrations around 1998, followed by decreasing levels (Lind et al., 2003).

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 -100 at 0.5 and 0.4 ng/g lw, respectively. The sum of the concentrations of 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. 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. was 61 ng/g lw. PBDEs included in this study were tetraBDE-47; pentaBDE-85, -99, and -100; and hexaBDE-153 and -154. Median concentrations of these individual PBDE congeners are given in Table 3-1 (Sjodin et al., 2004).

Serum samples from 24 pregnant Mexican immigrant women living in an agricultural community in California were collected during 1999 and 2001 (Bradman et al., 2007). Tetra-, penta-, hexa-, and hepta-BDE congeners were measured in the serum samples. The median concentration of the sum of tetra-, penta-, hexa-, and heptaBDE congeners was 21 ng/g lw with a median concentration for BDE-47, -99, -100, and -153 of 11, 2.9, 1.8, and 1.5 ng/g lw, respectively. There was no clear association between 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, -100, and -99. 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) 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 sum of the concentration of these PBDEs in serum was highest for the 0- to 4-year-old children (12 ng/g lw) but was about one-third lower and relatively constant for the different age groups above 4 years. Except for the 0- to 4-year-olds who seemed to experience elevated exposure, there was a lack of an age-related trend of PBDE body burdens. This may be explained by the fact that PBDEs are relatively new contaminants in the environment; the time period for human exposure is therefore relatively short, and different age groups (except the 0- to 4-year-old group) may thus have experienced a similar exposure period (Thomsen et al., 2002). 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 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). Commercial PBDEs used as flame retardants in the electronic industry are usually decaBDE and to a lesser extent octabromodiphenyl ether (octaBDE). The median concentration of BDE-47 in serum was about the same in the controls and computer clerks (~1.5 ng/g lw) but almost double that level in the electronic-dismantling personnel. Serum concentrations of all PBDE congeners decreased in the 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 in Indiana were analyzed for 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 Tables 3-1 and 3-2 for comparison with a Swedish study (Guvenius et al., 2003) described below. TetraBDE-47 was the most abundant congener, followed by BDE-99 and BDE-100. 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 the concentration of PBDE congeners in maternal and fetal sera with increasing degree of bromination.

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

PBDE congener	Maternal serum (ng/g lw)		Fetal serum (ng/g lw)	
	Mazdai et al. (2003)[a]	Guvenius et al. (2003)[b]	Mazdai et al. (2003)	Guvenius et al. (2003)
BDE-47	28	0.8	25	1.0
BDE-99	5.7	0.2	7.1	0.07
BDE-100	4.2	0.2	4.1	0.07
BDE-153	2.9	0.6	4.4	0.17
BDE-154	0.3	0.04	0.7	<0.01
BDE-183	0	0.06	0	0.01
Σ PBDEs	37	2.1	39	1.7

[a]U.S.: year of sampling, 2001; number of donors = 12.
[b]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 the 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, indicating that the higher brominated PBDEs do not pass through the placenta to the same extent as the lower brominated congener BDE-47. This trend was not apparent in the Mazdai et al. (2003) study, where comparable levels were found in maternal and fetal sera for all PBDE congeners studied. The concentrations of PBDEs found in maternal and fetal blood samples in Indiana women (Mazdai et al., 2003) were substantially higher than those found in Swedish women (Guvenius et al., 2003).

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). The concentration profiles in the U.S. 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 and -100, with current median concentrations in human biological samples of approximately 25, 7, and 4 ng/g lw, respectively. 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

The animal data on BDE-99 distribution are limited but more quantitative than the human data because they represent the distribution after deliberate dosing studies.

In a study by Hakk et al. (2002a), tissue distribution of BDE-99 was assessed in young adult male Sprague-Dawley rats. ^{14}C-BDE-99 (>98% purity) in corn oil was given as a single oral dose of 8 mg/kg (1.0 μCi/rat) to groups of conventional (three/group) and bile-duct-cannulated (five/group) rats. Adipose tissue, adrenals, blood, carcass, gastrointestinal tract, heart, kidney, liver, lung, testes, and thymus were analyzed for radioactivity on day 3 after exposure. In the conventional rat, BDE-99 was preferentially found in lipophilic tissues, with 39% of the administered dose being found in the carcass, 6% in the gastrointestinal tract, and 4% in adipose tissue. No other tissues in the conventional or bile-duct-cannulated rats contained greater than 1% of the ^{14}C on day 3. The tissue data support the hypothesis that bile salts are necessary for the intestinal absorption of BDE-99, since tissue levels of ^{14}C for the bile-duct-cannulated rats were much lower when compared with those of conventional rats (2% in the carcass, 1.5% in the gastrointestinal tract, and 0.8% in adipose tissue).

The remaining carcasses from conventional rats were fractionated into skin, bone, brain, eyes, and muscle. An estimated 21% of the BDE-99 dose was found in the skin. When the tissue distribution data were expressed on a concentration basis, the most lipophilic tissues (adipose, adrenal, gastrointestinal tract, and skin) contained the highest concentrations of ^{14}C. The lipid content of selected tissues was determined, but the observed distribution pattern for BDE-99 did not consistently correlate with tissue lipid content. Adipose tissue had the highest lipid content and the highest BDE-99 concentration. However, kidney and lung with higher lipid content than liver had lower concentrations of BDE-99 than liver, an indication that selectivity in hepatic retention was not occurring.

In the study by Chen et al. (2006), the tissues that contained the largest portion of the radiolabel from a 0.6 mg/kg-day oral dose in both F344 rats and B6C3F1 mice were the adipose deposits, muscle, skin, and liver. Adipose tissue contained the highest percentage of the dose in rats and mice. In rats, the next-to-the-highest percentage was in the skin, while in mice it was the muscle tissue. All other tissues evaluated contained less than 1% of the dose. Following i.v. injection of 1 mg/kg uniformly ^{14}C-labeled BDE-99 (1.5 Ci) to C57BL/6 mice, Staskal et al. (2006) found that the percentage in muscle was greater than that in skin 5 days after exposure. This is in agreement with the Chen et al. (2006) finding for mice and may suggest a species difference in distribution.

After oral dosing, the radiolabel in adipose tissues as well as in kidney and lung (Hakk et al., 2002a) appeared to be totally the parent compound. The apparent requirement for bile in absorption and the extrahepatic tissue data indicating the presence of parent compound rather

than hydroxylated metabolites in peripheral tissues could be interpreted as suggesting initial postabsorption distribution of a substantial portion of the parent compound by way of the chylomicrons. This absorption route would explain the presence of unmetabolized parent compound in the adipose and other high-affinity tissues. Hakk et al. (2002a) found there was some binding of nonextractable metabolites to proteins in the liver. The lower extractability of the label from the liver was presumably the result of binding to cellular biomolecules.

Chen et al. (2006) compared tissue levels of ^{14}C-labeled BDE-99 (95.6% purity; 35.6 mCi/mmol) in male F344 rats at 24 hours after a single exposure to 0.6 or 6 mg/kg with the tissue levels at 24 hours after 10 days of daily exposure to 0.6 mg/kg (a total of 6 mg/kg). For all tissues except adipose tissue, the single 6 mg/kg dose resulted in a higher concentration (nmol-eq/g) than the 10 single 0.6 mg/kg-day doses. In adipose tissues, the level of label 24 hours after the last of the 10 0.6 mg/kg-day single doses was greater than that after the single 6 mg/kg dose, illustrating the potential for accumulation of BDE-99 in body lipids.

Hakk et al. (2006) conducted a study comparable to their 2002 study of BDE-99 in Sprague-Dawley rats, using BDE-100. About 73% of the radiolabel (0.9 μCi/rat) remained in the body of conventional rats after 72 hours and was found in the adipose tissue, gastrointestinal tract, skin, liver, and lungs. The other tissues evaluated contained less than 0.1% of the label after 72 hours. The results with BDE-100 were similar to those from the study of BDE-99.

Eriksson et al. (2002) demonstrated that radiolabeled BDE-99 can be taken up and retained in the neonatal mouse brain. Male NMRI mice (five/group) were administered 8 mg/kg of ^{14}C-BDE-99 (purity >98%; 40.5 μCi/kg) in a fat emulsion on PND 3, 10, or 19 and were sacrificed 24 hours or 7 days after administration. The amount of radioactivity in the brain was between 0.4 and 0.5% of the administered dose, 24 hours after administration. Seven days after the administration, ^{14}C-BDE-99 (or its metabolites) could still be detected in the brain, decreasing to between 0.1 and 0.3% of the administered dose in mice exposed on PND 3, 10, or 19. The amount of radioactivity in the brain was similar in mice exposed on PND 3 or 10 compared with mice exposed on PND 19 and therefore does not appear to explain the different behavioral effects seen in adult mice exposed to BDE-99 on PND 3, 10, or 19 (see section 4.3.1.5).

The overall qualitative distribution of ^{14}C-labeled pentaBDE-85 and -99 was studied in C57BL mice by using whole-body autoradiography (Darnerud and Risberg, 2006). ^{14}C-BDE-85 or -99 (>95% purity) was administered to male and female C57BL mice by i.v. injection or by gavage at 20 μmol/kg (25 μCi/g body weight; ~11 mg/kg). The animals were sacrificed at time intervals varying from 1 hour to 16 days after administration. The distribution of radioactivity in mice after i.v. administration was characterized by a high initial uptake of radioactivity in fatty tissues. In addition, the liver, adrenal cortex, lung, ovaries, and nasal epithelium accumulated radioactivity. Initially, intermediate radioactivity levels were found also in the brain tissue. No

radioactivity was observed in the thyroid gland. At 4 and 16 days after the administration, the radioactivity concentration was weaker, indicating significant ^{14}C excretion. In the male mouse after 6 hours, the concentration of radioactivity in the testis was low; in females, labeling in the ovaries was localized to the follicular structure. At 16 days postinjection, labeling was still visible in the fat tissues, liver, lung, and adrenal cortex; elimination from the lungs seemed to be slower than from the liver. Some faint labeling remained in the brain. The distribution pattern after oral administration of BDE-85 and -99 was similar to what was found after i.v. injection, which showed that the gastrointestinal uptake is effective. In spite of the lipophilic nature of these PBDEs, retention in the body fat depot was only moderate, probably because substantial metabolism and/or excretion occurred in mice.

The qualitative distribution of ^{14}C-BDE-85 and -99 was also studied in pregnant mice sacrificed 1 day after i.v. administration of 11 mg/kg of ^{14}C-BDE-85 or -99 on gestational days (GDs) 16–17 or 4 days after i.v. administration on GDs 13–17. In general, the uptake of radioactivity observed in pregnant mice was comparable to that in nonpregnant mice. Radiolabel was observed in the membranes surrounding the fetus, and labeling of fetal liver and intestinal contents was higher than in surrounding tissues. Faint radiolabeling was observed in the fetal brain.

Darnerud and Risberg (2006) also studied the partition of ^{14}C-BDE-85 and ^{14}C-BDE-99 to maternal milk in lactating C57BL mice. The lactating dams were injected intravenously with 2.0 μmol/kg (1 mg/kg) of each of the pentaBDE congeners on day 11 postpartum. Quantitative measurements were made of ^{14}C-BDE-85 and ^{14}C-BDE-99 radioactivity in the liver, kidney, fat, and plasma from lactating dams and their offspring on day 12 postpartum. No significant differences in ^{14}C levels of the studied tissues were observed between the two congeners. In the dams, fat contained about 10 times as much ^{14}C as did the liver, and the liver had higher ^{14}C concentrations than both the kidney and the plasma. In the offspring, liver and kidney radioactivity levels were similar to what was found in corresponding tissues from dams, whereas a two to four times higher plasma concentration was found in offspring.

Radioactivity was also measured in breast milk of lactating dams 1 and 4 days after i.v. administration of 1 mg/kg of ^{14}C-BDE-85 or -99 at day 11 postpartum (Darnerud and Risberg, 2006). Breast milk collected on days 12 and 15 postpartum contained a substantial amount of radioactivity, the levels decreasing with time after administration. Results from this distribution study indicate that, in spite of the lipophilic nature of these PBDEs, retention in the body fat depot is only moderate, probably because substantial metabolism and/or excretion occur in mice. Breast milk transport of these pentaBDE congeners was substantial, and radioactivity was found in the milk and in tissues of the suckling offspring 4 days after administration of a single dose of radioactive pentaBDEs to the dam. However, neonatal excretion seems to prevent accumulation and high levels in offspring compared with maternal levels.

Adult male and female offspring of pregnant Long-Evans rats exposed to BDE-99 (purity >99%) by nine subcutaneous (s.c.) injection (1 or 10 mg/kg-day) on GDs 10–18 were found to have detectable parent compound in the brain, plasma, and adipose tissues 120 days after birth. For the 1 mg/kg-day dose, the level in the adipose tissue was about 400 times greater than that in plasma. For the 10 mg/kg dose, the level in adipose tissue was about 1,500 times greater than that in plasma. There was considerable variability in the levels found in the adipose tissue among the individual samples analyzed (Ceccatelli et al., 2006).

Darnerud et al. (2005) examined whether an infection of Coxsackie virus B3 (CB3), a common human virus, changes tissue distribution of ^{14}C-BDE-99. On day 0, adult female Balb/c mice were infected with CB3; on day 1 of the infection, they were dosed orally with 0.2 mg/kg of ^{14}C-BDE-99; and on day 3 of the infection, they were sacrificed for studies of ^{14}C-BDE-99 distribution. Clinical signs of disease started to appear on day 2. In comparison with control values, there was no change in distribution of ^{14}C-BDE-99 in the brain, heart, spleen, kidney, blood, or thymus. However, ^{14}C-BDE-99 concentrations were increased in the liver (186%) and decreased in the lung (47%) and pancreas (51%). This correlated with decreased enzyme activities of CYP-450-mediated ethoxyresorufin O-dealkylase (EROD) and pentoxyresorufin O-dealkylase (PROD) in mice (see section 3.3), possibly suggesting decreased metabolism of BDE-99 and increased retention of unmetabolized compound by the liver during the acute phase of the viral infection.

In the study of Branchi et al. (2005), BDE-99 at 0 or 18 mg/kg-day was administered to CD-1 Swiss mice (nine/group) from GD 6 to PND 21. Two modes of administration of BDE-99 were investigated: by gavage or by letting the mouse drink BDE-99 dissolved in corn oil from a syringe (see also section 4.3.1.7). The brains collected from treated male pups on PND 22 (n = 2 per group) showed significantly elevated BDE-99 concentration: 640 μg/kg compared to <5 μg/kg for controls. No effects of administration routes were seen in the concentration of BDE-99 in the brain of treated mice.

Kodavanti et al. (2005) carried out a study using cultures of cerebellar granule cells from 7- to 8-day-old Long-Evans rat pups. The cultures were treated with ^{14}C-labeled PBDE-99 (0.05 μCi/mL) combined with different concentrations of unlabeled compound (0–30 μM) for 15 minutes to 1 hour. For each concentration tested there was a linear increase in percent uptake of the label from the culture medium over the 1-hour exposure period. When time was held constant and concentration varied, the percent accumulation increased linearly for the 0.67 and 3.67μM concentrations at 15, 30, and 60 minutes and then decreased linearly between the 3.67 and 30.67 μM concentrations, suggesting saturation of uptake.

3.3. METABOLISM

BDE-99 and -100 appear to be the most extensively metabolized of the individual PBDE congeners. Staskal et al. (2006) estimated that about 70% of the BDE-99 in urine and 80% in fecal matter were present as metabolites in mice, following i.v. injection of a 1 mg/kg dose. For BDE-100 the percentages were similar. When comparing urinary excretion and tissue loads across PBDE congeners, there appeared to be an inverse relationship (Staskal et al., 2006): tissue retention was higher when urinary excretion was low. Urinary excretion in male mice appears to exceed that in male rats (Chen et al., 2006).

Data on the metabolites present in bile and excreta in rats from the Hakk et al. (2006, 2002a) and Chen et al. (2006) studies and in mice from the Chen et al. (2006) and Staskal et al. (2006) studies support a metabolic pathway that involves epoxidation as the initial reaction for BDE-99 and -100. This can be followed by debromination in some cases and possibly by conjugation of the hydroxylated derivatives with GSH, glucuronate, and/or sulfate. Hydrolysis of the ether linkage between the two phenyl rings may also occur, producing brominated phenols. There is consistency among these studies of metabolites with regard to the production of mono- and dihydroxylated metabolites as well as hydroxylated/debrominated metabolites. The data for conjugation with GSH, glucuronate, and/or sulfate are more variable.

Chen et al. (2006) have proposed a metabolic pathway for BDE-99 (Figure 3-1) that is consistent with much of their data as well as those from the Staskal et al. (2006) and Hakk et al. (2006, 2002a) studies. There are some differences among the studies that relate to the extent to which metabolism occurs for the different congeners (BDE-99 and -100) and in different species.

According to the proposed metabolic pathway, BDE-99 can undergo two primary reactions (Figure 3-1). Both involve CYP-450 epoxidation of the phenyl ring. In one case, epoxidation targets carbons 5 and 6 of the disubstituted phenyl ring. This reaction favors BDE-99 to a greater extent than BDE-100 because there is less steric hindrance of unsubstituted carbon 6 of the disubstituted ring by the bromines on the trisubstituted phenyl ring in BDE-99. The 5,6-epoxy intermediate can undergo three subsequent reactions as follows (Figure 3-1): (1) rearrangement of the epoxy by way of a 1,2-hydride shift, (2) protonation of the epoxide generating a carbocation intermediate, and (3) conjugation with GSH.

The 1,2-hydride shift generates monohydroxylated pentaBDEs with the hydroxyl group located on the dibrominated phenyl ring. Hydrolysis of the epoxide, although not proposed as part of the Chen et al. (2006) pathway, could also occur and would produce dihydroxylated pentabromo metabolites.

Protonation of the 5,6-epoxide would lead to an unstable carbocation with the capability of accepting an electron pair from a nucleophile, providing an additional route for formation of dihydroxypentabromodiphenyl ethers and for protein binding in the liver. A

1,2-dihydroxypentabromodiphenyl ether formed via this route could fragment, generating 3,5-dibromo-1,2-dihydroxybenzene and 2,4,5-tribromophenol (Figure 3-1). Chen et al. (2006) tentatively identified 2,4,5-tribromophenol in rat feces and its sulfate and glucuronate conjugates in urine.

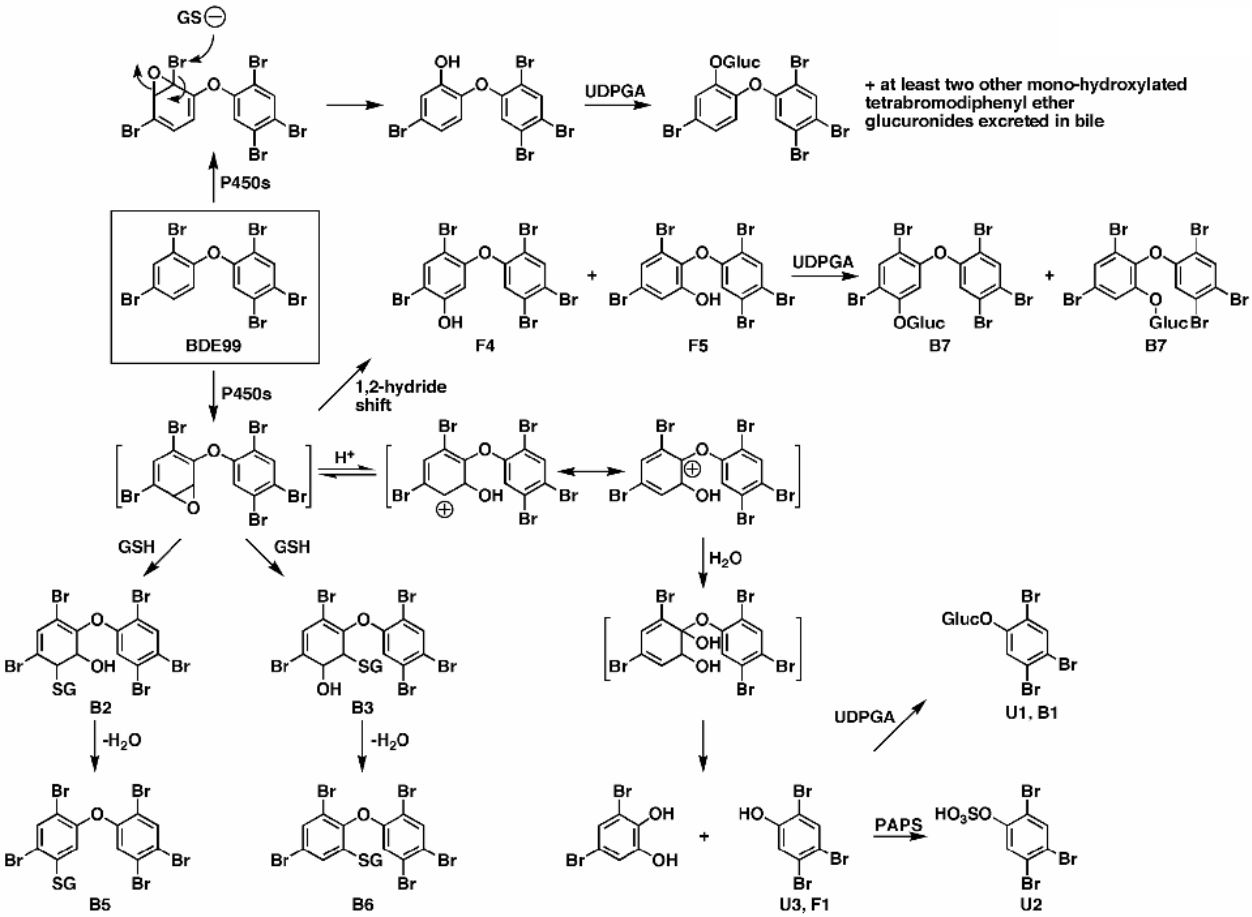

Figure 3-1. Proposed metabolic pathway for BDE-99 in male rats.

Note: PAPS = 3'-phosphoadenosine-5'-phosphosulfate; UDPGA = uridine diphosphate glucuronic acid.

Source: Chen et al. (2006).

Conjugates of metabolites with GSH have been identified as potential metabolites in bile and thereby in fecal matter by Hakk et al. (2002a) and Chen et al. (2006). GSH conjugates could be modified through the activity of γ-glutamyl transpeptidase, carboxypeptidase, and cysteine β-lyase to produce the thiols occasionally identified in urine (Hakk et al., 2002a).

In the alternate primary pathway, the epoxide would form between a brominated and nonbrominated carbon (Figure 3-1). Chen et al. (2006) proposed that this would lead to formation of tetrabromo-hydroxylated metabolites. Any brominated carbon with a neighboring

nonbrominated carbon is liable to this reaction, permitting generation of several tetrabromo-hydroxylated derivatives. Tetrabromo-hydroxylated metabolites have been identified in rats for both BDE-99 and -100 by Hakk et al. (2006, 2002a) and in mice by Staskal et al. (2006).

Additional research is needed to support the metabolic pathway proposed by Chen et al. (2006). The primary steps involving the formation of arene oxide intermediates, leading to hydroxylated and hydroxylated/debrominated metabolites, are consistent with the available data on metabolites in feces and urine of rats and mice. Support for the mechanisms of reaction and the extent and variability in conjugate formation and protein binding are fertile areas for additional research.

There have been no whole animal studies of the CYP-450 isozymes that participate in the epoxidation of BDE-99 or -100, but involvement of the CYP-1A1/2 and -2B isozymes has been investigated in vitro with BDE-99 because of their link to the activation of the aryl hydrocarbon (Ah) receptor (Sanders et al., 2005; Chen and Bunce, 2003; Chen et al., 2001). The activity of CYP-1A1/2 and -2B is generally evaluated through analysis of the phase I enzymes EROD for CYP-1A1/2 activity and PROD for CYP-2B activity. In the study by Darnerud et al. (2005) of the effects of a viral infection on the distribution of ^{14}C-BDE-99 (see section 3.2.2), both EROD and PROD were active in the noninfected control adult female Balb/c mice treated with ^{14}C-BDE-99, EROD to a greater extent than PROD. On day 3, in the infected mice treated with ^{14}C-BDE-99, the enzyme activities of EROD and PROD were about 17 and 31%, respectively, of those in the noninfected ^{14}C-BDE-99-treated mice.

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, 0.57, 5.7, or 57 mg/kg-day BDE-99 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) by using real-time polymerase chain reaction (PCR). Target gene amplification was evaluated by using specific probes for CYP-1A1, -2B, and -3A. These analyses indicated that CYP-1A1 expression was significantly up-regulated (eightfold) only with the 57 mg/kg-day dose of BDE-99. On the other hand, BDE-99 doses up-regulated expression of CYP-2B in a dose-related fashion at the 5.7 and 57 mg/kg doses in one assay and at the highest dose in another assay. At the highest dose, CYP-2B mRNA levels were 14–25 times those in the corn oil controls. These data conflict with the data from the control mice from Darnerud et al. (2005), where EROD appeared to be up-regulated to a greater extent than PROD. The expression of CYP-3A was up-regulated (four- to fivefold) with the highest dose.

C57BL/6 (10 weeks of age) mice were injected with doses of 0, 10, or 100 μmol/kg (0, 5.7, or 57 mg/kg) BDE-99 in corn oil for 4 days in a study by Pacyniak et al. (2007). The livers were removed 24 hours after the last dose and the levels of mRNA measured by Northern blot

and branched DNA (bDNA) analyses. The bDNA was considered to be the more accurate of the two assay systems. Northern blot analysis indicated that the levels of CYP-2B10 were induced 4 and 74 times, respectively, at the two doses tested, while the bDNA results indicated 51- and 38.9-fold inductions. CYP-3A11 did not show a difference with respect to the dose administered but was induced fivefold by the Northern blot analysis and 1.8-fold by the bDNA analysis.

Kester et al. (2002) evaluated whether or not the human estrogen sulfotransferase and the human phenol sulfotransferase were able to conjugate sulfate from 3'-phosphoadenosine-5'-phosphosulfate to several hydroxylated PBDEs. The highest degree of sulfation was observed with the tetraBDE hydroxy congener with both enzymes; the values for the pentaBDE hydroxy congener fell between those for the tested tri- and tetraBDE hydroxy congeners. Estrogen sulfotransferase was approximately 20 times more active toward 4-OH-3,5,2',4',6'-pentaBDE than was phenol sulfotransferase. Studies of excreted pentaBDE metabolites do not indicate that sulfate conjugation is a major metabolic process.

3.4. ELIMINATION

Elimination of BDE-99 and -100 occurs by way of the fecal matter and urine. Urinary excretion appears to be more substantial in mice than rats. Both parent compound and metabolites are identified in the excreta; the amounts of each vary among the studies. Radiolabel in fecal matter represents absorbed and unabsorbed material. Bile is an important contributor to the radiolabel in fecal matter.

In the study by Hakk et al. (2002a), a single oral dose of 8 mg/kg ^{14}C-BDE-99 (1.0 μCi/rat) was given to conventional and bile-duct-cannulated male Sprague-Dawley rats. Parent BDE-99 compound and metabolites were analyzed in urine, feces, and bile collected at daily intervals for 3 days. Excretion in urine was low and amounted to 0.9% of the dose in the conventional rat and 0.4% in the bile-duct-cannulated rat within 3 days; biliary elimination was only 4% over the same period of time. Excretion in both urine and bile of bile-duct-cannulated rats peaked at 1–2 days after exposure. Feces were the major route of elimination of BDE-99. Approximately 43% of the dose in conventional rats and 87% in bile-duct-cannulated rats were found in the feces within 3 days. In both conventional and bile-duct-cannulated rats, fecal radioactivity was highest on the first day (22 and 53%, respectively) and then declined steadily thereafter, suggesting that absorption is increased by bile emulsion and that enterohepatic circulation of BDE-99, if it occurs, plays a minor role in the male rat.

The Hakk et al. (2006) study of ^{14}C-BDE-100 in male Sprague-Dawley rats found only 0.1% of the dose in urine of the conventional rat at 72 hours. This is lower than the 0.9% observed with BDE-99. The bile contained about 1.7% of the radiolabel after 72 hours, which is also lower than that observed with BDE-99 in the study described above (Hakk et al., 2002a). There was evidence of glucuronidation of some of the biliary material but no evidence of sulfur-

containing metabolites. Most of the biliary label was protein bound. Approximately 20–30% of the extractable fecal radiolabel was unmetabolized in the conventional and bile-duct-cannulated rats. Large amounts of label were not extractable.

In F344 rats receiving an oral dose of 0.6 mg/kg BDE-99, 43% of the dose was present in the feces on day 1; the cumulative amount excreted in feces was 56% by day 10 after exposure. Urinary excretion was 1.6% on day 1 and 2.9% by day 10 (Chen et al., 2006). Over the 10-day period, about half of the single dose had been excreted; the remainder was retained by the tissues.

Biliary and fecal excretion appear to be important in C57BL mice, based on the results from the whole-body autoradiography study by Darnerud and Risberg (2006). Radiolabel was observed in both the bile and intestinal contents after both oral and i.v. administration of 10 mg/kg BDE-99 or -85. Intestinal radiolabel was more intense for the oral route of exposure, suggesting the presence of unabsorbed material in the feces as well as radiolabel derived from bile.

Hakk et al. (2002b) published an extension of their study of BDE-99 in male Sprague-Dawley rats to determine whether BDE-99 can bind to endogenous carrier proteins in the urine and bile, either as the parent compound or as metabolites. Such binding may facilitate the elimination of lipophilic xenobiotics. Because of the low amount of ^{14}C in urine in the conventional- and cannulated-treatment groups, urine collected from each rat group was pooled over 3 days. Bile from cannulated rats was pooled on a daily basis. Chromatographic analysis of urine revealed that the majority (76%) of the ^{14}C in conventional rat urine was not associated with protein, while 7% was bound to an 18-kDa monomeric protein characterized as α_{2u}-globulin. In the cannulated rat urine, none of the ^{14}C was bound to protein (100% unbound). Presumably, BDE-99 metabolites formed in cannulated rat urine were sufficiently polar and did not require a carrier system for excretion via the urine.

The pooled bile sample 1 day after dosing indicated that 61% of the biliary ^{14}C was unbound, decreasing steadily to 43% by day 3 after dosing (Hakk et al., 2002b). Approximately 28% of the biliary ^{14}C was associated with a 79-kDa protein, increasing steadily to 47% by day 3. Extractability of the bound radioactivity from rat bile protein ranged from 27–85%. In the day 1 bile sample, 21% of bound, extractable radioactivity was parent compound and the remainder was polar metabolites. At days 2 and 3, only metabolites were observed to be bound to the 79-kDa protein. Metabolite identification was not possible. The authors concluded that, although this study demonstrated the ability of BDE-99 and/or its metabolites to associate or tightly bind with urine proteins, it was unknown whether BDE-99 exposure could lead to nephropathy in rats.

In mice, urinary excretion of BDE-99 and -100 appears to involve binding to a major urinary protein (MUP) (Staskal et al., 2006). These proteins are synthesized in the liver, secreted

into serum, and eliminated in urine. Male mice secrete more protein than females. Analysis of pooled urine samples from BDE-99 and -100 intravenously dosed female mice indicated that 59.6 and 55.1%, respectively, was protein bound to an MUP. The two congeners appeared to bind to different MUP isoforms, with BDE-99 binding to MUP-1 and BDE-100 binding to MUP-1 and MUP-3.

3.4.1. Half-life Determinations

In the study by Hakk et al. (2002a), BDE-99 preferentially deposited in lipophilic tissues. BDE-99 was slowly mobilized from skin and fat deposits. In the conventional rat, an estimated 21% of the dose was deposited in skin at 3 days after exposure. At 6 days from exposure, over 18% of the dose to the conventional rat was still in the skin, declining to 12% by day 12. A substantial portion of the dose in the conventional rat remained in adipose tissue, 14 and 10% for the 6- and 12-day rats, respectively. Based on the disposition and excretion results obtained in this study, the estimated whole-body half-life of BDE-99 in male Sprague-Dawley rats was about 6 days, indicating that BDE-99 has bioaccumulation potential.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Limited information is available on the absorption, distribution, metabolism, and elimination of BDE-99 in experimental animals and in humans. A model for human metabolism has not been established. Extrapolation of results from laboratory animals to humans by using physiologically based pharmacokinetic models is not possible at this time.

4. HAZARD IDENTIFICATION

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

Epidemiological studies of BDE-99 are 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 (see also section 3.2.1). 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 3-2. 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 among 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, the available limited human studies do not permit any conclusions to be made concerning a possible association between exposure to PBDEs or BDE-99 and adverse health outcome in humans.

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

Short-term, subchronic, or chronic inhalation toxicological studies of BDE-99 are not available.

4.2.1. Short-term and Subchronic Studies

4.2.1.1. *Mice*

The aim of the study by Skarman et al. (2005) was to determine the effects on plasma T_4 levels and hepatic enzyme activities in juvenile mice following maternal gestational and lactational exposure to BDE-99. Groups of 22 or 13 dams received 0 or 0.08 mmol/kg (45 mg/kg) of BDE-99 (>99% purity) in corn oil every third day from GD 4 through PND 17, on a total of 10 occasions. The total dose of BDE-99 administered was, therefore, 0.8 mmol/kg or

450 mg/kg. Parallel groups of dams were similarly treated with a total dose of 0.8 mmol/kg (~450 mg/kg) of a commercial pentaBDE (Bromkal 70-5DE with main constituents of 37% BDE-99 and 35% tetraBDE-47) or with a total dose of 0.8 mmol/kg (260 mg/kg) Aroclor 1254. On GD 17, four dams from each treatment group were sacrificed and liver and blood samples collected. On PND 3, the size of the litters was adjusted to 10 pups. On PNDs 11, 18, and 37, three to four pups from each litter were sacrificed and liver and plasma samples collected.

Dam and offspring body weights were not affected by BDE-99, Bromkal, or Aroclor treatment. Significantly increased liver-to-body-weight ratio was seen on PND 20 in dams treated with BDE-99 but not in their offspring on PND 11, 18, or 37. Pregnancy rate, gestation length, and litter size were not statistically different from controls. Plasma total and free T_4 in the pregnant dams on GD 17, in the postweaning dams on PND 20, and in the offspring on PNDs 11, 18, and 37 were unaffected by BDE-99 treatment. On the other hand, plasma total and free T_4 were significantly reduced in the offspring of the Bromkal groups on PND 11 but returned to control levels by PND 18.

Hepatic microsomal CYP-450 enzyme activity was measured by means of the EROD activity assay, a marker of CYP-1A1 activity. Hepatic EROD activity in pregnant dams sampled on GD 17 and in postweaning dams sampled on PND 20 was unaffected by treatment with BDE-99. Induced EROD activity was seen in the Bromkal group in dams on GD 17 but returned to control levels on PND 20, while Aroclor treatment increased EROD activity in the dams on GD 17 and PND 20. Offspring sampled on PNDs 11 and 18 showed increased hepatic EROD activity in all treatment groups relative to controls but returned to control levels by PND 37. The increase in EROD activity was highest for the Aroclor group, while the increase was similar for the Bromkal and BDE-99 groups.

Hepatic uridine diphosphoglucuronosyl transferase (UDPGT) activity was studied in offspring on PNDs 11 and 18. UDPGT activity was not different from that in controls in the BDE-99 group at both time points. A significant increase in UDPGT activity was observed in the Aroclor group at both time points, while the Bromkal group showed an increase in enzyme activity of borderline significance on PND 18 only.

Based on the above, the study of Skarman et al. (2005) shows that BDE-99 had no effect on plasma T_4 levels in dams and their offspring relative to controls at any sampling occasion, suggesting that other components in Bromkal are responsible for the reduction of T_4 levels in offspring on PND 11. One of the components of Bromkal is tetraBDE-47, which has been shown to cause a decrease in T_4 levels in mice and rats (Hallgren and Darnerud, 2002; Hallgren et al., 2001). These results indicate that interference with thyroid hormone homeostasis can vary significantly between PBDE homologs.

4.2.1.2. *Rats*

Hakk et al. (2002a) examined the effect of BDE-99 on total T_4 plasma levels in young adult male Sprague-Dawley rats. A single oral dose of 8 mg/kg of ^{14}C-BDE-99 (>98% purity) in corn oil was given to groups of conventional (three/group) and bile-duct-cannulated (five/group) rats. The rats were housed in steel metabolism cages and sacrificed 3, 6, or 12 days after exposure. Average total plasma T_4 concentration (bound and free) was 1.7 µg/dL in the control rats. In the treated conventional rats, the average total T_4 levels increased approximately twofold to 3.2 µg/dL at 3 days after exposure, remained elevated at 3.0 µg/dL at 6 days after exposure, but by day 12 returned to control levels at 1.9 µg/dL. TetraBDE-47, which has been shown to cause a decrease in T_4 levels in mice and rats (Hallgren and Darnerud, 2002; Hallgren et al., 2001), may be the component responsible for the reduction of T_4 levels seen with commercial pentaBDE mixtures (Skarman et al., 2005; Zhou et al., 2002).

4.2.2. Chronic Studies and Cancer Bioassays

Chronic toxicity/carcinogenicity studies of BDE-99 are not available.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

4.3.1. Mice

4.3.1.1. *Eriksson et al. (2001)*

This study was carried out to determine whether exposure to BDE-99 during the period of rapid brain growth in neonatal mice could lead to disruption of the adult brain function. Single doses of 0, 0.8, or 12 mg/kg BDE-99 (>98% purity) in a 20% fat emulsion (1:10 egg lecithin to peanut oil in water) were administered by gavage to NMRI male mice on PND 10. Mice serving as controls received 10 mL/kg of the 20% vehicle. Spontaneous motor behavior tests (locomotion, rearing, and total activity) were measured over three 20-minute periods, at ages 2 and 4 months, in groups of eight mice randomly selected from three to four different litters, and the mice were tested once only. Habituation capability was evaluated in 2- and 4-month-old mice. Habituation is defined as the ability of the animals to adapt to a new environment and is characterized as initial investigation and exploration of their surroundings followed by gradual aclimitization and acceptance of the new area. It was evaluated in terms of the ratio of the motor behavior measures from the 40- to 60-minute observation period divided by the measures from the 0–20 minute period and multiplied by 100 (habituation ratio). Swim maze performance, a measure of learning and memory ability, was tested in groups of 16–18 mice, at age 5 months, given the high dose of BDE-99 (12 mg/kg).

There were no clinical signs of dysfunction throughout the experimental period nor any significant deviations in body-weight gain in the BDE-99-treated mice compared with the vehicle-treated mice. The spontaneous motor behavior data showed, for all three variables

(locomotion, rearing, and total activity), a dose-related disruption in mice treated with BDE-99, significant at both doses, and the aberrations were more pronounced in 4-month-old mice than in 2-month-old mice, indicating worsening with increasing age. Mice receiving 0.8 and 12 mg/kg of BDE-99 displayed significantly less activity (hypoactive) during the first 20-minute period (0–20 minutes), while during the third 20-minute period (40–60 minutes) they were significantly more active (hyperactive) in relation to control animals and for all three behavioral variables. The habituation capability significantly decreased with age in mice exposed to BDE-99 at 0.8 and 12 mg/kg. Performance of 5-month-old mice in the swim maze learning/memory test was significantly worse in mice exposed to 12 mg/kg BDE-99 than in control mice. The lowest-observed-adverse-effect level (LOAEL) in this study was 0.8 mg/kg for effects on spontaneous motor behavior and decreased habituation capability.

4.3.1.2. *Eriksson et al. (2002)*

This study was undertaken to investigate whether behavioral disturbances observed in adult mice following neonatal exposure to BDE-99 are induced during a defined neonatal brain developmental window of unique biological susceptibility. On PND 3, 10, or 19, male and female NMRI mice were given a single oral dose of 0 or 8 mg/kg BDE-99 by gavage in a 20% fat emulsion (1:10 egg lecithin to peanut oil in water). There were no effects on body weight or body-weight gain nor clinical signs of dysfunction in the BDE-99-treated mice at any time during the experimental period. Spontaneous motor behavior tests (locomotion, rearing, and total activity) were measured over three 20-minute periods in 4-month-old male mice (10 mice randomly selected from three to five different litters in each treatment group). Control mice receiving the 20% fat emulsion on PND 3, 10, or 19 showed normal habituation (i.e., a decrease in the variables locomotion, rearing, and total activity) in response to the diminished novelty of the test chambers over a 60-minute period, divided into three 20-minute periods. Mice neonatally exposed to BDE-99 on PND 3 or 10 showed decreased activity during the first 20-minute interval of the 60-minute period for all three behavioral variables compared with the control groups. During the last 20-minute period, a significantly increased activity compared with the controls was seen for all three behavioral variables. The most pronounced effects were seen in mice exposed to BDE-99 on PND 10, with significant hypoactive behavior during the first 20-minute period and significant hyperactive behavior for all three spontaneous behavior variables (locomotion, rearing, and total activity) during the last 20 minutes of the 60-minute test period. In mice neonatally exposed to BDE-99 on PND 19, there were no changes in the three behavioral variables compared with controls (i.e., a decrease in activity in all variables was evident over the 60-minute observation period). In conclusion, the behavioral disturbances observed in adult mice, following neonatal exposure to BDE-99, are induced during a defined

critical period of neonatal brain development, and mice exposed on PND 10 are most susceptible to the neurotoxic effects of BDE-99.

Uptake and retention of radiolabeled BDE-99 in the mouse brain were also measured in this study after exposure of NMRI male mice (five/group) to 8 mg/kg of ^{14}C-BDE-99 on PND 3, 10, or 19; the animals were sacrificed 24 hours or 7 days after administration (see also section 3.1). The amount of radioactivity in the brain was between 0.4 and 0.5% of the administered dose 24 hours after administration. Seven days after the administration, ^{14}C-BDE-99 (or its metabolites) could still be detected in the brain, decreasing to between 0.1 and 0.3% of the administered dose. The amount of radioactivity in the brain was similar in mice exposed on PND 3 or 10 compared with mice exposed on PND 19 and therefore does not appear to explain the difference in behavioral effects seen in adult mice exposed to BDE-99 on PND 3, 10, or 19.

4.3.1.3. *Viberg et al. (2002)*

A study was conducted to determine whether changes in spontaneous behavior in adult mice neonatally exposed to BDE-99 would include effects on the cholinergic system and thereby would alter the response in the adult animal to the cholinergic agent nicotine. On PND 10, male NMRI mice received a single dose of BDE-99 (>98% purity) by gavage at 8 mg/kg in a 20% fat emulsion. Control mice received fat emulsion in the same manner. At the age of 2 months, 12 mice per group, randomly picked from three to four different litters, were subjected to spontaneous behavior testing (locomotion, rearing, and total activity) for a 60-minute period (0–60 minutes), divided into three 20-minute periods. Directly after the spontaneous behavior test, the mice were given a single s.c. injection of saline solution (control) or 0.08 mg/kg nicotine base and were tested again immediately for spontaneous motor behavior during another 60-minute period (60–120 minutes). This amount of nicotine is known to cause an increased activity in normal adult NMRI mice.

There were no clinical signs of toxicity in the treated mice at any given time during the experimental period, and no difference was observed in body weights or body-weight gains between controls and treated animals. A decrease in locomotion, rearing, and total activity over the 60-minute test period was observed in control mice in response to the diminished novelty of the test chamber, but BDE-99-treated animals displayed significantly less activity (hypoactivity) for all three variables during the first 20-minute period (0–20 minutes), while during the last 20-minute period (40–60 minutes) the BDE-99-treated animals had significantly increased activity (hyperactivity) for all three variables, compared with controls. Pair-wise testing between the nicotine-injected and saline-injected mice showed, as expected, a significant increase in response to nicotine in the neonatally vehicle-treated mice during the first 20-minute period (60–80 minutes) for all three variables, locomotion, rearing, and total activity. In contrast, animals treated with BDE-99 on PND 10 and injected with nicotine at the age of 2 months

showed significantly decreased activity during the first 20-minute period (60–80 minutes) compared with BDE-99-treated animals injected with saline. The authors concluded that neonatal exposure to BDE-99 on PND 10 can affect the cholinergic system (see section 4.4.2.5), seen as changes in the adult mouse response to the cholinergic agent nicotine.

4.3.1.4. *Viberg et al. (2004a)*

This study was carried out to determine whether exposure to BDE-99 during a period of rapid brain growth in neonatal mice could lead to disruption of the adult brain function. Single oral doses of BDE-99 (purity >99%) of 0, 0.4, 0.8, 4.0, 8.0, or 16 mg/kg in a 20% fat emulsion (1:10 egg lecithin to peanut oil) were given by gavage to male and female C57/B1 mice on PND 10. Control mice received 10 mL/kg of the 20% fat emulsion only. Spontaneous motor behavior was tested at ages 2, 5, and 8 months in eight male and eight female mice, randomly selected from three to five different litters in each treatment group, at each testing occasion. Spontaneous motor behavior was measured for a 60-minute period, divided into three 20-minute periods, at each dose. Spontaneous motor behavior tests used measured locomotion (horizontal movement), rearing (vertical movement), and total activity (all types of vibration within the test cage [i.e., those caused by mouse movements, shaking/tremors, and grooming]). In order to study time-dependent changes in habituation (2-month-old versus 8-month-old mice), data from the spontaneous motor behavior tests were used.

The habituation ratio was used to analyze alteration in habituation between 2-month-old and 8-month-old mice, within each treatment group, in comparison with their respective controls. Data for the three spontaneous behavior variables (horizontal movement, vertical movement and total activity) are only available in graphic form and could not be used for quantitative assessment.[1] Numerical values suitable for dose-response assessment are only available for the habituation ratio.

There were no clinical signs of toxicity or effects on body-weight gain or body weight at any of the dose groups. Control mice showed habituation over the three 20-minute test periods. There were significant dose-related changes in spontaneous motor behavior (locomotion, rearing, and total activity) at 0.8 mg/kg and above in male and female mice at ages 2, 5, and 8 months. These disturbances were also worse with increasing age. Male and female mice receiving doses of 0.8 mg/kg and higher showed significantly decreased activity during the first 20-minute period (hypoactive) and significantly increased activity during the last 20-minute period (hyperactive) compared with control animals. Male and female mice exposed to the lowest dose (0.4 mg/kg) did not significantly differ in activity in any of the three behavioral variables during any of the three 20-minute periods. The habituation capability for the

locomotion and rearing variabiles was signicantly decreased in the 2- and 8-month-old male and female mice at 0.8 mg/kg and above, as evidenced by dose-related increases in the habituation ratio. The decline in habituation capability (i.e., the increase in the habituation ratio) was more pronounced in the 8-month-old mice than in the 2-month-old mice.

The habituation ratio for rearing (ratio between the performance periods 40–60 minutes and 0–20 minutes for rearing), which provided a good fit in the benchmark dose (BMD) modeling (see section 5.1.2), was 0.24, 0.51, 1.49, 45.8, 94.2, and 217 for the control, 0.4, 0.8, 4.0, 8.0, and 16 mg/kg dose groups, respectively, in 2-month-old female mice; the habituation ratio for rearing in 8-month-old female mice was 0.22, 0.33, 2.83, 43.8, 118, and 271 for the control and five different doses, respectively, indicating that the capability of the animals to habituate to a new environment decreased with increasing BDE-99 dose and with age. No major gender differences in spontaneous motor behavior responses or habituation capability were seen in this study.

The no-observed-adverse-effect level (NOAEL) for spontaneous motor behavior effects in this study was 0.4 mg/kg. The LOAEL was 0.8 mg/kg for significant changes in spontaneous motor behavior and decreases in the rearing and locomotion habituation capability in both male and female mice, worsening with increasing age.

4.3.1.5. *Viberg et al. (2004b)*

A study was conducted to determine effects on spontaneous behavior in adult male mice neonatally exposed to BDE-99 and whether these effects would include changes in the density of cholinergic nicotinic receptors in the hippocampus of the adult animal. Such changes have been proposed to affect learning and memory functions. (See section 4.4.2.5 for the discussion of the results of the receptor binding studies.) Single oral doses of 0, 0.2, 0.4, or 12 mg/kg of BDE-99 (>98%) in a 20% fat emulsion were given by gavage to male NMRI mice on PND 10. Spontaneous motor behavior was measured over three 20-minute periods in groups of mice at the age of 4 months. Ten mice were tested, randomly picked from three to five different litters in each treatment group. Spontaneous motor tests evaluated locomotion, rearing, and total activity behaviors. There were no clinical signs of toxicity or significant differences in body-weight gain or adult weight between controls and mice treated with BDE-99 at any time during the experimental period.

Habituation, defined as a decrease in the three behavioral variables (locomotion, rearing, and total activity) in response to the diminished novelty of the test chamber over the 60-minute period, was observed in the control animals. Mice exposed neonatally to 12 mg/kg of BDE-99 displayed significantly less activity (hypoactive) for all three behavioral variables during the first

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

20-minute period (0–20 minutes) compared with the controls, while during the third 20-minute period (40–60 minutes), they were significantly more active (hyperactive) than the control animals in relation to all three behavioral variables. Mice receiving 0.2 or 0.4 mg/kg BDE-99 showed no significant differences in activity for any of the three behavioral variables compared with the control animals at any of the 20-minute periods. The NOAEL in this study was 0.4 mg/kg and the LOAEL 12 mg/kg for effects on spontaneous motor behavior.

4.3.1.6. *Ankarberg (2003)*

The objective of the study by Ankarberg (2003) was to determine whether neonatal exposure to nicotine could affect the susceptibility of adult mice to BDE-99. The motor behavior response of adult mice after exposure to BDE-99 was used as a measure of the impact of the nicotine on the neonatal nervous system. Ten-day-old male NMRI mice received s.c. injections of saline (10 mL/kg) or nicotine base at 0.033 mg/kg, twice daily for 5 consecutive days (total daily dose, 0.066 mg/kg-day). Studies of brain development in rodents previously identified 10 days as the peak period for the developmental "brain growth spurt," during which mice and rats acquire many sensory and motor functions. At the age of 5 months, the 8–10 mice received 8 mg/kg BDE-99 in 20% fat emulsion or 10 mL/kg of 20% fat emulsion by gavage and were tested for spontaneous motor activity (locomotion, rearing, and total activity) for three 20-minute periods, 24 hours after exposure to BDE-99. Control animals, animals that received 0.066 mg/kg-day nicotine base neonatally but were not given the BDE-99, and animals that received only 8 mg/kg BDE-99 as adults showed a normal decrease in activity over the 60-minute test period, indicating a normal habituation pattern in response to the diminished novelty of the test chamber. However, the mice that received nicotine on PND 10 and BDE-99 as adults showed a lack of habituation. They displayed hypoactive behavior in the beginning of the test period (0–20 minutes) but became hyperactive toward the end of the period (40–60 minutes), indicating that the neonatal nicotine exposure had affected their susceptibility to BDE-99 as adults. At the age of 7 months, the animals were again tested for spontaneous motor behavior. The lack of habituation in the nicotine-BDE-99-treated mice was even more pronounced, indicating a disturbance that worsened with age. Overall, this study indicates that neonatal nicotine exposure affected the response of the adult animals to BDE-99.

4.3.1.7. *Branchi et al. (2002)*

In order to examine the neurobehavioral effect of perinatal exposure, BDE-99 dissolved in corn oil was administered by gavage at 0, 0.6, 6, or 30 mg/kg-day to groups of female CD-1 Swiss mice (four/group) from GD 6 through PND 21, at which time the pups were weaned.

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successful.

Effects on pregnancy and somatic and neurobehavioral development of pups were assessed. Body-weight gain of pregnant females, pregnancy duration, proportion of successful deliveries, pup sex ratio, and body-weight gain of pups from birth to weaning were not affected by treatment with BDE-99.

Six to eight male and female pups from each litter of each treatment group were used in a series of tests to assess somatic and neurobehavioral development from PNDs 2–20. These tests were carried out every 2 days and included hair growth; day of eyelid and ear opening and of incisor eruption; righting, forelimb stick grasp, and forelimb-placing reflexes; level and vertical screen tests; screen climbing test; and pole grasping test. Ultrasonic vocalization on PNDs 4, 8, and 12 and homing tests on PND 11 were carried out on one male and one female not previously handled or tested from each litter of each treatment group. In addition, an open-field apparatus was used to test locomotion (horizontal movement), rearing (vertical movement), and thigmotaxis (time and distance traveled close to the walls) in one male and one female from each litter of each treatment group for 30-minute sessions on PNDs 22 and 34 and for 60-minute sessions on PNDs 60 and 120.

In the battery of tests carried out from PNDs 2–20, BDE-99 treatment did not affect somatic development (hair growth and day of eyelid and ear opening and incisor eruption). There was a statistically significant 2-day delayed appearance of screen-climbing response in the high-dose group (30 mg/kg-day); all other responses based on neuromotor coordination from PNDs 2–20 were not affected by BDE-99 treatment. No effects were seen in pups from any of the treatment groups on ultrasonic vocalization or homing performance assessed on PND 11 both for distance traveled and latency to reach the scent area. In the open-field test, there was no statistically significant difference in activity between controls and treatment groups on PND 22. However, BDE-99 exposure affected several behavioral/activity parameters in the open-field arena on PNDs 34, 60, and 120, indicating that behavioral alterations due to perinatal BDE-99 exposure seem to worsen with increasing age, becoming clearly evident around 1 month of age. On PND 34, mice were hyperactive in the 0.6 and 6 mg/kg-day dose groups but not in the high-dose group. Mice exhibited a statistically significant increase in rearing frequency, with mice in the 0.6 mg/kg-day dose group being more hyperactive than mice in the 6 mg/kg-day dose group. The mice in the 6 mg/kg-day dose group also exhibited signs of increased locomotion as evidenced by increases in distance traveled, although this was not apparent in the low- and high-dose groups. Thigmotactic response, considered an index of anxiety, was not affected at any dose. On PND 60, mice in the 0.6 mg/kg-day group, but not in the 6 and 30 mg/kg-day groups, displayed significantly more locomotion compared with controls. Thigmotactic behavior on PND 60, measured as percent of time spent near the walls, was significantly lower at the medium dose only (6 mg/kg-day) in comparison with control mice, indicating a less marked fearful response in this treated group.

At adulthood (PND 120), the 0.6 and 6.0 mg/kg-day groups displayed significantly lower levels of locomotion (in contrast to hyperactivity measured prior to PND 120) than controls during the last part of the 60-minute test session. At this age, rearing and thigmotaxis were not affected at any BDE-99 dose. The authors concluded that prenatal and postnatal exposure of mice to BDE-99 produced a transient hyperactivity that was characterized by an inverted dose-response relationship, ending around 4 months of age.

The behavioral/activity changes observed in mice treated with BDE-99 did not consistently show a dose-response relationship. Effects were generally observed in mice administered the medium dose and not observed in the others. The lack of a clear dose-response relationship in the behavior/activity changes in this study does not permit clear identification of the NOAEL/LOAEL for alteration in behavioral or activity parameters. Additionally, the magnitude of variation in responses among the low, medium, and high doses cannot be determined with any precision because all motor activity data are presented in graphic forms and do not show a consistent relationship to dose.

4.3.1.8. *Branchi et al. (2005)*

It has been reported that gavage administration of a test compound can in itself produce stress in the animal. In this study, BDE-99 at 0 or 18 mg/kg-day was administered to CD-1 Swiss mice (nine/group) from GD 6 to PND 21, except for PND 0 (day of birth), when dams were left undisturbed. Two modes of administration of BDE-99 to the dams were investigated for their effects on neurobehavioral development in male offspring: by gavage in corn oil or by self-administration, consisting of letting the mouse spontaneously drink BDE-99 dissolved in corn oil from a modified syringe (without the needle and with a larger hole).

Pregnancy duration, body-weight gain of dams, proportion of successful deliveries, litter size, pup weight, and sex ratio were not affected by treatment with BDE-99 or by the method of administration in comparison with the respective control groups.

On PNDs 34, 60, 90, and 120, male mice (one from each litter, seven to nine mice/group) were tested in an open-field apparatus that measured locomotion (horizontal movement), rearing (vertical movement), and thigmotaxis (time and distance traveled close to the walls). Testing sessions were 30 minutes (three 10-minute blocks) on PND 34 and 60 minutes (six 10-minute blocks) on PNDs 60, 90, and 120. Each animal was tested only once. Distance traveled and frequency of rearing were not affected by the exposure methods (gavage or self-administration), and, therefore, the groups were pooled together. On PND 34, offspring of dams treated with BDE-99 showed hyperactivity for distance traveled and frequency of rearing during the third 10-minute testing period. On PNDs 60, 90, and 120, behavioral parameters (distance traveled and frequency of rearing) in treated mice were not different from those of controls at any time point during the 60-minute testing period.

With regard to thigmotactic behavior, an index of anxiety, mice administered BDE-99 by gavage spent more time near the wall than self-administered BDE-99 mice, when tested on PNDs 34, 60, and 90, with the percent of time spent near the wall in the gavage group reaching statistical significance on PND 34 only. On PND 120, the difference in thigmotactic behavior was minor, suggesting that the effect of the gavage route of administration was temporary.

On PND 22, two male mice from each of the control, BDE-99 gavage, and self-administered groups were sacrificed, and BDE-99 levels were determined in the brain. No effect of administration route was found on level of BDE-99 in the brain. The mean BDE-99 level in treated animals was 640 μg/kg compared to 5 μg/kg for the controls.

Serum total and free T_4 levels were also measured in BDE-99-treated male mice (eight to nine/group) on PND 22 and were not found to be statistically different from control levels. No effect of method of administration was found on T_4 levels.

4.3.2. Rats

4.3.2.1. *Kuriyama et al. (2005)*

The effect of in utero exposure to BDE-99 on locomotor activity and male reproductive health was investigated in rat offspring. Groups of 16–20 Wistar rats were given single doses of 0, 0.06, or 0.3 mg/kg of BDE-99 (98% purity) by gavage in peanut oil on GD 6. Since PBDEs may interfere with thyroid hormone homeostasis, a reference group for thyroid-mediated effects was included in which dams were treated from GDs 7–21 with 5 mg/L of the goitrogen 6-n-propyl-2-thiouracil (PTU) in drinking water (approximate dose: 0.9 mg/kg-day).

Developmental landmarks (eruption of incisors, fur development, eye opening, and testes descent) and postnatal reflexes (development of spontaneous cliff-drop aversion reflex starting on PND 3 and ability to stay on a rotating rod for 3 minutes at seven revolutions/minute starting on PND 18) were evaluated in 163–218 male and female pups. The eruption of incisors was significantly delayed in the PTU-treated group and in the 0.3 mg/kg BDE-99-treated group. The development of spontaneous cliff-drop aversion reflex was significantly delayed in the PTU-treated male and female offspring and in male offspring exposed to 0.3 mg/kg BDE-99. No other effects on developmental landmarks or spontaneous reflexes were seen.

On PNDs 36 and 71, the circadian locomotor activity of one male and one female per litter per group (16 litter groups, controls; 20 litter groups, 0.06 mg/kg; 19 litter groups, 0.3 mg/kg), housed individually, was evaluated over 24-hour periods. Locomotor activity was measured in individual offspring by using a device that monitors the locomotion of the animal at 5-minute intervals over a 24-hour period. Locomotor activity included total activity measured as light-beam interruption (LBI) counts per day, duration (hours) of activity per day, LBI counts per active phase (an active phase is defined as the time period from when the animal begins to move until it pauses moving), and duration of activity (minutes) per active phase. There was no

difference between the sexes for all groups, and therefore the data for males and females were pooled. On PND 36, the total activity (LBI count) was significantly increased in the offspring of dams treated with 0.3 mg/kg BDE-99 and in the PTU-treated group. The number of active hours per day was also higher in the 0.3 mg/kg group, an effect not seen in the PTU group. LBI counts/active phase and duration of activity/active phase were also significantly increased in the BDE-99 group at 0.3 mg/kg and in the PTU-treated group.

PTU-treated animals, while temporarily hyperactive on PND 36, restored to normal levels on PND 71. On PND 71, both total activity and duration of activity per day were significantly increased at 0.06 and 0.3 mg/kg BDE-99 but not in the PTU-treated groups. When the locomotor activity was expressed as LBI counts/active phase and duration of activity/active phase, hyperactivity was not seen on PND 71.

The effects of in utero exposure to BDE-99 on body and organ weights and the male reproductive system of adult offspring (PND 140) were also investigated in this study (Kuriyama et al., 2005). Twelve males per treatment group (from different litters) were sacrificed on PND 140, and thymus, liver, spleen, testis, epididymis, ventral prostate, and seminal vesicle weights were recorded. Spermatids and sperms were counted, sperm morphology were examined, and testosterone and luteinizing hormone (LH) levels were measured. No effects were seen on body weight or absolute and relative liver, thymus, seminal vesicle, or prostate weights in BDE-99-treated animals. Sperm morphology, LH, and testosterone were unaffected. Absolute and relative spleen weights were increased but not in a dose-dependent manner. Relative testis weight was significantly decreased at 0.3 mg/kg BDE-99 and in the PTU group, and relative epididymis weight was significantly decreased at 0.06 and 0.3 mg/kg BDE-99 and in the PTU-treated group. Sperm numbers were significantly decreased compared with those in controls at both BDE-99 doses but not in a dose-dependent manner, with sperm numbers (in millions) being 190, 135, and 156 in the controls, 0.06, and 0.3 mg/kg dose groups, respectively. Daily sperm production and spermatid count were significantly decreased at both doses in a dose-dependent manner: daily sperm production (in millions) was 44, 30, and 29 and spermatid counts (in millions) were 266, 183, and 175 in the control, low-, and high-dose groups, respectively. The percentage of abnormal sperm was within normal limits in all groups.

Reproductive effects were also examined in this study. Adult male offspring approximately 150 days old (5–19/group) from the BDE-99- and PTU-treated groups were mated with untreated females (1:1) daily for 14 days to determine whether the males were fertile and could produce normal offspring. The dams were sacrificed on GD 21. Uterine and fetal weights, number of implantations, implantations per litter, viable fetuses per litter, percent total resorptions, and male/female sex ratio were all within the normal range of control in all treatment groups.

Sexual behavior (ejaculatory, mounting and intromission latencies, intromission frequency, and number of penetrations before the first ejaculation) in 160-day male offspring (20/group) were also normal in all treatment groups compared with controls. The only effect seen was a significant decrease in the 0.3 mg/kg BDE-99 group in the number of animals that had two or more ejaculations during 20 minutes of mating. Approximately 50% of controls had a second ejaculation, while 70% of the PTU-treated animals achieved a second ejaculation. In the 0.06 and 0.3 mg/kg BDE-99 groups, only 39 and 21%, respectively, of the males achieved a second ejaculation. Therefore, PTU treatment improved the sexual performance of mice, while BDE-99 decreased it. The biological significance of this effect is uncertain.

In summary, treatment of rats with BDE-99 at 0.06 and 0.3 mg/kg on GD 6 resulted in a dose-dependent decrease in daily sperm production, spermatid count, and relative epididymis weight in adult male offspring on PND 140. No effects were seen on male fertility or sperm morphology at these doses. However, in rodent species, sperm number has to be substantially reduced before fertility is compromised, while relatively small changes in sperm production in men may affect human reproduction. The decreased sperm production, spermatid count, and epididymis weight warrant additional studies to determine their significance for reproductive functions in humans.

The LOAEL in this study was 0.06 mg/kg, based on increases in certain locomotor activity parameters on PND 71. A NOAEL for absence of hyperactivity was not identified in this study. The LOAEL for decreased sperm production, spermatid count, and relative epididymis weight on PND 140 was 0.06 mg/kg, the lowest dose tested.

4.3.2.2. *Viberg et al. (2005)*

The objective of this study was to determine whether the changes in spontaneous behavior and cholinergic receptors observed in adult mice neonatally exposed to BDE-99 (Viberg et al., 2004a, b) could also be induced in another species, namely the rat. Results from the receptor assay are reported in section 4.4.2.5.

Single oral doses of 0, 0.8, 8.0, or 16 mg/kg BDE-99 (purity >98%) in a 20% fat emulsion (1:10 egg lecithin to peanut oil) were given by gavage to male Sprague-Dawley rats on PND 10. Control rats received 10 mL/kg of the 20% fat emulsion. Spontaneous motor behavior was tested in nine 2-month-old rats randomly selected from three to five different litters in each treatment at each testing occasion. Spontaneous motor behavior was measured for a 60-minute period, divided into three 20-minute periods, at each dose. Spontaneous motor behavior tests used measured locomotion (horizontal movement), rearing (vertical movement), and total activity (all types of vibration within the test cage [i.e., those caused by rat movement, shaking/tremors, and grooming]).

There were no clinical signs of toxicity at any time during the experimental period nor significant differences in body weight gain or adult weight between controls and rats treated with BDE-99. Two-month-old control rats showed habituation (i.e., a distinct decrease in locomotion, rearing, and total activity over the three 20-minute test periods) in response to the diminishing novelty of the test chamber. Rats exposed on PND 10 to 8.0 and 16 mg/kg BDE-99 displayed significantly less activity for all three behavioral variables during the first 20-minute period, while, during the third 20-minute period (40–60 minutes), they were significantly more active than the control animals for all three behavioral variables. Rats receiving BDE-99 at 0.8 mg/kg did not show any difference from controls in locomotion or rearing activities over the three 20-minute test periods. A slight decrease in the total activity variable was seen only during the first 20-minute period that returned to control levels during the second and third 20-minute periods.

The NOAEL in this study was 0.8 mg/kg. The LOAEL was 8.0 mg/kg for significant changes in spontaneous motor behavior in 2-month-old rats exposed to BDE-99 on PND 10. These changes in behavior were characterized by hypoactive behavior, followed by hyperactive behavior, for all three variables (locomotion, rearing, and total activity) during the 60-minute test period. The NOAEL/LOAEL values in this study indicate that rats are equally or perhaps less sensitive than mice to the spontaneous motor behavior effects of BDE-99. In the study in mice by the same research group (Viberg et al., 2004a), the NOAEL was 0.4 mg/kg and the LOAEL was 0.8 mg/kg for significant changes in spontaneous motor behavior in 2-month-old mice exposed to BDE-99 on PND 10.

4.3.2.3. *Talsness et al. (2005)*

The effect of BDE-99 on the female reproductive system was evaluated by the same research group as that of the Kuriyama et al. (2005) study. A single dose of 0.06 or 0.3 mg/kg BDE-99 (98% purity) was administered by gavage to Wistar rats on GD 6. The controls received the peanut oil vehicle. A reference control was treated with PTU at a concentration of 5 mg/L in drinking water on GDs 7 through 21. At approximately 5 months of age, 20 virgin female F_1 offspring from each group were mated with untreated males to evaluate fertility.

Pregnancy rate, total implantation sites, mean implantation sites per gravid dam, total live fetuses per dam, resorption rate, and percentage of dams with resorptions in the F_1 females were not statistically different from controls at both doses of BDE-99. The only statistically significant effect noted was an increase in mean fetal weights at 0.06 mg/kg BDE-99, but not at 0.3 mg/kg BDE-99, and in the PTU-treated group. Pregnancy rate in the PTU-treated group was also significantly lower than in controls.

Histologic evaluation by electron or light microscopy of the ovary, uterus, and vagina was performed in the F_1 female offspring on PND 90. Electron micrographs and

photomicrographs revealed qualitative ultrastructural changes in the ovaries and hyperplastic vacuolar degeneration of the vaginal epithelium in the F_1 offspring from the 0.06 and 0.3 mg/kg BDE-99 and PTU-exposed groups. No significant changes were observed in the different ovarian follicle types following exposure to either BDE-99 or PTU, indicating that follicle numbers and maturation of follicles were unaffected. Skeletal anomalies were observed in two animals from the F_2 generation from two different litters, following exposure of the F_0 dams to 0.3 mg/kg BDE-99 on GD 99. The possible causes for these anomalies remain unknown, and the authors suggested they may be either spontaneous or substance related.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Subcutaneous Exposures

4.4.1.1. *Lilienthal et al. (2005)*

BDE-99 was administered by s.c. injections to pregnant Long-Evans rats from GDs 10–18 at doses of 1 or 10 mg/kg-day. Controls received the olive oil vehicle. For comparison, an additional group was exposed to Aroclor 1254 at 30 mg/kg-day. Dissections were conducted on GD 19 and in male offspring on PNDs 21 and 160 for organ examination and analyses of circulating levels of estradiol and testosterone. Neurobehavioral measurements in male offspring included sweet preference on PND 120 and haloperidol-induced catalepsy on PND 240. In addition, activity in the open field was studied in male offspring on PNDs 30, 90, and 400.

On PND 160 a slight but significant reduction in anogenital distance, a marker of sexual development, was reported in male offspring (eight/group) after maternal exposure to the high dose of BDE-99 (10 mg/kg-day) and also in the Aroclor-exposed group. Testes weights were not affected by BDE-99 or Aroclor treatment. Circulating levels of estradiol and testosterone were significantly decreased by exposure to BDE-99 at both doses of BDE-99 and by exposure to Aroclor 1254. Sweet preference, measured as the ratio of saccharin to water consumption, showed a significant increase (which may indicate behavioral feminization) at 10 mg/kg-day BDE-99 but was not altered by Aroclor treatment (10–12/group). Locomotor activity on PND 30, 90, or 400 of male rats in the open field was not changed by BDE-99 or Aroclor treatment. By contrast, in the catalepsy test, all exposed groups, in comparison with the control group, exhibited increased retraction latencies of the hind legs in the box 30 minutes after the injection with haloperidol. The authors concluded that these results, taken together, suggest an endocrine-modulating activity of BDE-99.

4.4.1.2. *Lilienthal et al. (2006)*

This follow-up study used the same protocol as Lilienthal et al. (2005) and was conducted to determine reproductive and developmental effects following BDE-99

administration by s.c. injections to pregnant Long-Evans rats from GDs 10–18 at doses of 1 or 10 mg/kg-day. Body weight of dams and body weight gain during gestation were not influenced by exposure to BDE-99 or Aroclor 1254. The numbers of implantations per litter and pups per litter and the percentage of male pups were not different from controls. At weaning there were no significant differences in weights of brain, thymus, testis, ventral prostate, and uterus between BDE-99-exposed and control rats. There was a tendency for decreased pituitary weights in male offspring on PND 21 at the high-dose level (10 mg/kg-day) compared with controls. In contrast, females on PND 21 showed a statistically significant increase in pituitary weight at the low dose only (1 mg/kg-day). The most notable effect seen was a significant reduction in thyroid weights in adult male and female offspring, compared with controls, after exposure to 1 and 10 mg/kg-day BDE-99.

Puberty onset was not affected in males but was delayed in females at 10 mg/kg-day. A slight but significant reduction in anogenital distance was reported in adult males (eight/group) on PNDs 21 and 160 after maternal exposure to the high dose of BDE-99 (10 mg/kg-day). A dose-related decrease in the number of secondary ovarian follicles was seen in females exposed to BDE-99 that was significant at 10 mg/kg-day.

Concentrations of BDE-99 in the brain tissues of dams and offspring were highest on GD 19 but decreased at weaning (PND 21) and returned to control levels on PND 160. A decline in adipose tissue concentration occurred in dams during lactation and in male offspring from weaning to adulthood. Circulating levels of estradiol on PNDs 21 and 160 were significantly decreased in male offspring exposed to BDE-99 at both doses. Levels of testosterone were significantly reduced in males on PND 160 at both doses of BDE-99. Sweet preference, measured as the ratio of saccharin to water consumption in male rats, showed a significant increase (which may indicate behavioral feminization) at 10 mg/kg-day BDE-99 but was not altered by Aroclor treatment (10–12/group).

In summary, gestational exposure to BDE-99 did not affect reproductive success in dams or development of body weights in offspring at the doses tested. The weights of reproductive and nonreproductive organs were largely unchanged. There was a significant dose-related decrease in circulating estradiol in male offspring at PNDs 21 and 160 and in testosterone on PND 160. A marked effect was the decrease in thyroid weights in adult offspring, which was more pronounced in the high-dose group.

4.4.1.3. *Ceccatelli et al. (2006)*

Ceccatelli et al. (2006) examined the effects of prenatal exposure to BDE-99 (purity >99%) on several developmental endpoints and gene expression in adult uteri of exposed female rats. Groups of six to nine time-pregnant Long-Evans dams were given daily s.c. injections of 0, 1, or 10 mg/kg BDE-99 from GDs 10–18. There were no clinical signs of adverse effects during

pregnancy, and the body weights of the dams were not significantly affected by the BDE-99 exposure. There were no significant differences between the control and exposed pups related to litter size, sex ratio, and body weight/litter on PND 2 or 14 and anogenital distance on PND 2. Litters were culled to 8–10 pups per litter on PND 2 and the male and female litter mates were kept separated. There was no additional exposure to BDE-99 after birth. Monitoring of the day of vaginal opening as the females reached puberty showed no significant delays on a per litter basis but showed a slight dose-related significant delay ($p < 0.05$) for individual rats.

The female pups were sacrificed at 12 weeks of age. Body weights as well as absolute and relative liver, uterine, and ovarian weights were measured. There were no significant differences among groups except for a slight but significant increase in absolute and relative ovarian weights in the animals prenatally exposed to 10 mg/kg-day. Analysis of plasma, brain, and adipose tissue for the presence of BDE-99, 120 days after exposure ceased, identified small amounts in the brain and plasma and substantially higher levels in the adipose tissues for both dose groups.

The uteri were collected from the now adult female pups. Levels of mRNA for insulin-like growth factor (IGF)-1, progesterone receptor (PR), estrogen receptor (ER)-α, and ER-β were measured after amplification by using real-time PCR and targeting of the resultant cDNA by using appropriate probes. There was a dose-dependent decrease in PR. In the case of ER-α and ER-β, the levels were elevated compared with controls for the 1 mg/kg dose but were comparable with controls for the 10 mg/kg-day dose. The IGF-1 results were more difficult to evaluate. Distributions within dose groups were highly skewed. The levels for the 1 mg/kg dose group were significantly higher than those for controls, while those for the 10 mg/kg group, although still elevated compared with controls, were not significantly different and were lower than those for the 1 mg/kg dose group.

The data from the first phase of the Ceccatelli et al. (2006) study suggested that BDE-99 might have subtle developmental impacts on the endocrine status of the uterus in female adult rats exposed to BDE-99 only during prenatal development. The authors then conducted a second phase to their experiment by examining the response of IGF-1, PR, ER-α, and ER-β biomarkers in prenatally BDE-99-exposed adult females after a single s.c. injection of estradiol-17β. The rats used in this part of the study were ovariectomized at 10 weeks, injected with estradiol-17β at 12 weeks, and sacrificed 6 hours later. The purpose of the ovariectomies was to reduce exposure to endogenous estrogen.

The results of these analyses were complex because, when compared with untreated controls, the ovariectomized controls had differing baseline levels of the hormonal biomarkers. Baseline levels for all of the biomarkers except ER-β were decreased compared with the nonovariectomized controls. Baseline levels of PR in the low-dose BDE-99-treated ovariectomized animals were significantly higher than those in the ovariectomized controls. In

the high-dose ovariectomized BDE-99-treated animals, IGF-1 and ER-β levels were significantly higher than those in ovariectomized controls.

After treatment with the estradiol, the levels of IGF-1 were increased in all groups, but the magnitude of the increase was less than that of the controls for both BDE-99-treated groups, and the difference relative to the controls increased with BDE-99 dose. The levels of PR also increased in response to estrogen; the increase in the high-dose BDE-99-treated group was significantly higher than in the controls. In response to the estradiol, the levels of ER-α and ER-β decreased in both the controls and the BDE-99-treated animals. There were no significant differences between groups for ER-α, but for ER-β there was a significant, dose-related increase in the magnitude of the response. Overall, the results of the estradiol challenge demonstrated that there were significant differences in the hormonal responses of prenatally exposed BDE-99-treated mice when they became adults.

4.4.2. Receptor Site Interactions

There is considerable evidence from studies of PCBs, chlorinated dibenzo-p-dioxins (CDDs), and chlorinated dibenzofurans (CDFs) that halogenated aromatic compounds exert an influence on cells by interacting with membrane receptor sites and activating cellular transcription factors. Transcription factor complexes then initiate DNA synthesis, allowing the cell to respond to the extracellular signal by producing a series of mRNAs that in turn produce a variety of proteins. This process is termed signal transduction. The structural similarities between PBDEs and PCBs suggest that PBDEs might activate the Ah receptor, ER, and androgen receptor. Based on the data from the well-studied PCBs, CDDs, and CDFs, the activation of these receptor sites is associated with immunosupression, reproductive effects, and carcinogenesis (Klaassen, 1996; Bock, 1994), all endpoints of interest for PBDEs. Table 4-1 provides a summary of the pentaBDE congeners that have been evaluated in a variety of receptor interaction studies.

Table 4-1. Receptor interaction studies of pentaBDE congeners

Congener evaluated	85	99	100	105	119	126	Findings
Ah receptor	X[a]	X	X	X	X	X	Effect levels are 10^{-2} to 10^{-5} that of TCDD[b]. Receptor binding and CYP-1A1/2 results are not always consistent for the different assays that have been completed.
ER	X	X	X	X	X	X	No activity for BDE-85 and -99. Weak activity for BDE-100 and -119.
Androgen receptor		X	X				BDE-100 has a greater antiandrogenic effect than BDE-99.
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, but the up-regulation is not concentration related.

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

4.4.2.1. *Aryl Hydrocarbon Receptors*

Transcription of the genes for CYP-1A1, -1A2, and -1B1 is linked to a signal transduction cascade that is initiated by activation of the Ah receptor by an appropriate ligand. The CYP-1 family of enzymes is highly conserved in mammals and is responsible for the oxidative metabolism of a variety of planar and near-planar compounds (Lewis et al., 1998). The CYP-1 family of enzymes metabolically activates and metabolizes polycyclic aromatic hydrocarbons and aromatic amines as well as PBDEs. Many substrates for the CYP-1 family enzymes are also Ah receptor ligands. Differences in Ah receptor affinity are correlated with variations in CYP-1 induction. 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 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. PentaBDE congeners BDE-85, -99, -100, -119, and 126 (>98% purity) had Ah receptor-binding affinities approximately 2×10^{-2} to 8×10^{-5} that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). BDE-85, which is not a major constituent in environmental samples, was the most active, but its relative binding affinity was only 2×10^{-2} that of TCDD. The binding affinities of the pentaBDEs were not influenced by the planarity of the molecule. The authors hypothesized that the large atomic volume of bromine distorted the Ah-binding site so that the coplanarity of the rings was less important in Ah binding than it was for the PCBs.

Quantitative measures of EROD induction were reported for BDE-85, -99, -100, -119, and -126. EROD induction was strongest in all cell lines for BDE-100, 119, and 126, although their relative induction potencies in the different cell cultures were approximately three to four orders of magnitude lower than the potency of TCDD. BDE-85 was a very weak inducer in rat hepatocytes and inactive in the other cells. The environmentally prominent congener BDE-99 was not an inducer in any cell line. These structurally related pentaBDE congeners were found to have differing responses in the in vitro test systems studied, and all were considerably less potent than TCDD, a strong Ah activator (Chen et al., 2001).

Peters et al. (2006) examined the interaction of BDE-99 and -100 as well as other PBDEs on the Ah receptor in primary hepatocyte cultures from four healthy cynomolgus monkeys (three

males, one female) by using EROD activation as a biomarker for receptor activation. Both compounds were weak Ah agonists when coexposures of TCDD and the respective PBDE were tested, as evidenced by a decrease in the activation caused by TCDD alone. The action of the PBDEs was receptor mediated rather than through inhibition of the enzyme 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 primary hepatocytes from the four monkeys, likely reflecting individual differences in the animals.

Using hepatocyte cultures from Sprague-Dawley rats, Chen and Bunce (2003) investigated whether PBDE congeners, including pentaBDEs, 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 among strength of Ah receptor binding, CYP-1A induction, and toxicity for many halogenated aromatic compounds.

There were four components to this study (Chen and Bunce, 2003): (1) the binding of the PBDE congener to the Ah receptor, (2) the binding of the receptor/PBDE complex to an oligonucleotide segment of the dioxin response element, (3) the induction of EROD, and (4) the production of CYP-1A mRNA and CYP-1A protein. The pentaBDE congeners evaluated in the study were BDE-85, -99, -100, -119, and -126.

BDE-119 and -126 were the most active of this group when compared to TCDD. They were moderately active in dioxin response element binding and induced responses of both CYP-1A1 mRNA and CYP-1A1 protein equivalent to the maximal response of TCDD in primary Sprague-Dawley rat hepatocytes, although at concentrations three to five orders of magnitude greater than TCDD. BDE-85 was inactive, and BDE-100 was a very weak activator of dioxin response element binding. When tested in combination with TCDD, BDE-119 and -126 tended to enhance the activity of a nonsaturating concentration of TCDD and slightly inhibit a saturating TCDD concentration.

The environmentally prominent congener BDE-99 was inactive at all stages of signal transduction. BDE-99 did not have an additive relationship with nonsaturating TCDD concentrations and acted as an antagonist in combination with a saturating TCDD concentration. The authors concluded that, at present, the current concentrations of PBDEs in the biota, including those of the environmentally predominant congeners BDE-99 and -100, contribute negligibly to dioxin-like toxicity compared with other environmental contaminants such as PCBs and TCDD but cautioned that this may change as the concentrations of PCBs decline and those for PBDEs increase.

Villeneuve et al. (2002) examined the ability of several pentaBDE congeners (BDE-99, -100, -105, and -126) to induce Ah receptor-mediated gene expression in vitro, using H4IIE-luc

(luciferase) recombinant rat hepatoma cells. The cells were grown in culture well-plates and then exposed to PBDE concentrations ranging from 2–500 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 (SDs) above the mean value for the control. BDE-99, -100, and -105 failed to induce Ah receptor-mediated gene expression in H4IIE-luc cells. BDE-126 induced significant Ah receptor-mediated gene expression at 500 ng/mL, but the magnitude of induction was only 1.7%-TCDD-max. 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-99 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-99 (96% purity) 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 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 Ah receptor activation.

BDE-99 had a significant effect on the level of CYP-1A1 (8.1 times the vehicle-treated controls) only at 100 μmol/kg-day (57 mg/kg-day), making it a weak activator of the Ah receptor. When BDE-99-induced CYP-1A1 expression was compared with induction by tetraBDE-47 and hexaBDE-153, the impact on the Ah receptor seemed to be correlated to the levels of contaminant polybrominated dibenzofurans in the mixtures, which in turn correlated with increasing 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.

4.4.2.2. *Other CYP-450 Inducing Receptors*

The study of CYP-450 mRNA expression in rat liver by Sanders et al. (2005) (see section 3.3) found that expression of CYP-2B was up-regulated by BDE-99 in F344 rats to a greater extent than was CYP-1A1, a biomarker for the activation of the Ah receptor. Up-regulation of CYP-3A was also observed. CYP-2B and -3A are biomarkers for activation of the constitutive androstane receptor (CAR) and pregnane X receptor (PXR), respectively. In the case of BDE-99, the effect on CAR was greater than that on PXR. The CAR and PXR are both involved in the metabolism of xenobiotics and are stimulated by phenobarbital. The CAR receptor is also involved in steroid metabolism. The impact of BDE-99 on these receptors is similar to the

impact of noncoplanar PCBs on the same receptors. Little is known about the physiological effects of PXR and CAR receptors.

Pacyniak et al. (2007) carried out additional work with the PXR and its human counterpart, the steroid X receptor (SXR), by using HepG2 cells transvested with the appropriate cDNA, the receptor response elements, and a luciferase reporter vector. The cultured cells were exposed to 0, 0.1, 1, 10, or 100 μM concentrations of BDE-99. With the PXR there was an increase in relative luciferase activity that showed a significant increase above the control for all tested concentrations; however, the increase above control was generally similar for all and was not concentration related. With the SXR, there was a linear significant response to dose for both the 10 and 100 μM concentrations but not for the 0.1 and 1 μM concentrations. The authors also compared the response in PXR knock-out mice (10–12 weeks old) with the wild-type mice and found that CYP-3A11 was induced to a similar extent in the wild-type and control animals, suggesting that the PXR was not activated by BDE-99. The authors suggested that the fact that CYP-3A11 protein was up-regulated even in the PXR knock-out mice could be related to activation of the CAR.

4.4.2.3. *Estrogen Receptors*

Studies have also been conducted to evaluate the interaction between PBDEs and the ER sites. 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 BDE-85, -99, -100, and -119 and three hydroxylated PBDEs, were investigated in a human T47D breast cancer cell line stably transfected with an ER-dependent luciferase reporter gene or human embryonic kidney cells stably transfected with an ER-α or ER-β luciferase reporter gene (Meerts et al., 2001). Cells were trypsinized and seeded in 96 well-plates for the ER-CALUX (Chemical-Activated LUciferase eXpression) assay. After allowing for cell growth, the wells were exposed to solutions containing the test compounds or estradiol and were incubated. The luciferase activity was measured with a luminometer. BDE-100 and -119 showed estrogenic potencies in the assay, with concentrations leading to 50% induction (median effective concentration [EC_{50}]) of 2.5 and 3.9 μM, respectively, in comparison to an EC_{50} value of 1.0×10^{-5} μM for estradiol. These pentaBDEs were, respectively, 250,000 and 390,000 times less potent than estradiol. BDE-85 and -99 did not show any estrogenic activity in the ER-CALUX assay (Meerts et al., 2001).

Several hydroxylated derivatives of PBDEs (≥99% purity) were also evaluated in the CALUX assay described above. 2,4,6,3',5'-Pentabromo-4'-hydroxy-DE (2,6-dibromo-4-(2,4,6-tribromophenoxy-phenol), a T_4-like hydroxylated-BDE, demonstrated no estrogenic activity up

to concentrations of 10 μM. Antiestrogenic potency was determined in the ER-CALUX assay by treating T47D.Luc cells with various concentrations of PBDEs in the presence of estradiol. The four pentaBDEs (BDE-85, -99, -100, and -119) and the T_4-like hydroxylated-BDE compound did not show antiestrogenic activity (Meerts et al., 2001).

Villeneuve et al. (2002) examined the ability of 10 different PBDEs, including BDE-99, -100, -105, and -126 (99% purity), to initiate ER-mediated gene expression in vitro. At concentrations up to 500 ng/mL, all pentaBDEs tested failed to induce ER-mediated gene expression in MVLN recombinant human breast carcinoma cells, using a luciferase response element for detection. Overall, the PBDEs tested were found to be 50,000 times less potent than estradiol for inducing ER-mediated gene expression.

Villeneuve et al. (2002) also studied the ability of PBDEs to displace steroid hormones from serum proteins. At concentrations up to 833 ng/mL, the pentaPBDEs tested in this study 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.

Another aspect of the possible impact of PBDEs on estrogen (estradiol) was investigated by Kester et al. (2002). In this instance, the authors studied the effect of hydroxylated PBDEs on the activity of the human sulfotransferases that metabolically inactivate estrogen. Inhibition of the sulfotransferases would increase the half-life of estradiol and facilitate increased opportunities for receptor site stimulation. In this study, the human sulfotransferase that is active in liver, endometrium, mammary gland, and testes was incubated with various concentrations of 4-hydroxy PBDE congeners. Tri-, tetra-, and pentaBDE hydroxy congeners were evaluated by using concentrations of 0–1,000 nM. All three compounds tested acted as inhibitors of the enzyme. The pentaBDE hydroxy congener (2,4,6,3',5'-pentabromo-4'-hydroxy-DE) was the most effective inhibitor of the three tested compounds, causing approximately 90% inhibition at the highest concentration. The authors hypothesized that the presence of bromine residues on the two carbons adjacent to the hydroxyl grouping increased the likelihood of inhibition. A Lineweaver-Burk analysis of the penta-compound data suggests that the inhibition was noncompetitive (i.e., the interaction with the enzyme did not involve the active site). The median inhibitory concentration (IC_{50}) for the inhibition was 150 nM.

In summary, the mechanistic studies of the ER indicate that the activities of the pentaBDEs are much lower than the activities of dioxin and PCBs. Receptor-site-mediated activity via the ER site appears to be minimal for the pentaBDEs.

4.4.2.4. *Androgen Receptors*

DE-71, a commercial pentaBDE mixture, was found by Stoker et al. (2004) to delay puberty and suppress the growth of androgen-dependent tissues in male Wistar rats exposed to doses of 30 or 60 mg/kg during the peripubertal 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 examined competitive binding of BDE-99 (98% purity) and BDE-100 (100% purity) congeners 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-99 and -100 were incubated in the presence of 1.0 nM R1881 (an agent that blocks the progesterone and glucocorticoid receptors) and 10 μM triamcinolone acetonide. Both congeners acted as competitive inhibitors for the binding of R1881, but the activity of BDE-100 was more potent than that of BDE-99. The approximate IC_{50} for BDE-99 was 33 μM, while BDE-100 had 98% inhibition at the same concentration.

In the assay using the MDA-kb2 cell line, BDE-99 and -100 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 (DHT). BDE-100 demonstrated a concentration-dependent antiandrogenic activity in this assay, with a 50% decrease in DHT activity at the 5 μM concentration. BDE-99 did not exhibit antiandrogenic activity in this assay.

4.4.2.5. *Acetylcholine Receptors*

Several studies have examined the impact of BDE-99 on acetylcholine receptors in the hippocampus. Data have been collected on the activity of both the nicotinic and muscarinic acetylcholine receptors. Nicotinic receptors are located in skeletal muscle and neurons. The muscarinic receptors are found in smooth muscle, glands, and the central nervous system (Klaassen, 1996). Interaction of acetylcholine with the appropriate receptor is responsible for neuronal activation of muscle contraction along with learning and memory (Ankarberg, 2003).

In rats and mice, the most active period for development of the cholinergic system occurs in the 3-week period after birth. There are several subfamilies of both muscarinic and nicotinic receptors. Some display high-affinity binding properties and others low-affinity binding (Ankarberg, 2003). Acetylcholine receptors are found in a number of areas in the brain, including the cortex, cerebellum, hippocampus, striatum, and thalamus (Ankarberg, 2003). The BDE-99 studies that have examined acetylcholine receptor binding have primarily utilized the hippocampal tissues.

A study of the impact of postnatal nicotine exposure by Ankarberg (2003) evaluated the hypothesis that nicotine exposure during the developmental brain growth spurt period would affect the development of the cholinergic system and change adult responses to cholinergic agents. Using nicotine as the cholinergic binding agent, Ankarberg (2003) found that the maximum impact on the cholinergic system occurred with exposures on PNDs 10–14 and not on PNDs 3–7 or 19–23. Hippocampal tissues from the nicotine-treated animals were evaluated 24 hours after exposure of the pups and adult animals by using radiolabeled α-bungarotoxin, a nicotinic receptor antagonist, and quinuclidinyl benzilate (QNB), a muscarinic receptor antagonist. Nicotine is an acetylcholine receptor stimulant. There was a decrease in the low affinity receptor-binding sites in all adult animals compared with pups exposed to nicotine 24 hours after exposure, suggesting a decline in receptors with age. However, only animals that had been exposed to nicotine on PNDs 10–14 had receptor levels as adults that were significantly lower than those of adult controls. Low-affinity-binding sites appeared to be affected to a greater extent than high-affinity sites (Ankarberg, 2003).

The results from the study by Ankarberg (2003) and knowledge of the plasticity of the cholinergic system in mice and rats during the postnatal period provided an incentive for examining cholinergic receptors in BDE-99-exposed mice. Viberg et al. (2004b) evaluated the nicotinic receptors in the hippocampus of adult mice after postnatal exposure to BDE-99 as part of a neurobehavioral study. Single oral doses of 0, 0.2, 0.4, or 12 mg/kg of BDE-99 in a 20% fat emulsion were given by gavage to male NMRI mice on PND 10, and the habituation response of the animals was evaluated at 4 months of age. One week after completion of the behavioral tests, the mice in the control and 12 mg/kg groups were sacrificed, and measurement of nicotine-binding sites in the hippocampus was performed by using ^{3}H-labeled α-bungarotoxin. Specific binding was determined by calculating the difference in the amount bound in the presence versus absence of α-bungarotoxin. There was significant decrease (31%) in α-bungarotoxin binding in the hippocampus of adult mice given 12 mg/kg BDE-99 on PND 10 compared to the density in control animals, indicating effects on the nicotinic receptors in the brain.

As part of another neurobehavioral study, Viberg et al. (2005) examined the binding of tritium-labeled QNB to muscarine-like binding sites in the hippocampus. Single oral doses of 0, 0.8, 8.0, or 16 mg/kg BDE-99 (purity >98%) in a 20% fat emulsion (1:10 egg lecithin to peanut oil) were given by gavage to male Sprague-Dawley rats on PND 10. Control mice received 10 mL/kg of the 20% fat emulsion. Behavioral tests were administered at 2 months of age. One week after completion of the behavioral tests, the rats in the 0, 8.0, and 16 mg/kg BDE-99 groups were sacrificed, and measurement of muscarine-like binding sites was performed. Specific binding was determined by calculating the difference in the amount of QNB bound in the presence versus absence of atropine, a known inhibitor of muscarinic receptors (Klaassen, 1996).

There was a significant decrease in the density of specific [^{3}H]-QNB binding sites in the hippocampi in rats given 16 mg/kg, while no difference was seen in rats treated with 8.0 mg/kg.

The results of the studies by Viberg et al. (2005, 2004b) in conjunction with the work by Ankarberg (2003) support the concept that exposure to BDE-99 during a critical window in postnatal development may result in a decrease in selected acetylcholine receptors in the brain and that these changes may contribute to some or all of the observed neurobehavioral responses exhibited in exposed animals as adults. Additional studies of receptor responses to the PBDEs, using the hippocampus and other regions of the brain, are warranted. The limited data available indicate that the effects on habituation were only seen at doses that also cause decreased binding of the cholinergic receptor antagonists.

4.4.3. 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 pentaBDEs 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 four pentaBDE congeners evaluated (BDE-85, -99, -100, and -119) did not compete with T_4-TTR binding (Meerts et al., 2000).

Meerts et al. (2000) also tested these four pentaBDEs before and after incubation with differently induced hepatic microsomes to examine the ability of their hydroxylated metabolites to displace T_4 from TTR. The pentaBDEs were individually incubated with liver microsomes prepared following induction with phenobarbital (a CYP-2B inducer), β-naphthoflavone (a CYP-1A inducer), or clofibrate (a CYP-4A3 inducer). Incubation of the pentaBDEs with CYP-2B-enriched rat liver microsomes resulted in the formation of metabolites that were able to displace ^{125}I-T_4 from TTR. The metabolites of BDE-100 and -119 were able to displace more than 60% of the ^{125}I-T_4 from TTR. BDE-85 and -99 showed a lower ability to displace ^{125}I-T_4 from TTR (20–60%). No T_4-TTR displacement by pentaBDEs occurred after incubation with liver microsomes enriched with CYP-1A or -4A3. PentaBDEs are therefore able to compete with T_4-TTR binding only after metabolic conversion by induced rat liver microsomes, suggesting an important role for hydroxylation. The relevance of this observation for humans has yet to be resolved. T_4-binding globulin, rather than TTR, is the major T_4-binding protein in humans.

As part of the Darnerud et al. (2005) study of the impact of a CB3 infection on the distribution of BDE-99, plasma total T_4 levels were monitored. On day 3 of the infection, decreases were seen in T_4 levels (33%). However, infections seem to be associated with a

decreased release of circulating T_4; thus, the decrease in T_4 seen in this experiment could be unrelated to ^{14}C-BDE-99 exposure.

4.4.4. Neurotoxicity

The effects of BDE-99 on the developing brain were investigated by Alm et al. (2006). Neonatal NMRI mouse pups were given a single 12 mg/kg dose of emulsified BDE-99 or vehicle (a 20% emulsion of egg lecithin and peanut oil, 1:10, in water) by gavage on PND 10, during the rapid brain growth spurt. Both groups were sacrificed 24 hours after dosing, and brains were rapidly excised. Striatal and hippocampal tissues from the brains of three mice were separately pooled, homogenized, and cleaned to remove lipids and nucleic acids. Samples were analyzed to determine total protein content and labeled using different cyanine dyes for the controls and for the treated tissues. There were three pooled tissue samples from the controls and from treated animals for both the striatal and hippocampal tissues. Two additional pooled replicate samples were prepared from controls and treated animals with unlabeled protein from the pool of control animals added to assist in protein identification. Four gels were run per brain region. The proteins were separated by two-dimensional fluorescence difference gel electrophoresis. Separation in the first dimension employed isoelectric focusing. Separation in the second dimension was done with polyacrylamide gel electrophoresis. There was considerable similarity in the gels from the striatum and hippocampus. There were 685 spots common to the four striatal gels and 651 spots common to the four gels from the hippocampus. From these common protein spots, 40 differentially expressed striatal proteins and 56 proteins from the hippocampus were selected for further analysis.

The gel spots selected for further analysis were removed; the protein was extracted and subjected to trypsin digestion. The resultant peptides were analyzed by using time-of-flight mass spectrometry and identified by means of the National Center for Biotechnology Information nonredundant database and the MASCOT search engine. Nine spots from the striatum and 10 from the hippocampus were identified in this fashion. Of the nine spots identified in the striatum, three were up-regulated and six were down-regulated. All 10 proteins identified in the hippocampus were up-regulated. Mortalin, a heat-shock protein up-regulated in the striatum and in the hippocampus, was the only protein common to both tissues. Two of the striatal spots were neuromodulins and three were stathmins. The neuromodulins play a role in guiding the growth of axons and forming new neural connections. Both neuromodulins were up-regulated in the BDE-99 exposed mice. Stathmins are also associated with neurite growth. They were down-regulated in the BDE-99 exposed mice. Both of these proteins are substrates for protein kinase C (PKC), an enzyme that functions in neuronal growth, learning, and memory. In the hippocampus, two γ-enolases, α-enolase, adenosine triphosphate synthase, a mitochondrial hydrogen ion transporter, and isocitrate dehydrogenase were all up-regulated. Several of these

proteins participate in PKC signaling complexes and/or are involved in cellular energy production. The enolases have also been observed in brain synaptic terminals.

Identification of some of the differentially expressed proteins in the brains of BDE-99-treated mice compared with controls does not fully explain the mode of action for the neurodevelopmental effects of BDE-99. It does indicate that brain proteins in the hippocampus and striatum of the exposed animals were different from those in the controls during the brain growth spurt period. Several of the differentially expressed proteins are linked to the PKC signaling cascade, a system that functions in neuronal growth, learning, and memory. The authors suggested that the proteins identified may be biomarkers that will be useful in additional studies of the early-life changes in brain development precipitated by BDE-99 exposure.

Activation of PKC has been suggested to be involved in the neurotoxicity of PCBs. Madia et al. (2004), therefore, examined whether BDE-99 and Aroclor 1254 would cause translocation of PKC α, ε, and ζ from cytosol to the membrane in astrocytoma cells. BDE-99 caused translocation of the three PKC isozymes present in astrocytoma cells, while Aroclor 1254 affected only PKC α and ε translocation. The ability of BDE-99 and Aroclor 1254 to induce apoptosis in astrocytoma cells was also investigated. BDE-99, but not Aroclor 1254, caused apoptotic cell death in astrocytoma cells. These results indicate that the overall pattern of cytotoxicity of BDE-99 to human astrocytoma cells is different from that of Aroclor 1254, suggesting that these two compounds may also have different effects in vivo.

As described in section 3.2.2, Kodavanti et al. (2005) carried out a study using cultures of cerebellar granule cells from 7- to 8-day-old Long-Evans rat pups. The cultures were treated with ^{14}C-labeled PBDE-99 (0.05 μCi/ml) combined with different concentrations of unlabeled compound (0–30 μM) for 15 minutes to 1 hour. For each concentration tested, there was a linear increase in percent accumulation over the 1-hour exposure period. When time was held constant and concentration varied, the percent accumulation increased only at the low concentrations, suggesting saturation of uptake. A similar pattern was observed for PKC translocation in the cerebellar granule neurons where ^{3}H-phorbol ester binding was increased at a PBDE-99 concentration of 10 μM but then remained fairly constant at 30 and 60 μM.

4.4.5. Immunotoxicity

Mitogen-induced DNA synthesis and immunoglobulin G (IgG) synthesis by human lymphocytes were examined after exposure to BDE-85 (≥98% purity) in vitro in order to determine the immunotoxic potential of this substance (Fernlof et al., 1997). Human peripheral lymphocytes were isolated from blood donated by 15 healthy females. The lymphocytes were cultured and utilized to assay radiolabeled-deoxythymidine uptake in response to pokeweed mitogen stimulation. In addition, the supernatants from the culture media were examined for the presence of immunoglobulin by using an antihuman IgG from goats. No effects on pokeweed

mitogen-induced DNA proliferation or IgG synthesis were observed in human lymphocytes after exposure of cells to 10^{-9} to 10^{-5} M BDE-85, indicating that this congener was not immunotoxic in this assay.

4.4.6. Cytotoxicity

The cytotoxicity of BDE-99 was assessed in human astrocytoma cells and compared with that of Aroclor 1254 (Madia et al., 2004). The mitochondrial activity that reduces 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was used to assess cell survival in a quantitative colorimetric assay. Cytotoxicity was also evaluated by measuring the release of lactate dehydrogenase (LDH) in the culture medium. To further determine the effects of treatments on cell survival, cells were treated with trypan blue and counted with a hemocytometer. BDE-99 and Aroclor 1254 caused comparable concentration-dependent inhibition of MTT reduction. Aroclor 1254 caused significant release of LDH at the two highest concentrations (50 and 100 μM), while BDE-99 did not cause any change in this parameter. Direct counting of dead cells with trypan blue staining provided similar results (i.e., Aroclor 1254 was toxic at high concentrations, while BDE-99 was not).

4.4.7. Genotoxicity

Evandri et al. (2003) studied the reverse mutation activity of BDE-99 by using *Salmonella typhimurium* strains TA98 and TA100, *Escherichia coli WP2 uvrA,* and the chromosome aberration test in *Allium cepa*. BDE-99 was nontoxic in bacteria at the highest dose tested (0.305 mg/plate). The number of revertant colonies was comparable to the solvent control group, both with and without S9. BDE-99 was also not clastogenic in the *A. cepa* test at concentrations up to 100 μM (56 mg/L). The number of structural chromosome aberrations induced by BDE-99 was not significantly different from that of the control.

4.5. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.5.1. Oral

Alterations of behavioral parameters (i.e., impaired motor functions worsening with age) have been shown to occur in male and female mice and rats orally exposed prenatally and neonatally to BDE-99 (Kuriyama et al., 2005; Viberg et al., 2005, 2004a, b; Branchi et al., 2002; Eriksson et al., 2002, 2001). Effects on spontaneous motor behavior were not species, gender, or strain specific and were induced during a defined and narrow developmental window in which rodents seemed to be uniquely susceptible to the neurodevelopmental effects of BDE-99 (Eriksson et al., 2002). Similar neurodevelopmental effects have been observed in studies of the tetra (BDE-47), hexa (BDE-153), and deca (BDE-209) congeners. 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-99, in the absence of human evidence, behavioral changes in experimental animal studies are assumed to indicate concern in humans.

Treatment of rats with BDE-99 on GD 6 resulted in a dose-dependent decrease in daily sperm production, spermatid count, and relative epididymis weight in rat offspring at a dose as low as 0.06 mg/kg BDE-99. However, no effects were seen on male fertility or sperm morphology at these doses (Kuriyama et al., 2005). The decreased sperm production, spermatid count, and epididymis weight warrant additional studies to determine their potential significance to humans.

The effects of BDE-99 on the female reproductive system were evaluated in rats (Talsness et al., 2005). Histologic changes in the ovaries and vaginal epithelium were seen at a dose as low as 0.06 mg/kg BDE-99 but were not associated with statistically significant effects on fertility (pregnancy rate, mean implantation sites per dam, live fetuses per dam, and resorption rate).

Data from the studies of Ceccatelli et al. (2006) and Lilienthal et al. (2006), conducted utilizing the subcutaneous exposure route and doses of 0, 1, or 10 mg/kg-day, suggest that BDE-99 may have a subtle impact on sexual development in males and females, possibly mediated through the steroid sex hormones. However, the effects observed were not consistently dose related.

BDE-99 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-99. Whether such exposures constitute a health risk at adulthood for neurodevelopmental dysfunction or adverse reproductive effects is not known at this time. An association between prenatal or neonatal exposures to BDE-99 and neurobehavioral or reproductive effects in humans has not been established.

4.5.2. Inhalation

No data are available on the toxicity of BDE-99 by the inhalation route of exposure.

4.5.3. Mode-of-Action Information

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, 2004b, 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). In rats and mice the critical postnatal brain growth period occurs within the first few weeks after birth,

while in humans it occurs in the last trimester of pregnancy and continues throughout the first year of life. PNDs 10–14 appear to be a period of maximum vulnerability for the developing cholinergic system that coincides with the most pronounced neurodevelopmental effects from BDE-99 exposure.

There is evidence that BDE-99 interacts with brain tissues from studies reported by Alm et al. (2006) in which the striatum and hippocampus of mice exposed to BDE-99 on PND 10 showed distinct differences in the proteins expressed from those seen in controls. Several of the proteins that seemed to be biomarkers for BDE-99 exposure are linked to the PKC signaling cascade. PKC signaling plays a role in neuron development, memory, and learning. Additional neurological interactions were observed in an in vitro study of BDE-99 uptake by cultured cerebellar granule neurons from Long-Evans rat pups that demonstrated that low BDE-99 concentrations caused translocation of PKC (Kodavanti et al., 2005). Although evidence exists that BDE-99 and other PBDEs interact at the neurological level, data are inadequate to determine the mode of action for BDE-99.

Exposure of rats to BDE-99 resulted in an increase of total T_4 plasma levels 3–6 days following exposure but returned to normal levels by 12 days after exposure (Hakk et al., 2002a). Serum total and free T_4 levels of male mouse offspring of dams treated with BDE-99 from GD 6 to PND 21 were not found to be statistically different from control levels on PND 22 (Branchi et al., 2005). In a study in mice (Skarman et al., 2005), BDE-99 administered from GD 4 to PND 17 had no effect on plasma T_4 levels in dams and their offspring relative to controls at any sampling occasion. The only effect noted in these in vivo studies was, therefore, a transient increase in T_4 plasma levels (Hakk et al., 2002a).

It is known that thyroid hormones are essential for brain development and that decreases in thyroid hormone levels during fetal and early neonatal life may have profound adverse effects on the developing brain (Morreale de Escobar et al., 2000). The limited data in humans (Mazdai et al., 2003) and the available data in mice and rats (Branchi et al., 2005; Skarman et al., 2005; Hakk et al., 2002a) do not seem to indicate that BDE-99 interferes with thyroid hormone homeostasis. However, thyroid hormone levels and behavioral activity were not comeasured in any of the developmental toxicity studies in mice or rats.

Hydroxylated pentaBDE metabolites have been shown in vitro to compete with T_4 for binding with high affinity to TTR. Meerts et al. (2000) indicated that pentaBDEs are 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. Despite the possibility that BDE-99 interacts with TTR, there are no mode-of-action data that link thyroid effects to the reported neurobehavioral effects observed in rodents

(Kuriyama et al., 2005; Branchi et al., 2005, 2002; Viberg et al., 2005, 2004a, b, 2002; Ankarberg, 2003; Eriksson et al., 2002, 2001).

Other observations include the absence of effects on pokeweed mitogen-induced DNA proliferation or IgG synthesis in human lymphocytes after exposure of cells to the pentaBDE-85 congener, indicating that this congener was not immunotoxic in this assay. BDE-99 was not mutagenic in *S. typhimurium* or *E. coli* assays, with and without S9, or in the *A. cepa* chromosome aberration test (Evandri et al., 2003). Studies of pentaBDE interactions with the Ah, estrogen, and androgen receptors indicate that these compounds are considerably less potent than dioxins and PCBs (Chen and Bunce, 2003; Villeneuve et al., 2002; Chen et al., 2001). The implications of these results are unknown.

4.6. EVALUATION OF CARCINOGENICITY

There is *inadequate information to assess the carcinogenic potential* of BDE-99 (U.S. EPA, 2005a,b). Epidemiological studies of exposure to BDE-99 and cancer occurrence in humans are not available. Animal chronic toxicity/carcinogenicity studies have not been conducted for BDE-99. BDE-99 was not mutagenic in *S. typhimurium* or *E. coli* assays, with and without S9, or in the *A. cepa* chromosome aberration test (Evandri et al., 2003). Additional in vitro or in vivo studies are not available to determine the full genotoxic potential of BDE-99.

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 mice and rats exhibiting alterations of neurobehavioral functions following prenatal and neonatal exposure to BDE-99 (Kuriyama et al., 2005; Viberg et al., 2004a, b; Branchi et al., 2002; Eriksson et al., 2001). The neonatal stage is a period of rapid development of the nervous system and is considered a critical window for brain 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-99 in human milk, umbilical cord serum, and children's serum (Mazdai et al., 2003; Schecter et al., 2003; Thomsen et al., 2002) implies humans are exposed to BDE-99 during a critical window of rapid development of the brain, indicating a potential for susceptibility. Whether such exposure constitutes a health risk for adverse neurodevelopmental effects in children is not known at this time because of the limited toxicological database for BDE-99. An association between prenatal or neonatal exposures to BDE-99 and neurobehavioral dysfunction in humans has not been established.

4.7.2. Possible Gender Differences

Most of the neurobehavioral studies were conducted in male rodents. In the neurobehavioral studies conducted in both sexes of mice and rats (Kuriyama et al., 2005; Viberg et al., 2004a; Branchi et al., 2002), there was no difference in neurobehavioral response in male and female animals from exposure to BDE-99. There is no indication that susceptibility to BDE-99 differs in male and female humans or in experimental animals.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

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

Table 5-1 summarizes the oral reproductive and developmental toxicity studies on BDE-99 that were candidates for use in the derivation of an RfD for this chemical. Other toxicity studies were also considered for use in deriving the RfD but were rejected for various reasons. These studies included a short-term toxicity study in mice (Skarman et al., 2005) and an acute toxicity study in rats (Hakk et al., 2002a). In these studies, hepatic mixed-function oxidase system enzyme activities and/or plasma thyroid hormone levels were measured following exposure to BDE-99 (see section 4.2.1). Changes in the activity of the mixed-function oxidase system enzymes, however, often accompany exposure to xenobiotic compounds and are not definitively adverse since enzyme activities are inducible and usually return to normal levels following cessation of exposure. In regard to hormone levels, the transient increase in T_4 levels seen in the single-dose study of Hakk et al. (2002a) was not confirmed in the longer duration study of Skarman et al. (2005). Thus, neither of these studies is a good candidate for use in dose-response assessment, and additional studies of the impact of BDE-99 on thyroid hormones are warranted.

Of the studies summarized in Table 5-1, Viberg et al. (2004a) was selected as the principal study, and neurobehavioral developmental effects were identified as the critical effect for deriving an RfD for BDE-99. The principal study and critical effect were selected after careful evaluation of all the available toxicity studies, including consideration of whether the data were amenable to BMD modeling (see section 5.1.2). The primary reasons for selecting Viberg et al. (2004a) were as follows: (1) several different dose levels of BDE-99 were employed, (2) quantitative dose-response data were available with which to conduct BMD modeling, (3) good model fits were obtained in subsequent BMD modeling, (4) a clear NOAEL was identified from this study, and (5) the results of this study are supported by several other studies in mice. In Viberg et al. (2004a), male and female mice were administered single oral doses (0, 0.4, 0.8, 4.0, 8.0, or 16 mg/kg) of BDE-99 on PND 10. The NOAEL identified in this study was 0.4 mg/kg. Adverse effects noted in 2-, 5-, and 8-month-old mice at the next highest dose, 0.8 mg/kg, included hypoactive spontaneous motor behavior in the beginning of the test period, hyperactive behavior at the end of the test period, and decreases in habituation capability, with these behavioral disturbances becoming more pronounced with increasing age. By administering BDE-99 on PND 10, animals were exposed during a period of maximum vulnerability of the developing mouse brain (Eriksson et al., 2002). Exposures to BDE-99 at

older ages (i.e., 2 months and up) did not cause such behavioral changes in mice (Branchi et al., 2005).

Table 5-1. Summary of oral reproductive/developmental toxicity studies of BDE-99

Species, strain, sex	Duration, purity	Dose levels (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Observed effects	Reference
Mouse, C57/B1, M & F	Single dose on PND 10, >99%	0, 0.4, 0.8, 4.0, 8.0, or 16	0.4	0.8	Neurobehavioral developmental effects; significant dose-related changes in spontaneous motor behaviors; decreased habituation capability with increasing age	Viberg et al. (2004a)
Mouse, NMRI, M	Single dose on PND 10, >98%	0, 0.2, 0.4, or 12	0.4	12	Neurobehavioral developmental effects and effects on the cholinergic system; significant dose-related changes in spontaneous motor behavior; decreased habituation capability with increasing age	Viberg et al. (2004b)
Mouse, NMRI, M	Single dose on PND 10, >98%	0, 0.8, or 12	Not identified	0.8	Neurobehavioral developmental effects; significant dose-related changes in spontaneous motor behavior & learning/memory ability; decreased habituation capability with increasing age	Eriksson et al. (2001)
Mouse, CD-1 Swiss, M & F	GD 6 to PND 21, purity not specified	0, 0.6, 6, or 30	Not identified	Not identified	Transient hyperactivity in absence of dose-response relationship	Branchi et al. (2002)
Rat, Wistar, M & F	Single dose on GD 6, 98%	0, 0.06, or 0.3	Not identified Not identified	0.06 0.06	Neurobehavioral developmental effects; effects on locomotion (hyperactivity), increasing with increasing age Decreased daily sperm production, spermatid count, and relative epididymis weight in offspring; no effect on fertility at any dose	Kuriyama et al. (2005)
Rat, Sprague-Dawley, M	Single dose on PND 10, >98%	0, 0.8, 8.0, or 16	0.8	8.0	Neurobehavioral developmental effects; significant dose-related changes in spontaneous motor behavior	Viberg et al. (2005)
Rat, Wistar, F	Single dose on GD 6, 98%	0, 0.06, or 0.3	Not identified 0.3	0.06 Not identified	Qualitative histologic changes in ovaries and vaginal epithelium in offspring No significant effect on fertility at any dose	Talsness et al. (2005)

Although Viberg et al. (2004a) was selected as the principal study, several concerns exist regarding the design of this study. First, the protocol was unique and did not conform to current

health effects testing guidelines for neurotoxicity screening batteries or developmental neurotoxicity studies (U.S. EPA, 1998b). Second, the dosing regimen did not encompass exposure via gestation and/or lactation (U.S. EPA, 1998a), with only single doses being administered. In some respects, the fact that effects occurred with such limited dosing argues for the sensitivity of this study. While dosing appears to have been performed during a critical window of developmental susceptibility, this design is inadequate to determine the effects of longer-term dosing. Extrapolating the results of this study to more traditional dosing regimens is problematic, particularly with regard to the potential impact of in utero and postnatal exposure. Another concern is that, based on the methodology provided in the published report, more than one pup per litter was used for the behavioral testing (eight mice were randomly selected from three to five different litters in each treatment group). This methodology may increase the number of pups from the same litter, which may bias the analyses towards false positives, resulting in observed neurobehavioral effects attributable to differences that are not treatment related in pups born to a single dam. Another concern regarding the study design was the limited number of neurobehavioral parameters that were assessed. The absence of a full functional observational battery (FOB) limits the ability of this study to correlate the reported effects with other FOB parameters, data which 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 animals will pose a potential hazard to humans. For BDE-99, in the absence of human evidence, data from experimental animal studies are used as the basis for the RfD.

While study design issues limit the utility of Viberg et al. (2004a), several additional considerations support the use of these data. Supporting data that exposure in Viberg et al. (2004a) occurred during the period of maximum vulnerability of the developing mouse brain come from Eriksson et al. (2002), who demonstrated that vulnerability of adult mice to neurodevelopmental effects of BDE-99 exposure occurred during a narrow phase of neonatal brain development. Furthermore, acute exposure to a highly lipophilic chemical that possesses an extended half-life, such as BDE-99, will result in systemic exposure that lasts much longer than suggested by the exposure duration. In addition, there are a wide variety of brain structures that have very limited critical windows during development. These critical windows result in susceptible periods of exposure that can be very short in duration. The concept that exposure during critical periods of development can induce functional neurological effects later in development has been demonstrated with structurally related PBDE congeners, including tetra-, hexa-, and deca-BDEs (Rice et al., 2007; Kuriyama et al., 2005; Viberg et al., 2004a, b, 2003a, b; Branchi et al., 2002; Eriksson et al., 2001). Therefore, the observed neurobehavioral effects are biologically plausible, and thus exposure to BDE-99 may pose a potential hazard to humans

(U.S. EPA, 1998a). Taken together, these considerations support the use of Viberg et al. (2004a) for deriving the RfD for BDE-99.

Supporting studies for neurobehavioral effects in mice from BDE exposure include Viberg et al. (2004b) in which male mice were administered single oral doses (0, 0.2, 0.4, or 12 mg/kg) of BDE-99 on PND 10. The NOAEL identified in this study was 0.4 mg/kg and the LOAEL was 12 mg/kg for dose-related changes in spontaneous motor behavior and effects on the cholinergic system. A similar study by Eriksson et al. (2001) identified a LOAEL for neurobehavioral developmental effects of 0.8 mg/kg in male mice treated with BDE-99 at doses of 0, 0.8, or 12 mg/kg on PND 10. In a study by Branchi et al. (2002), transient hyperactivity in the absence of a clear dose-response relationship occurred in male and female offspring of mice treated with BDE-99 at 0.6, 6, or 30 mg/kg-day on GD 6 through PND 21.

A supporting study that observed the neurobehavioral developmental effects of BDE-99 in rats include Viberg et al. (2005). In this study, male rats were administered single oral doses (0.8, 8.0, or 16 mg/kg) of BDE-99 on PND 10. The NOAEL in this study was 0.8 mg/kg and the LOAEL was 8.0 mg/kg for dose-related changes in spontaneous motor behavior in adult rats. In a study by Kuriyama et al. (2005), Wistar rat dams were exposed to 0, 0.06, or 0.3 mg/kg BDE-99 on GD 6. Male and female offspring from these dams showed significant increases in locomotor activity on PNDs 36 and 71 at both doses. The LOAEL for hyperactivity was 0.06 mg/kg.

Reproductive effects were also examined in the Kuriyama et al. (2005) study. Male offspring of Wistar rat dams exposed to 0, 0.06, or 0.3 mg/kg BDE-99 on GD 6 showed significant decreases on PND 140 in daily sperm production and spermatid count at both doses, but fertility was not affected. However, in rodents, sperm number has to be substantially reduced before fertility is affected.

The effects of BDE-99 on the female reproductive system have also been evaluated in rats by Talsness et al. (2005). In this study, qualitative histologic changes in the ovaries and vaginal epithelium were seen at both doses of BDE-99 tested, 0.06 and 0.6 mg/kg, but were not associated with significant effects on fertility (i.e., pregnancy rate, mean implantation sites per dam, live fetuses per dam, and resorption rate).

5.1.2. Methods of Analysis

The RfD for BDE-99 was derived by using the BMD approach by fitting the continuous models available in BMD software (BMDS) version 1.3.2 to the neurobehavioral data in mice from Viberg et al. (2004a). In the case of motor activity, no specific magnitude of change exists that is generally regarded as indicative of an adverse effect. In the absence of consensus on the level of response that is considered to be adverse, the benchmark response (BMR) selected was a change in the treatment mean equal to 1 SD of the control mean. In addition, changes in the

critical effect mean equal to 0.5 and 1.5 of the control SDs (0.5 and 1.5 SD, respectively) were also employed as BMRs to evaluate the impact of BMR choice on the BMD and the 95% lower bound on the BMD (BMDL).

Several BMD analyses were conducted by using relevant endpoints from Viberg et al. (2004a). The specific data sets used included locomotion habituation ratio for males and females at 2 and 8 months and rearing habituation ratio for males and females at 2 and 8 months. The BMD modeling results based on Viberg et al. (2004a) are summarized in Table 5-2, with further detail provided in Appendix B. No satisfactory model fits were obtained from the habituation ratio data based on locomotion activity in male and female mice from Viberg et al. (2004a). The BMD modeling results for the models that exhibited the best fit to the data for rearing habituation ratio are displayed in Table 5-2.

Based on data from Viberg et al. (2004a), the best-fit model was the power model, fit to data on rearing habituation in 8-month-old female mice following exposure to BDE-99. Based on the power model, the resulting BMD_{1SD} was estimated to be 0.41 mg/kg and the corresponding $BMDL_{1SD}$ was estimated to be 0.29 mg/kg. The BMDs and BMDLs corresponding to BMRs of 0.5 SD and 1.5 SD were also estimated in order to evaluate the impact of BMR selection on these model-derived BMDs and BMDLs. Employing the same data as before, the $BMD_{0.5SD}$ and $BMDL_{0.5SD}$ were estimated to be 0.29 and 0.20 mg/kg, respectively, while the $BMD_{1.5SD}$ and $BMDL_{1.5SD}$ were estimated to be 0.50 and 0.37 mg/kg, respectively.

Table 5-2. Summary of BMD modeling output results with good data fit in mice

Reference	Endpoint	Model	BMD_{1SD} (mg/kg-day)	$BMDL_{1SD}$ (mg/kg-day)
Viberg et al. (2004a)	Rearing habituation in 2-month-old male mice	Power	0.59	0.44
Viberg et al. (2004a)	Rearing habituation in 2-month-old female mice	Power	0.70	0.47
Viberg et al. (2004a)	Rearing habituation in 8-month-old female mice	Power	0.41	0.29

Sand et al. (2004) also applied the BMD method to the Viberg et al. (2004a) spontaneous motor behavior data observed in 2-, 5-, and 8-month-old male and female mice exposed orally on PND 10 to different doses of BDE-99. Additional data not available in the published study of Viberg et al. (2004a) were used to quantify spontaneous behaviors (i.e., locomotion, rearing, and total activity) in terms of a fractional response defined as the cumulative response after 20 minutes of the test divided by the cumulative response produced over the whole 1-hour test period. This fractional response contains information about the time-response profile (which differed among treatment groups) and was found to have appropriate statistical characteristics. In this analysis, male and female mice could be characterized by a common dose-response model (i.e., each sex responded similarly to exposure to BDE-99). By fitting the Hill model to

spontaneous motor behavior data observed in 2-month-old male and female mice, the BMDs and BMDLs corresponding to a BMR of 10% were estimated. Total activity was found to be the most sensitive neurobehavioral endpoint, with the BMD_{10} and $BMDL_{10}$ for this endpoint estimated to be 0.61 and 0.42 mg/kg, respectively. These values are similar to the BMD_{1SD} of 0.41 and the $BMDL_{1SD}$ of 0.29 mg/kg estimated from the published data in Viberg et al. (2004a) on rearing habituation in 8-month-old female mice. Values for the $BMD_{0.05}$ and $BMDL_{0.05}$ corresponding to a BMR of 5% were also estimated by Sand et al. (2004) and were 0.33 and 0.21 mg/kg, respectively.

For comparison purposes, all relevant endpoints from Eriksson et al. (2001) and Kuriyama et al. (2005) were also modeled. The specific data sets used and detailed BMD modeling results are presented in Appendix B. No satisfactory model fits were obtained for any of the endpoints from Eriksson et al. (2001). For the Kuriyama et al. (2005) study, additional information on the data points, as well as standard deviations of the means for locomotor activity on PNDs 36 and 71, were obtained from the study authors via e-mail (Ibrahim Chahoud, Charité University Medical School, Berlin, to Mary Manibusan, U.S. EPA, dated 2004). The data modeled as a continuous variable were locomotor activity on PND 36. Locomotor activity on PND 71 was not amenable to modeling in BMDS. The best-fit model for duration of activity per day (measured on PND 36) was the polynomial model, yielding a BMD_{1SD} of 0.28 mg/kg and a $BMDL_{1SD}$ of 0.22 mg/kg. The best-fit model for LBI counts per phase was the linear model, yielding a BMD_{1SD} of 0.16 mg/kg and a $BMDL_{1SD}$ of 0.11 mg/kg.

The $BMDL_{1SD}$ of 0.29 mg/kg estimated from data in mice (Viberg et al., 2004a) and the $BMDL_{1SD}$ of 0.22 mg/kg estimated from data in rats (Kuriyama et al., 2005) are very similar and suggest that mice and rats are equally susceptible to the neurobehavioral effects of BDE-99 and that no significant difference is apparent when the animals are exposed in utero versus perinatally to BDE-99. The mouse study by Viberg et al. (2004a) is selected for use in the derivation of the RfD because, as indicated above, in this study: (1) several different dose levels of BDE-99 were employed, (2) quantitative dose-response data were available with which to conduct BMD modeling, (3) good model fits were obtained in subsequent BMD modeling, (4) a clear NOAEL was identified from this study, and (5) the results of this study are supported by several other studies in mice. Conversely, the Kuriyama et al. (2005) neurobehavioral study in rats examined only two doses and is supported by only one other neurobehavioral study in rats, Viberg et al. (2005).

For the reproductive effects observed in the Kuriyama et al. (2005) study, none of the models in BMDS satisfactorily fit the data on spermatid count, sperm numbers, or daily sperm production. The only data that could be adequately modeled were percent of adult rats with less than two ejaculations (see Appendix B). However, no changes in fertility or other functional reproductive endpoints were observed as a result of these decreased ejaculations. In general, rat

fertility is less sensitive to changes in sperm count than human fertility. Therefore, without more information, the biological significance of this effect is uncertain. For this reason, the BMD modeling results based on the reproductive effects observed in Kuriyama et al. (2005) were not used in the derivation of the RfD.

The studies by Viberg et al. (2005, 2004b) of rats and mice, respectively, and the data from the Branchi et al. (2002) study were not amenable to a BMD approach because the data needed for dose-response modeling in BMDS (i.e., means and SDs) were not available from the published studies. Neurobehavioral data were only displayed graphically, and the needed means and SDs cannot be read with any accuracy from the graphs. Data from the study by Talsness et al. (2005) also could not be used for BMD modeling because the histologic changes observed in the ovaries and vaginal epithelium of rats were only qualitatively described. Neither incidence nor severity was quantified. In addition, no effect on fertility was observed in this study, and the NOAEL was at the highest dose tested.

5.1.3. RfD Derivation

Through use of BMD modeling, the estimated $BMDL_{1SD}$ of 0.29 mg/kg based on a decrease in rearing habituation in 8-month-old female mice exposed to BDE-99 on PND 10 (Viberg et al., 2004a) was selected as the point of departure for the RfD. To calculate the RfD, a total uncertainty factor (UF) of 3,000 was applied. This total UF was comprised of a factor of 10 to account for extrapolating animal data to humans (UF_A or interspecies variability), a factor of 10 to account for susceptible human subpopulations (UF_H or intrahuman variability), a factor of 3 to account for extrapolating from a single-dose exposure to a lifetime exposure (UF_S), and a factor of 10 to account for database deficiencies (UF_D). The rationale for application of each of these UFs is described below.

A 10-fold UF_A was used to account for laboratory animal to human interspecies differences. Although the toxicokinetics of BDE-99 in animals have been evaluated, no adequate description of toxicokinetics of BDE-99 in humans exists. The critical effect for deriving the RfD, altered behavior due to exposure during development, is expected to be relevant to humans. No quantitative data were identified to compare relative human and rodent sensitivity to these changes. However, given the longer period of brain development in humans as compared to rodents and the higher importance of cognitive function, it is appropriate to consider that humans may be more sensitive than rodents in the absence of specific data. Based on these considerations the default UF_A value of 10 was applied.

A default intraspecies UF_H of 10 was applied to account for variations in susceptibility within the human population (intrahuman variability). This factor accounts for the segment of the human population that may be more sensitive than the general population to exposure to

BDE-99. A default value is warranted because insuffiencient information is currently available to assess human-to-human variability in BDE-99 toxicokinetics or toxicodynamics.

A UF_S of 3 was used to account for extrapolating from effects seen in a single-exposure neurodevelopmental study to a lifetime exposure. Exposure on PND 10 occurred during a period of rapid brain development in mice. Brain development does not continue at an equivalent rate over a mouse's lifetime and is more quiescent during adult life stages. Many brain structures have a critical window during early life development. Following BDE-99 exposure, toxicokinetic data suggest that a mouse urinary protein becomes functional some time between PNDs 28 and 40, which leads to a dramatic increase in BDE-99 urinary excretion, especially in males. This increased excretion reduces the total body burden of BDE-99 in older mice compared with that in younger mice, including the levels of radiolabel reaching the brain 24 hours after dosing. These data 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-99 and/or its metabolites. Therefore, chronic exposure is not expected to result in more serious effects. However, because the mice received only a single dose rather than repeated doses over multiple days within the hypothesized critical window, a threefold UF was applied.

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

A UF_D of 10 was used to account for database uncertainty. The available oral database for BDE-99 lacks a full prenatal developmental neurotoxicity study, a multigeneration reproductive toxicity study, and conventional studies of subchronic and chronic toxicity. In addition, 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 this database UF.

In conclusion, application of a total UF of 3,000 to the $BMDL_{1SD}$ of 0.29 mg/kg results in an RfD for BDE-99 of 1×10^{-4} mg/kg-day or 0.1 μg/kg-day. For comparison, by using a NOAEL/LOAEL approach to derive the RfD, a total UF of 3,000 is applied to the NOAEL of 0.4 mg/kg for neurodevelopmental effects identified in the Viberg et al. (2004a) study, yielding an RfD for BDE-99 of 1.3×10^{-4} mg/kg-day or 0.1 μg/kg-day, essentially equivalent to the RfD derived by using the BMD approach.

5.1.4. Previous RfD Assessment

A previous IRIS assessment of commercial grade pentaBDE (CASRN 32534-81-9) is available (U.S. EPA, 1990). The composition of this commercial pentaBDE product was 58.1%

penta-, 24.6% tetra-, 13.3% hexa-, 2.6% hepta-, 0.8% deca-, 0.3% octa-, and 0.2% nonaBDE (Carlson, 1980a). An RfD of 2×10^{-3} mg/kg-day (2 µg/kg-day) was derived, based on a NOAEL of 1.8 mg/kg-day and a LOAEL of 3.5 mg/kg-day for induction of hepatic enzymes in a 90-day oral gavage study in rats (Carlson, 1980b), employing a UF of 1,000. This UF of 1,000 is comprised of a factor of 10 each for interspecies (i.e., animal to human) and intrahuman variability in lieu of specific data on this chemical and a factor of 10 to account for extrapolation from a subchronic effect level to its chronic equivalent. At the time of this previous assessment, insufficient information was available to derive an RfC or to assess the carcinogenicity of this commercial grade pentaBDE.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

No data are available for deriving an RfC for BDE-99.

5.3. CANCER ASSESSMENT

Inadequate information is currently available to assess the carcinogenic potential of BDE-99 (U.S. EPA, 2005a, b).

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

6.1. HUMAN HAZARD POTENTIAL

BDE-99 (CASRN 60348-60-9) is a component of commercial PBDE flame retardants. BDE-99 has been found in human milk, adipose tissue, and blood. As a result, fetuses and infants are potentially exposed to BDE-99. Although this information does not elucidate the effects of BDE-99 on the human general population, it does demonstrate that exposure can occur. For example, the presence of BDE-99 in human breast milk allows transfer of BDE-99 (and/or its metabolites) from a mother's body to her infant through breast-feeding.

No data are currently available regarding the potential toxicity of BDE-99 in humans exposed via the oral route. However, the available animal data indicate that the nervous system is a sensitive target. Neurobehavioral developmental toxicity has been identified as the critical endpoint of concern in mice following pre- and neonatal oral exposure to BDE-99. Specifically, BDE-99 appears to disrupt spontaneous behavior and causes hyperactivity in mice and rats, which appear to be permanent effects that worsen with age (Kuriyama et al., 2005; Viberg et al., 2004a, b; Branchi et al., 2002; Eriksson et al., 2001). Since fetuses and infants are exposed to BDE-99 via maternal/cord blood and human breast milk, such exposures may constitute a risk of adverse neurodevelopmental effects in these population groups. In addition to effects on spontaneous motor behavior, Kuriyama et al. (2005) reported impairment of spermatogenesis in adult rat offspring but no consequent effect on sperm morphology, sperm quality, testosterone, LH levels, or the ability to sire offspring. Histological changes in the ovaries and vaginal epithelium were seen in rats exposed to BDE-99. However, these changes were not associated with statistically significant effects on fertility indices (Talsness et al., 2005).

No studies currently exist on the potential carcinogenicity of BDE-99 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-99 at this time.

6.2. DOSE RESPONSE

The RfD of 0.1 μg/kg-day was derived from a $BMDL_{1SD}$ of 0.29 mg/kg-day, based on the effects of BDE-99 on spontaneous motor behavior in mice (Viberg et al., 2004a). A total UF of 3,000 was applied to this $BMDL_{1SD}$ to derive the RfD. This total UF of 3,000 was comprised of a factor of 10 to account for interspecies variability, a factor of 10 to account for intrahuman variability, a factor of 3 to account for extrapolation from a single-dose to a chronic exposure, and a factor of 10 to account for database deficiencies.

No data are currently available regarding the potential toxicity of BDE-99 in humans exposed 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-99 among humans is unknown, representing another important area of uncertainty in the RfD. However, subpopulations expected to be more susceptible to BDE-99 toxicity include fetuses and children. Chronic studies relevant to BDE-99 toxicity in these subpopulations have not yet been performed in experimental animals.

The principal study used in the derivation of the RfD (Viberg et al., 2004a) evaluated a number of behavioral parameters in a limited number of male and female mice ages 2, 5, and 8 months, exposed to BDE-99 on PND 10 at five different oral dose levels. Supporting studies for the neurobehavioral developmental effects of BDE-99 include a study in male mice exposed on PND 10 to three doses of BDE-99 (Viberg et al., 2004b), a study in which neonatal male mice were exposed orally to BDE-99 at two dose levels (Eriksson et al., 2001), a study that examined a number of neurobehavioral parameters in male and female neonatal mice pre- and postnatally exposed to three oral dose levels of BDE-99 (Branchi et al., 2002), a study on effects on locomotor activity in rats exposed in utero to two dose levels of BDE-99 (Kuriyama et al., 2005), and a study in rats exposed on PND10 to three oral doses of BDE-99 (Viberg et al., 2005).

The toxicological database for BDE-99 is sparse. No standard reproductive, developmental, subchronic, or chronic studies exist in rats or mice, nor is there a much needed developmental neurotoxicity study. In addition, several concerns regarding the experimental design of the Viberg et al. (2004a) study used in deriving the RfD have been raised (see section 5.1.1). Thus, the overall confidence in the RfD is low.

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

The "Toxicological Review" for pentabromodiphenyl ether (BDE-99) 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-99 was conducted in concert with the external peer review of other PBDE congeners (i.e., BDE-47, -153, and -209), and some external peer review charge questions were specific to congeners other than BDE-99. 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 follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. Synthesis of comments from individual peer reviewers resulted in summaries that combine similar statements from peer reviewers that were mentioned in conjunction with more than one charge question. In such cases, the comment and its response have been placed under the most relevant charge question. Some of the peer review comments were not directly related to charge questions. Those comments are categorized as miscellaneous and placed after those related to the charge questions. EPA also received scientific comments from the public. These comments and EPA's responses are included in a separate section of this appendix.

The peer review of the "Toxicological Review" of BDE-99 was coupled with the review of the documents for BDE- 47, -153, and -209. Accordingly, most of the charge questions address all four congeners. The responses to the charge questions in this appendix apply primarily to comments related to BDE-99. 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-99 IRIS assessment. One reviewer identified 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
Schecter, A; et al. (2006) Environ. Health Perspect. 114:1515-1520
Fischer, D; et al. (2006) Environ. Health Perspect. 114:1581-1584
Bradman, A; et al. (2007) Environ. Health Perspect. 115:71-74
Kodavanti, PRS; Derr-Yellin, EC. (2002) Toxicol. Sci. 68:451-457
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 reviewers and has included the relevant studies for BDE-99. Fischer et al. (2006), Bradman et al. (2007), and Kodavanti et al. (2005) were found to be relevant and were added to the document. The remaining studies suggested by the reviewer fell outside the scope of the IRIS assessment (i.e., exposure data, commercial mixtures). Additionally, a new literature search was conducted to ensure recently published, relevant studies are included in the IRIS assessment. A description of these studies (Pacyniak et al., 2007; She et al., 2007) has been added to the "Toxicological Review" in sections 3 and 4.

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 based on the neurobehavioral toxicity studies was clearly and transparently described.

Response: No response needed.

Comment 2: Two reviewers stated that the neurobehavioral effects are the only toxic effects that have been observed consistently in PBDE rodent studies. One of these reviewers stated that the neurobehavioral studies appeared to provide the most appropriate dose-response data on which to base the health assessment. Three reviewers felt that some consideration should be given to other studies that provide data suitable for deriving an RfD, specifically the studies of Kuriyama et al. (2005) and Branchi et al. (2002).

Response: The Agency reviewed and evaluated several studies of neurobehavioral endpoints in rats (Kuriyama et al., 2005) and mice (Branchi et al., 2002) and reproductive endpoints in rats (Kuriyama et al., 2005) in the IRIS health assessment for BDE-99. BMD analyses were conducted using all relevant endpoints in the Viberg et al. (2004a) and Kuriyama et al. (2005) studies. See the Agency's response to Comment 1 under Charge Question 4 for further rationale for the selection of the study and endpoint on which to base the derivation of the RfD.

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: One reviewer supported the use of the Eriksson/Viberg neurobehavioral studies as the basis for the derivation of the RfDs, given the limited body of toxicological information available. Two reviewers noted that the studies 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-99 but make the confidence low. Another reviewer noted that the neurobehavioral findings of this laboratory have been corroborated in a study examining BDE-99 (Kuriyama et al., 2005). None of the reviewers stated that the studies could not be used as the basis for the derivation of the RfD.

Response: The "Toxicological Review" contains a detailed summary of the concerns with the study design and methods utilized in the principal study (see section 5.1.1). A discussion of the use of only male mice in the study by Viberg et al. (2004a) has been added to the discussion in section 5.1.1 of the "Toxicological Review." Additionally, the neurobehavioral effects reported in Viberg et al. (2004a) are supported by an expanding body of literature (Rice et al., 2007; Viberg et al., 2007, 2005, 2004b, 2003a, b, 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 of BDE-99 (Kuriyama et al., 2005; Branchi et al., 2002) using more traditional methodologies that have generated toxic effects similar to those reported by Viberg et al. (2004a). Altogether, these studies support the findings of Viberg et al. (2004a) 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. (2004a) (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. (2004a) study was appropriate for determining the point of departure. 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 several candidate studies were modeled for determining the point of departure, and the Viberg et al. (2004a) had the best dose-response data and provided the best model fit. All four reviewers also stated that data in Kuriyama et al. (2005) should be considered for determining the point of departure. Two of these reviewers noted that the lowest effective dose in the Kuriyama et al. (2005) study was lower than those reported in the Viberg and Eriksson studies. Another of the four reviewers stated that Kuriyama et al. (2005) provided good dose-response relationships for neurobehavioral and male reproductive effects. One of the reviewers stated that Branchi et al. (2002) should also be considered as the principal study and that the lack of a dose-response relationship at the highest dose was not a compelling reason to avoid modeling the data at the lower doses. One reviewer stated that the histologic changes that occurred in the ovaries and vaginal epithelium in Talsness et al. (2005) at very low doses of 0.06 mg/kg were not described with sufficient clarity to

determine whether they could be used because neither the incidence nor the severity are described.

Response: The Agency has reviewed and evaluated several neurobehavioral and reproductive endpoints in rats from the Kuriyama et al. (2005) developmental study in the IRIS health assessment for BDE-99. It is difficult to interpret the neurobehavioral effects reported by Kuriyama et al. (2005) at PND 71 at the low dose of 0.06 mg/kg-day. While these effects are similar to those observed in Viberg et al. (2004a), the LOAEL of 0.06 mg/kg-day is much lower than the NOAEL of 0.4 mg/kg-day in Viberg et al. (2004a), and the point of departure, combined with a UF of 3,000, produces an RfD that is the second lowest in the IRIS database. When BMD analyses were conducted for the data collected on PND 36, where 0.06 mg/kg-day is a NOAEL and 0.3 mg/kg-day is a LOAEL, the BMDs and BMDLs from the two studies are similar and indicate that rats and mice may be equally susceptible to the neurobehavioral effects of BDE-99 and that no significant difference is apparent when the animals are exposed, in utero or perinatally, to BDE-99. Because of the difficulty of interpreting the effects of the Kuriyama et al. (2005) study at the low dose, the Agency decided to retain Viberg et al. (2004a) as the principal study until such time as further research elucidates the effects of BDE-99 at very low doses. Viberg et al. (2004a) was selected for use in the derivation of the RfD because a clear NOAEL was identified, and the mouse study provided six data points compared with the four data points provided in Kuriyama et al. (2005) collected at PND 36. Additionally, an adequate fit to the data was obtained with BMD modeling and the critical effects are supported by several other studies in mice. For the reproductive effects in Kuriyama et al. (2005), the only data set that did not demonstrate significant lack of fit to the available models in the Agency's BMD modeling software was the percent of adult rats with less than two ejaculations. These results were not used to derive the RfD in the BDE-99 assessment, based on the lack of supporting data and the uncertainty surrounding the biological significance of this effect. The effects of BDE-99 on the female reproductive system were evaluated in rats (Talsness et al., 2005). Histologic changes in the ovaries and vaginal epithelium were seen at a dose as low as 0.06 mg/kg BDE-99 but were not associated with statistically significant effects on fertility (pregnancy rate, mean implantation sites per dam, live fetuses per dam, and resorption rate). Data from the study by Talsness et al. (2005) also could not be used for BMD modeling because the histologic changes observed in the ovaries and vaginal epithelium of rats were only qualitatively described. Neither incidence nor severity was quantified. In addition, no effect on fertility was observed in this study, and the NOAEL was at the highest dose tested. The modeling output for Kuriyama et al. (2005) and Viberg et al. (2004a) and a brief discussion of the results can be found in Appendix B.

The Branchi et al. (2002) study was not selected as the basis for the RfD based on the lack of a clear dose-response relationship in the behavioral and activity parameters. While changes in these parameters were observed, the changes were frequently noted in the low- and mid-dose groups with no change in high-dose-treated animals or observed in only one of the three dose groups. These responses do not allow for the identification of a NOAEL and/or LOAEL. Additionally, the magnitude of variation in responses among the low-, mid-, and high-dose animals cannot be determined with any precision because all motor activity data are presented in graphic forms and, thus, are not amenable to modeling (even if the high dose was dropped). The rationale for not selecting Branchi et al. (2002) as the basis for the RfD is described in further detail in section 4.3.1.7

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 as critical effects and that these effects were appropriate 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 of exposure and the concentration of the chemical in the brain. One reviewer added that 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 document. It was not clear to another reviewer 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 4.4.2.5 and are referenced in the principal study summary (section 4.3.1.4). The evidence of neurochemical interactions and the potential relationship with the neurobehavioral effects are highlighted in the mode-of-action section of the document (section 4.5.3.). The document 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, data are unavailable to adequately determine the complete relevance of the neurochemical effects or to establish a mode of action. Data by Eriksson et al. (2002) demonstrated the presence of radiolabel in the brain after administration on PND 3, 10,

or 19, and the data by Alm et al. (2006) showed that there were neurochemical differences in animals treated with BDE-99 on PND 10 and controls. There are no measurements of levels of any of the congeners in the brain or other tissues at the time of neurobehavioral testing at 2 or 4 months to show if any differences exist in the brain or other tissues at those time points.

The actual motor activity components (locomotion, rearing, and total activity) that gave rise to the habituation ratios were reported in graphical form only and could not be reasonably estimated as presented, and the Agency's attempts to obtain the raw data were unsuccessful. Thus, the habituation ratios, rather than the actual habituation data, served as the basis for determining the point of departure.

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.

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

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 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. The fourth reviewer felt that the document did not provide much explanation or justification for applying the default interspecies and intraspecies UFs.

Response: There is little information available on the effects of BDE-99 in humans, and in the absence of data there is no scientific rationale for moving away from the default values for the interspecies and intraspecies UFs. Additional explanation for applying default interspecies and intraspecies UFs was added to section 5.1.3.

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, pharmacokinetic, and human data that would sufficiently illustrate the effects of BDE-99 in animals and humans. 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 pharmacokinetics and mode of action of BDE-99 in humans. In the absence of human data, the effects in potentially susceptible populations exposed to BDE-99 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 felt that the accumulation of the chemical should be considered in the calculation of the RfD. A third 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. Another 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-99, the principal study identified endpoints that, for the most part, reflect specific aspects of developmental physiology. The hypothesized window of susceptibility, proposed by the Eriksson/Viberg research group study authors, is based on the observation that the developmental neurotoxic effects observed following postnatal exposure to BDE-99, with peak vulnerability from PNDs on PND 10–14, 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 et al. (2005, 2004b, 2003a) 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-99. 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 about possible effects as the result of a maternal pre-pregnancy body burden, the Agency notes that, although the principal study did not include prenatal exposure, the maternal uptake and retention of BDE-99 during the prenatal period would be lower than that of the pups during the postnatal period of vulnerability because of the differences in toxicokinetics for mature versus neonatal animals. Support for the UF_S is provided by the Branchi et al. (2005, 2002) studies, where the effects levels were comparable to those in the Viberg et al. (2004a) study and exposures extended from GD 6 to PND 21.

Comment 5: One reviewer recommended applying a threefold UF to the BMDL point of departure to account for uncertainty in extrapolating from a dose of non-negligible toxicity ($BMDL_{10}$) to a dose of negligible toxicity.

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

Comment 6: One reviewer disagreed with the use of a 10-fold database UF_D, stating "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-99 lacks 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 UFD to allow a risk manager to consider the impact of this UF during the risk management process.

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

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

C. Body Burden Approach

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

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

Response: EPA examined the data on BDE-99 to determine if a body burden approach could be used for this congener during the development of the "Toxicological Review." It was determined that existing half-life, exposure, metabolite, and mode-of-action data could not support a body burden calculation for this congener.

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: Available mode-of-action data that describe the developmental neurotoxicity of BDE-99 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 based on studies of BDE-99 (Viberg et al., 2005, 2004b) and supported by studies with BDE-153 (Viberg et al., 2003a) and BDE-209 (Viberg et al, 2007, 2003b). They have further hypothesized that the sensitivity of the cholinergic system occurs in the vicinity of PND 10 and have tested this hypothesis by varying the time of dosing and observing differences in the habituation effect for BDE-99 and -209 (Viberg et al., 2007; Eriksson et al., 2002). The resulting deficit in cholinergic receptors persisted across the duration of testing and could cause a hypoactive response to exposure to cholinergic stimulants in adulthood. The following statement has been added to the mode-of-action summary (section 4.5.3): "Although evidence exists that demonstrates BDE-99 as well as other PBDEs interact at the neurological level, data are inadequate to determine the mode of action for BDE-99."

Miscellaneous Comments

Comments1: Three reviewers felt that the assessment would benefit from the combination of the individual documents for the four congeners into one comprehensive document to compare and cohesively present the similarities and differences among the congeners.

Response: The Agency has recently completed IRIS assessments for four individual PBDE congeners: BDE-47, -99, BDE-153, and -209 (see Foreword). These congeners were selected based on frequent detection in human tissues and the environment, availability in 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, the available database is sufficiently different from one congener to another to support the separation of the four IRIS assessments. However, in response to the comments from the peer reviewers, the Agency increased the text that compares the data on BDE-99 to that of the other congeners evaluated using comparable methodological approaches.

Comment 2: One reviewer noted that the document failed to cite the purities of the radioactive chemicals in most of the studies, the position of the label, location of radioactivity in the brain, and the specific activities of the ^{14}C compounds. Another reviewer was confused by the reliance upon the ^{14}C data and the intermixing with direct chemical measures. The reviewer felt that the conclusions drawn were challenging.

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 was 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.

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, 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 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 the relative potency of the four congeners.

Comment 5: One reviewer suggested that the Agency provide conclusions on the extent of metabolism and the presence of metabolites in excreta for the PBDEs or provide a statement if conclusions cannot be drawn. One reviewer suggested the addition of a summary at the beginning of the toxicokinetics section to reduce potential confusion.

Response: An overview to the toxicokinetics section and summary paragraphs have been added to section 3. The data to determine the extent of metabolism are presently not available. There do appear to be some differences in metabolism and excretion between mice and rats. The available data are presented in sections 3.3 and 3.4.

Comment 6: One reviewer felt that the toxicokinetic information was not presented objectively.

Response: The toxicokinetic data were presented as they were reported. In synthesizing the data, the authors consulted with Agency researchers when there was uncertainty in the interpretation of results. Every attempt was made to accurately reflect the data in the published papers.

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

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

Comment 8: One reviewer recommended adding data for BDE-209 to Table 3-1, Median PBDE congener concentrations in human biological media in the U.S.

Response: These data for BDE-209 were not available for inclusion in Table 3-2.

Comment 9: One reviewer felt that the evolution of exposures that are different in the U.S. compared with other countries and the pattern of exposures are important issues and the studies need to be presented to support or refute these observations.

Response: The Agency has provided information on exposure in the U.S. and other countries for comparison purposes. While the Agency agrees that exposure analysis is a critical component of risk assessment, a comprehensive presentation and analysis of exposure data are outside the scope of the IRIS health assessment.

Comment 10: One reviewer stated that the large number of bromine atoms of the PBDEs can impart electrophilic and lipophilic properties to the aromatic ring of the chemical and also noted that oily vehicles (e.g., corn oil) were used in most of the in vitro and in vivo animal studies. This reviewer was concerned that the vehicle could significantly alter the distribution and tissue uptake of the PBDEs between the oily vehicle and the biological system. These conditions could lead to decreased absorption and distribution with subsequent alteration in metabolism and excretion.

Response: The lipophilicity of BDE-99 is acknowledged in the "Toxicological Review" as part of section 3 on toxicokinetics. It will be necessary to determine if absorption occurs via the chylomicrons along with the body lipids or via direct membrane transport before the full impact of the vehicle on absorption, distribution, metabolism, and excretion can be determined. The data are currently inadequate to determine the impact of the oily vehicle on the distribution and uptake of BDE-99.

Comment 11: 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 BDE-99) in Zhou et al. (2002) occurred at doses that are higher than those that produce the neurodevelopmental effects of BDE-47 and BDE-99, then it must be concluded that the neurodevelopmental effects cannot be linked to the antithyroid effects of these compounds. Additionally, the antithyroid effects have not been substantiated.

Response: The Agency acknowledges that data are insufficient to determine the mode of action; therefore, text was added to section 4.5.3 stating this conclusion.

Comment 12: One reviewer noted that the Hill model (utilized in modeling the rearing habituation data in 8-month-old female mice [Viberg et al., 2004a]) fits best in terms of its having the highest *p* value; however, it also has the highest Akaike Information Criterion (AIC).

This reviewer stated that this is not consistent with the approach utilized in the BDE-209 assessment, in which the lowest AIC is one of the criteria for model selection and suggested that AIC be used consistently.

Response: The Agency reevaluated the approach utilized in analyzing the modeling results for BDE-99 and determined that among the models that adequately fit the data, the power model yielded the lowest AIC, indicating that it is the "best-fit" model for the rearing habituation data in 8-month-old female mice (Viberg et al., 2004a). The rationale for this decision is explained further in section 5.1.2 and Appendix B.

Comment 13: One reviewer felt that the use of a BMDL rather than a BMD as the point of departure should be explicitly stated.

Response: The following statement can be found in section 5.1.3: "[T]he estimated $BMDL_{1SD}$ of 0.29 mg/kg based on a decrease in rearing habituation in 8-month-old female mice exposed to BDE-99 on PND 10 (Viberg et al., 2004a) was selected as the point of departure for the RfD."

Comment 14: One reviewer did not understand why it was assumed that eight animals per sex were tested behaviorally in Viberg et al. (2004a). Could this not be clarified by contacting the study authors?

Response: The Agency reevaluated the study methods described in Viberg et al. (2004a). The review of the study showed that the males and females were analyzed separately, and the description of the study methods indicated that eight animals per analysis were randomly selected from each treatment group. Consequently, eight males and eight females were analyzed for each dose. Therefore, the statement that "we *assume* that there were eight males and eight females exposed to each level" has been removed from the document.

Comment 15: One reviewer suggested raising the confidence in the RfD from low to medium, considering the existence of multiple studies corroborating the main conclusions.

Response: The Agency acknowledges the existence of multiple studies that support the principal study. However, considering the lack of a two-generation reproductive study and subchronic and chronic toxicity studies, the Agency maintains the low confidence in the RfD for BDE-99.

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.

Comments: 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.

Comments: One public commenter questioned the selection of Viberg et al. (2004a) 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-99 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-99 and that its limitations were transparently discussed in the "Toxicological Review." Additionally, the neurobehavioral effects reported in Viberg et al. (2004a) are supported by an expanding body of literature (Rice et al., 2007; Viberg et al., 2007, 2005, 2004b, 2003a, b, 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.

APPENDIX B: BENCHMARK DOSE MODELING FOR BDE-99

METHODS

All dose-response modeling for this assessment was conducted by using EPA's BMDS. Two different versions of this software were employed. BMDS version 1.3.2 was used to fit the power and Hill models, while BMDS version 1.4 beta was used to fit the linear and polynomial models. These two different versions were used because of specific types of errors encountered with each version, as follows. When fitting continuous models in version 1.3.2 (i.e., linear, polynomial, power, and Hill models), the degrees of freedom listed when the parameter estimates hit a boundary are incorrect. This error appears to have been corrected in version 1.4 beta. However, preliminary analyses revealed that, in fitting the power and Hill models with version 1.4 beta, the models occasionally failed to converge, while with version 1.3.2 no convergence issues were encountered. Therefore, version 1.4 beta was used to fit linear and polynomial models, while version 1.3.2 was used to fit the power and Hill models. With version 1.3.2, the degrees of freedom for the power and Hill models were corrected manually. A BMR of one estimated SD (1.0 SD) from the control mean was used for all endpoints, consistent with the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b).

A. Dose-Response Modeling Using the Data from Viberg et al. (2004a)

Rearing and locomotion habituation in male and female mice were each modeled separately by using the continuous dose-response models available in BMDS (i.e., linear, polynomial, power, and Hill models). All data sets failed the test for equality of variances across dose groups, so the variance was modeled as a power function of the mean. In some cases, this variance model failed to accurately estimate the observed variances; however, no alternative variance model is currently available in BMDS. Therefore, in those cases where the modeled variances yielded poor results, the differences between the observed and estimated standard deviations were examined, especially in the region of the BMR. In all cases, these differences were determined to be minimal, and thus the variance was modeled as a power function of the mean in all modeling runs.

For both rearing and locomotion habituation, when data from all six dose groups were included, all of the continuous models available in BMDS exhibited a significant lack of fit (i.e., goodness-of-fit p value <0.10) when default restrictions were placed on model parameters. For some endpoints, satisfactory goodness-of-fit p values (i.e., $p > 0.10$) were obtained by fitting a polynomial model with no restrictions on the parameters (Figure B-1). However, this unrestricted model yielded unreasonable behavior at low doses (i.e., in the region of the BMD) as shown in Figure B-2. As Figure B-2 shows, the fitted model exhibited an initial decrease in

the dose-response function, which is inconsistent with biological knowledge about the response to PBDE exposure. Therefore, default parameter restrictions were employed in all models to prevent such biologically implausible regions of decreasing dose response.

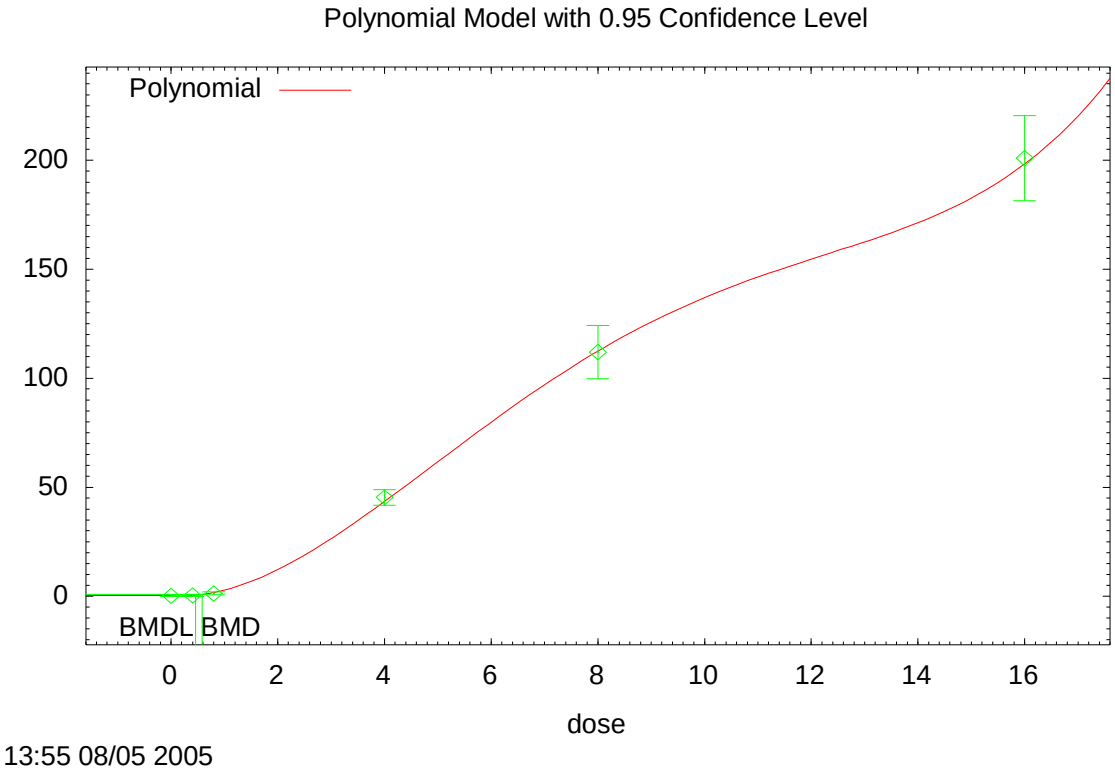


Figure B-1. Unrestricted fourth-order polynomial model fit to rearing habituation in 2-month-old male mice exposed to BDE-99.

Data source: Viberg et al. (2004a).

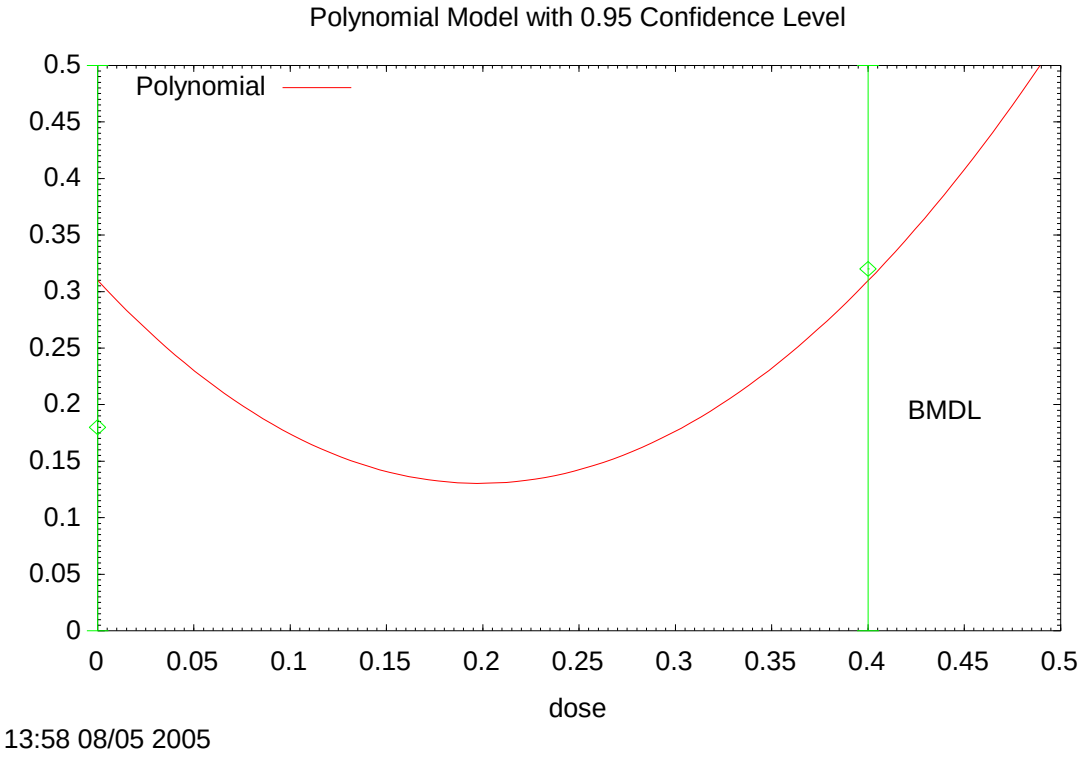


Figure B-2. Low-dose behavior of unrestricted fourth-order polynomial model fit to rearing habituation in 2-month-old male mice exposed to BDE-99.

Data source: Viberg et al. (2004a).

In most cases, when all six dose groups were included, the dose response trend at the higher doses was different from that in the low-dose range. Therefore, to obtain a better fit to the data at low doses (i.e., the region of interest), the highest dose group, 16 mg/kg body weight, was dropped from the analysis. This is a reasonable approach given that the estimated BMD is around 0.8 mg/kg, which is far below the high dose of 16 mg/kg. Among the four continuous models available in BMDS, only the Hill model provided an adequate fit to these truncated data sets, with adequate goodness-of-fit *p* values (i.e., $p > 0.10$) obtained for the following endpoints: rearing habituation in 2-month-old male mice and rearing habituation in 2- and 8-month-old female mice (Tables B-1, B-3, and B-4, respectively). Even with the dropping of the high dose, however, adequate model fits were not obtained for rearing habituation in 8-month-old male mice, so for this data set the 8 mg/kg dose group was dropped as well. All of the continuous models available in BMDS, except the Hill model, were fit to the remaining four dose groups. This approach still seems reasonable because the retained high-dose group at 4 mg/kg is still well above the region of the BMD. The Hill model was not fit to this truncated data set because this model requires at least five dose groups in order to compute goodness-of-fit *p* values. Finally, adequate model fits were not obtained for any of the continuous models fit to the locomotion habituation data employing either full or truncated data sets.

For many of the endpoints, the mean response at low dose (i.e., 0.4 mg/kg) showed no increase above the mean of the controls and also exhibited significantly smaller standard deviations than the high-dose groups. As a result, the monotonically increasing dose-response models in BMDS had difficulty fitting this low-dose mean, which resulted in poor goodness-of-fit because of the very small SD at this low dose, even though a plot of the dose-response curve appeared to fit the responses very well by visual examination. Despite the apparent good fit to the data, BMD estimates from models that lacked goodness of fit (i.e., $p < 0.1$) were not considered when determining the point of departure because the nonresponse at the 0.4 mg/kg dose is so critical in determining the shape of the curve in the low-dose region.

BMD modeling results for all endpoints from Viberg et al. (2004a) are summarized in Tables B-1 through B-4. In addition to goodness-of-fit tests, AIC was employed for model comparison and selection. The AIC is a function of the log-likelihood, and the numbers of parameters in the model, as well as each data point, contribute to the log-likelihood. Thus, the AIC can be used to compare model fits across models fit to the same data sets. The AIC was employed to make comparisons across the linear, polynomial, and power models fit to the same data sets in Tables B-1 through B-4.

Modeling Results

1. Rearing habituation in 2-month-old male mice

The BMD modeling results for rearing habituation in 2-month-old male mice are summarized in Table B-1. Based on the results of the chi-square goodness-of-fit tests, the Hill and power models did not exhibit significant lack of fit, while the linear and polynomial models did, and thus were not considered further in the derivation of the point of departure. Of the two models that did not exhibit significant lack of fit, the power model provided the best fit to the data because this model had a lower AIC value than the Hill model (i.e., 39 versus 96, respectively). Thus, for rearing habituation in 2-month-old male mice, the $BMD_{1.0SD}$ estimate selected is 0.59 mg/kg, and its corresponding $BMDL_{1.0SD}$ is 0.44 mg/kg.

Table B-1. Rearing habituation in 2-month-old male mice

Model	No. Groups	*p* Value	AIC	$BMD_{1.0SD}$ (mg/kg)	$BMDL_{1.0SD}$ (mg/kg)
Hill	5	0.84	96	0.63	0.48
Linear	4	<0.01	86	4.2	1.7
Polynomial	4	0.029	44	0.39	0.33
Power	4	0.71	39	**0.59**	**0.44**

Source: Viberg et al. (2004a).

Standard BMDS Output from Fitting the Power Model to Rearing Habituation in 2-Month-Old Male Mice (8 and 16 mg/kg-day Dose Groups Omitted)

```
Power Model
BMR = 1.0 SD
 ======================================================================
        Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
        Input Data File: S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\VMRTRUNC.(d)
        Gnuplot Plotting File:  S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\VMRTRUNC.plt
                                          Thu Aug 04 13:53:06 2005
 ======================================================================
 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = mg/kg
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = alpha*mean(i)^rho

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                         alpha =      5.05143
                           rho =            0
                       control =         0.18
                         slope =      1.46193
                         power =      2.47391


          Asymptotic Correlation Matrix of Parameter Estimates

                 alpha          rho      control        slope        power

   alpha             1        -0.38        -0.54       -0.053        0.056

     rho         -0.38            1         0.46        -0.12         0.11
 control         -0.54         0.46            1        -0.37         0.36

   slope        -0.053        -0.12        -0.37            1        -0.99

   power         0.056         0.11         0.36        -0.99            1


                       Parameter Estimates

      Variable          Estimate            Std. Err.
         alpha          0.670595             0.21373
           rho          0.818561            0.129962
       control          0.222551            0.112891
         slope           1.57485            0.418633
```

power 2.41986 0.191752

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	8	0.18	0.52	0.223	0.443	-0.0961
0.4	8	0.32	0.59	0.394	0.559	-0.132
0.8	8	1.27	0.76	1.14	0.864	0.15
4	8	45.3	4.36	45.3	3.9	-0.00486

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-39.778224	5	89.556448
A2	-13.995306	8	43.990611
A3	-14.484425	6	40.968849
fitted	-14.554490	5	39.108980
R	-111.525662	2	227.051325

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	d.f	p-value
Test 1	195.061	6	<.00001
Test 2	51.5658	3	<.00001
Test 3	0.978238	2	0.6132
Test 4	0.140131	1	0.7082

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

The p-value for Test 2 is less than .05. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .05. The modeled variance appears

to be appropriate here

The p-value for Test 4 is greater than .05. The model chosen seems
to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 0.591919

BMDL = 0.435548

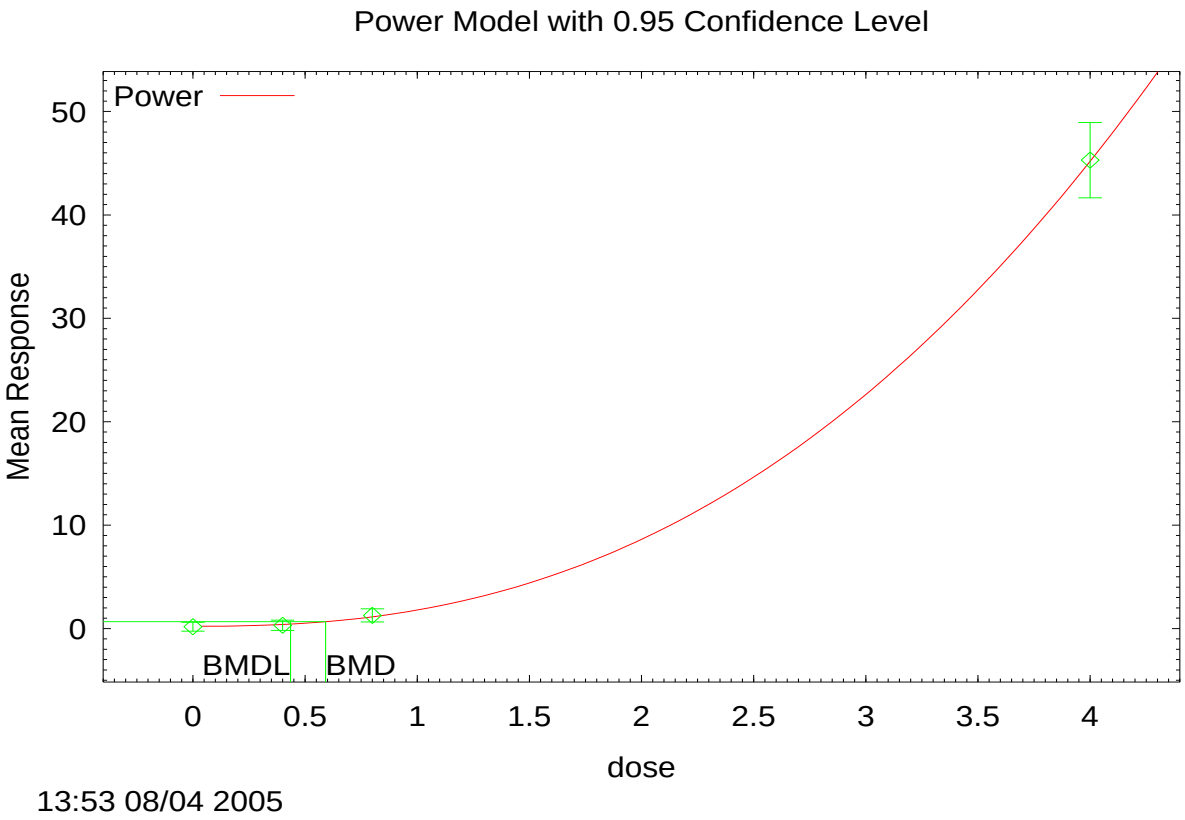


Figure B-3. Dose-response relationship based on rearing habituation in 2-month-old male mice.

Data source: Viberg et al. (2004a).

2. Rearing habituation in 8-month-old male mice

The BMD modeling results for rearing habituation in 8-month-old male mice are summarized in Table B-2. According to the results of the chi-square goodness-of-fit tests, none of the continuous models in BMDS provide satisfactory fits to these data because all of the models exhibited significant lack of fit ($p < 0.1$). This lack of fit may be due primarily to the mean response reported in the 0.4 mg/kg dose group, which was zero. The published manuscript from which these data were extracted did not indicate the basis for this zero value at 0.4 mg/kg (i.e., whether the calculated values were very small and reported as zero or whether no data were obtained for this dose group). Because this dose was in the vicinity of the BMD, modeling was not conducted with this dose group dropped from the data set. Therefore, because of this failure of the models to fit the data, no BMD or BMDL could be derived based on this endpoint.

Table B-2. Rearing habituation in 8-month-old male mice

Model	No. of groups	*p* Value	AIC	$BMD_{1.0SD}$ (mg/kg)	$BMDL_{1.0SD}$ (mg/kg)
Hill	5	<0.01	104	0.57	0.45
Linear	4	<0.01	160	0.055	0.019
Polynomial	4	<0.01	62	0.42	0.36
Power	4	<0.01	60	0.56	0.42

Source: Viberg et al. (2004a).

3. Rearing habituation in 2-month-old female mice

The BMD modeling results for rearing habituation in 2-month-old female mice are summarized in Table B-3. According to the results of the chi-square goodness-of-fit tests, the Hill, polynomial, and power models do not exhibit significant lack of fit. Among these three models that adequately fit the data, the power model yielded the lowest AIC, indicating that it is the best-fit model. Thus, for the endpoint rearing habituation in 2-month-old female mice, the $BMD_{1.0SD}$ estimate selected is 0.70 mg/kg, and its corresponding $BMDL_{1.0SD}$ is 0.47 mg/kg.

Table B-3. Rearing habituation in 2-month-old female mice

Model	No. of groups	*p* Value	AIC	$BMD_{1.0SD}$ (mg/kg)	$BMDL_{1.0SD}$ (mg/kg)
Hill	5	0.53	122	0.71	0.50
Linear	4	<0.01	130	0.065	0.034
Polynomial	4	0.12	73	0.45	0.38
Power	4	0.62	71	**0.70**	**0.47**

Source: Viberg et al. (2004a).

Standard BMDS Output from Fitting the Power Model to Rearing Habituation in 2-Month-Old Female Mice (8 and 16 mg/kg-day Dose Groups Omitted)

```
Power Model
BMR = 1.0 SD

 ================================================================
        Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
        Input Data File: S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\VFRTRUNC.(d)
        Gnuplot Plotting File:  S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\VFRTRUNC.plt
                                        Fri Aug 05 10:23:14 2005
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = mg/kg
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = alpha*mean(i)^rho

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                        alpha =      30.2634
                          rho =            0
                      control =         0.24
                        slope =      2.07452
                        power =      2.22846


          Asymptotic Correlation Matrix of Parameter Estimates

                 alpha          rho      control        slope        power
   alpha             1        -0.54        -0.64       -0.076        0.077

     rho         -0.54            1         0.52       -0.097        0.068

 control         -0.64         0.52            1        -0.41          0.4

   slope        -0.076        -0.097        -0.41            1        -0.98

   power         0.077        0.068          0.4        -0.98            1


                        Parameter Estimates

      Variable           Estimate            Std. Err.
         alpha            1.22736            0.477381
           rho            1.13527            0.149123
       control            0.37407            0.172425
         slope            1.52431            0.596555
```

power 2.44986 0.283549

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	8	0.24	0.69	0.374	0.634	-0.211
0.4	8	0.51	0.95	0.536	0.777	-0.0329
0.8	8	1.49	0.93	1.26	1.26	0.185
4	8	45.8	10.9	45.9	9.72	-0.00785

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-68.422509	5	146.845019
A2	-29.014179	8	74.028357
A3	-30.262812	6	72.525625
fitted	-30.387704	5	70.775407
R	-112.660182	2	229.320365

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

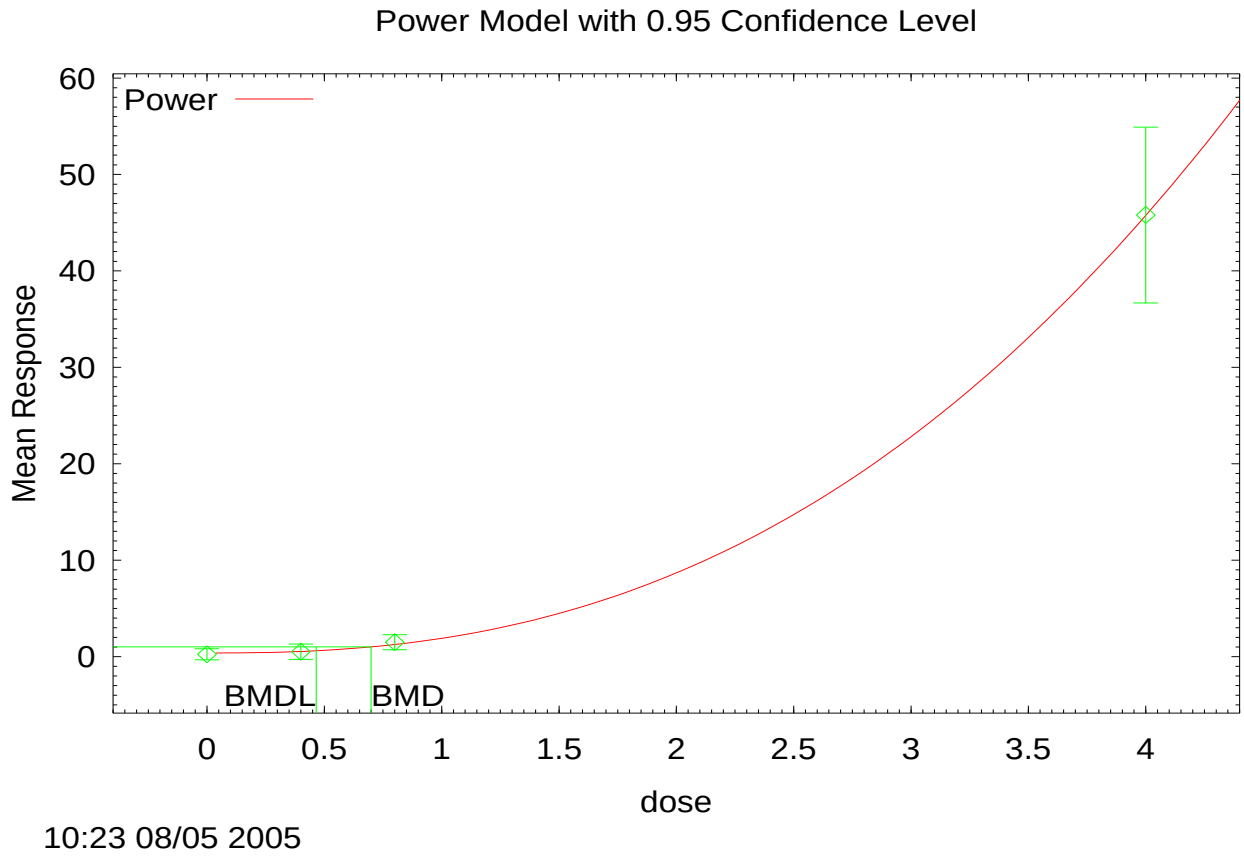
Tests of Interest

Test	-2*log(Likelihood Ratio)	d.f	p-value
Test 1	167.292	6	<.00001
Test 2	78.8167	3	<.00001
Test 3	2.49727	2	0.2869
Test 4	0.249782	1	0.6172

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

The p-value for Test 2 is less than .05. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .05. The modeled variance appears




```
 to be appropriate here
The p-value for Test 4 is greater than .05.  The model chosen seems
to adequately describe the data


 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =      0.699009

            BMDL =      0.465291
```

Figure B-4. Dose-response relationship based on rearing habituation in 2-month-old female mice.

Data source: Viberg et al. (2004a).

4. Rearing habituation in 8-month-old female mice

The BMD modeling results for rearing habituation in 8-month-old female mice are summarized in Table B-4. According to the results of the chi-square goodness-of-fit tests, the Hill and power models do not exhibit significant lack of fit. Between these two models that adequately fit the data, the power model yielded the lowest AIC, indicating that it is the "best-fit" model. Thus, for the endpoint rearing habituation in 8-month-old female mice, the $BMD_{1.0SD}$ estimate selected is 0.41 mg/kg, and its corresponding $BMDL_{1.0SD}$ is 0.29 mg/kg.

Table B-4. Rearing habituation in 8-month-old female mice

Model	No. of groups	*p* Value	AIC	$BMD_{1.0SD}$ (mg/kg)	$BMDL_{1.0SD}$ (mg/kg)
Hill	5	0.21	110	0.42	0.32
Linear	4	<0.01	100	1.9	0.56
Polynomial	4	<0.01	95	0.34	0.093
Power	4	0.16	63	**0.41**	**0.29**

Source: Viberg et al. (2004a).

Standard BMDS Output from Fitting the Power Model to Rearing Habituation in 8-Month-Old Female Mice (8 and 16 mg/kg-day Dose Groups Omitted)

Power Model
BMR = 1.0 SD

```
 ================================================================
        Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
        Input Data File: S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\VFRTRUNC.(d)
        Gnuplot Plotting File:  S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\VFRTRUNC.plt
                                        Fri Aug 05 10:29:26 2005
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = mg/kg
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = alpha*mean(i)^rho

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                        alpha =      14.0337
                          rho =            0
                      control =         0.22
                        slope =      1.46284
                        power =      2.44841


          Asymptotic Correlation Matrix of Parameter Estimates

                 alpha          rho      control        slope        power
     alpha           1        -0.51        -0.59        -0.13         0.13
       rho       -0.51            1         0.51        -0.13         0.11

   control       -0.59         0.51            1         -0.2         0.18

     slope       -0.13        -0.13         -0.2            1        -0.96

     power        0.13         0.11         0.18        -0.96            1


                        Parameter Estimates

       Variable          Estimate            Std. Err.
          alpha          0.931829            0.321871
            rho          0.985437            0.137401
        control          0.268288            0.124922
          slope           2.91602            0.525713
```

power 1.95332 0.131911

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	8	0.22	0.61	0.268	0.505	-0.0957
0.4	8	0.33	0.83	0.755	0.841	-0.506
0.8	8	2.83	0.56	2.15	1.41	0.48
4	8	43.8	7.4	44	6.23	-0.0323

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-56.126826	5	122.253652
A2	-19.791783	8	55.583565
A3	-25.553315	6	63.106629
fitted	-26.518839	5	63.037677
R	-110.453252	2	224.906503

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

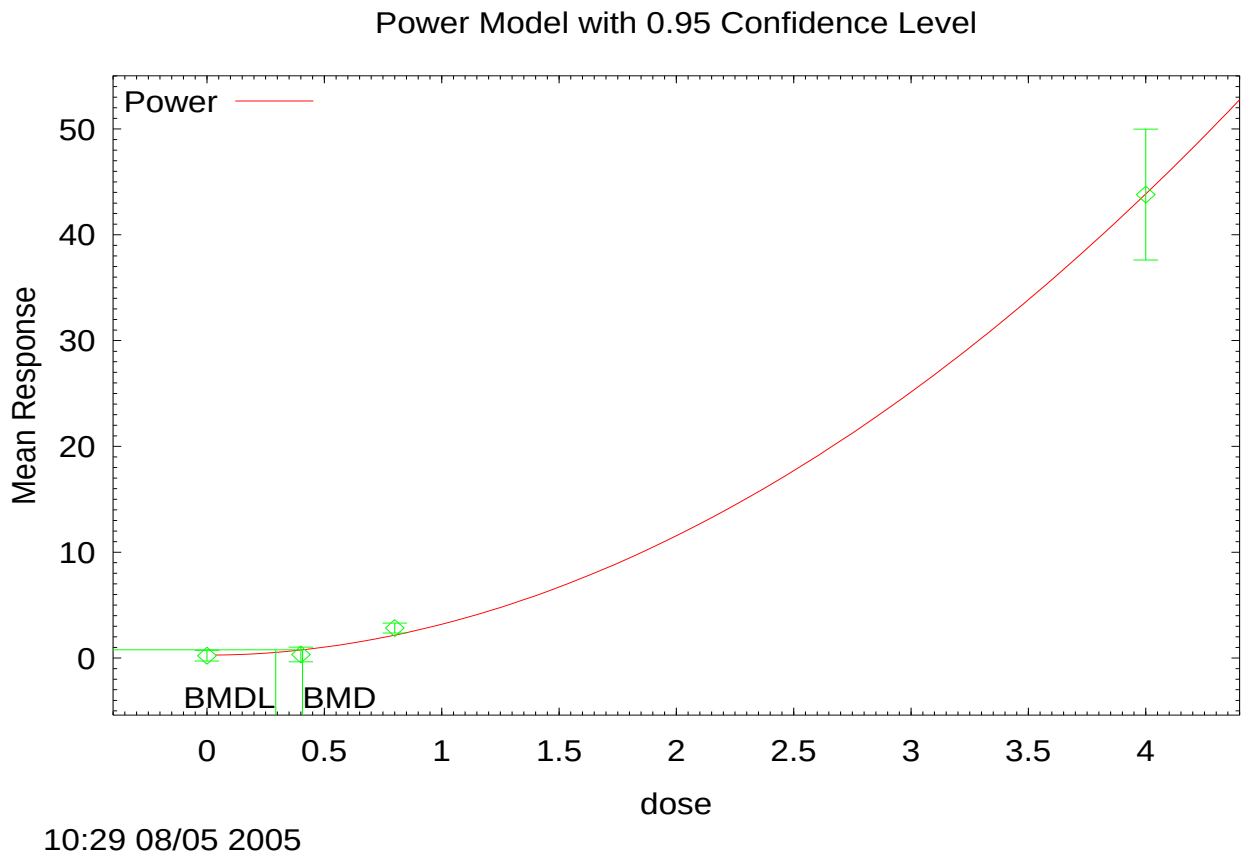
Tests of Interest

Test	-2*log(Likelihood Ratio)	d.f	p-value
Test 1	181.323	6	<.00001
Test 2	72.6701	3	<.00001
Test 3	11.5231	2	0.003146
Test 4	1.93105	1	0.1646

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

The p-value for Test 2 is less than .05. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .05. You may want to consider a




```
different variance model
The p-value for Test 4 is greater than .05.  The model chosen seems
to adequately describe the data


 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =      0.407446

            BMDL =      0.292715
```

Figure B-5. Dose-response relationship based on rearing habituation in 8-month-old female mice.

Data source: Viberg et al. (2004a).

5. Locomotion habituation in BDE-99-treated male and female mice

All of the continuous dose-response models available in BMDS (i.e., Hill, linear, polynomial, and power models) exhibited significant lack of fit ($p < 0.1$) when fit to the locomotion habituation data in either male or female mice from Viberg et al. (2004a), even when dose groups were dropped. Thus, BMD modeling results for this endpoint are not presented here.

6. Summary

The selected BMD and BMDL estimates based on each of the four endpoints under consideration are summarized in Table B-5. Based on these results, the lowest BMD_{1SD} and $BMDL_{1SD}$ estimates were derived from rearing habituation response in 8-month-old female mice, and the corresponding estimates were 0.41 and 0.29 mg/kg, respectively. As described above, the power model employing the rearing habituation data from four of the six dose groups in 8-month-old female mice from Viberg et al. (2004a) yielded the best fit. Figure B-5 displays the power model fit to the rearing habituation data in 8-month-old female mice. Following this graph, the standard BMDS output from the power model is presented.

Table B-5. Summary of BMD and BMDL results

Endpoint	Age	Species, sex	$BMD_{1.0SD}$ (mg/kg)	$BMDL_{1.0SD}$ (mg/kg)
Rearing habituation	2-month-old	Mouse, male	0.59	0.44
Rearing habituation	8-month-old	Mouse, male	–	–
Rearing habituation	2-month-old	Mouse, female	0.70	0.47
Rearing habituation	8-month-old	Mouse, female	**0.41**	**0.29**

Source: Viberg et al. (2004a).

B. Dose-Response Modeling Using the Data from Eriksson et al. (2001)

The locomotion, rearing, and total activity habituation data in male mice from Eriksson et al. (2001) were modeled in BMDS, using three of the four available continuous dose-response models (i.e., linear, polynomial, and power models). The Hill model was not used in modeling these data because this model requires at least five dose groups, and the Eriksson et al. (2001) study employed only three dose groups.

Similar to when the Viberg et al. (2004a) data were modeled, the test for equality of variances across dose groups failed for all data sets modeled, and so the variance was modeled as a power function of the mean. In some cases, this variance model failed to accurately

estimate the observed variances; however, no alternative variance model is currently available in BMDS. Therefore, in those cases where the modeled variances yielded poor results, the differences between the observed and estimated SDs were examined, especially in the region of the BMR. In all cases, these differences were determined to be minimal, and thus the variance was modeled as a power function of the mean in all modeling runs.

As was the case in modeling the Viberg et al. (2004a) data, restrictions were placed on model parameters to avoid biologically implausible dose-response relationships. More specifically, the parameter estimates for the polynomial model were restricted to be nonnegative, while the power parameter in the power model was restricted to be greater than or equal to 1. These restrictions were applied to the parameter estimates to prevent nonmonotonic dose-response for the polynomial model and supralinear curves for the power model. A supralinear dose-response curve was not considered plausible, given the responses observed in the Viberg et al. (2004a) study that employed tighter dose spacing (i.e., more data points in the low-dose region).

Modeling Results

All of the continuous models available in BMDS (i.e., linear, polynomial, power, and Hill models) exhibited significant lack of fit ($p < 0.1$) for all endpoints evaluated from the Eriksson et al. (2001) study. Although plots of the fits of the polynomial and power models to the 2-month rearing data appear good, with only three data points, these models possess too many parameters to evaluate goodness of fit. Also, Viberg et al. (2004a) employed an additional low dose at 0.4 mg/kg at which there was no response, while Eriksson et al. (2001) only tested two doses above controls (i.e., 0.8 and 12 mg/kg). Eriksson et al. (2001) observed responses at both of these doses. Therefore, Eriksson et al. (2001) likely missed the initial flat portion of the dose-response curve that was captured by Viberg et al. (2004a), leading to nearly linear fits through the data points using the polynomial and power models. Because no adequately fitting models were found among the available continuous models in BMDS, no BMD or BMDL estimates are reported from this study.

C. Dose-Response Modeling Using the Data from Kuriyama et al. (2005)

Kuriyama et al. (2005) exposed rat dams to a single PBDE dose on GD 6 (at dose levels of 0, 60, or 300 µg/kg) and observed the response in the male offspring of these exposed dams. Although this was a nested experimental design, the data were not presented in a form amenable to a nested analysis. Therefore, the data were simply modeled using the standard continuous and quantal models available in BMDS.

The effects modeled as continuous variables were locomotor activity on PNDs 36 and 71 (i.e., LBI counts per day, duration of activity [in hours] per day, LBI counts per active phase, and

duration of activity [in minutes] per active phase), spermatid count, daily sperm production, and sperm number on PND 140. Other endpoints assessed in the study were not evaluated using BMD modeling because inspection of the data revealed no clear dose-response relationship.

In fitting the continuous models, the test for equality of variances across dose groups failed for the locomotor activity data, and so the variance was modeled as a power function of the mean. In some cases, this variance model failed to accurately estimate the observed variances; however, no alternative variance model is currently available in BMDS. Therefore, in those cases where the modeled variances yielded poor results, the differences between the observed and estimated SDs were examined, especially in the region of the BMR. In all cases, these differences were determined to be minimal, and, thus, the variance was modeled as a power function of the mean in all modeling runs. For the spermatid data, however, variances were determined to be homogeneous across dose groups; therefore, a constant variance model was deemed appropriate for use in dose-response modeling.

As was the case in modeling the Viberg et al. (2004a) and the Eriksson et al. (2001) data, restrictions were placed on model parameters to avoid biologically implausible dose-response relationships. More specifically, the parameter estimates for the polynomial model were restricted to be nonnegative, while the power parameter in the power model was restricted to be greater than or equal to 1. These restrictions were applied to the parameter estimates to prevent nonmonotonic dose response for the polynomial model and supralinear curves for the power model.

The quantal data from this study were observations of the percent (or proportion) of adult animals with two or more ejaculations. In order to make these data amenable to modeling with quantal models, these data had to be converted so that adversity increased with increasing dose (i.e., the percent [or proportion] of adult animals with less than two ejaculations).

Modeling Results

1. Effects modeled as continuous variables

The effects modeled as continuous variables were locomotor activity on PNDs 36 and 71 (i.e., LBI counts per day, duration of activity [in hours] per day, LBI counts per active phase, and duration of activity [in minutes] per active phase [these data were presented in the published manuscript in graphical form, but the raw data were obtained from the authors]) as well as spermatid count, daily sperm production, and sperm number on PND 140. Among all of these continuous endpoints, only duration per day and LBI counts per phase on PND 36 were adequately fit by the linear and polynomial models. These modeling results are summarized in Table B-6.

For the endpoint duration per day and of the two models that showed adequate fit, the polynomial model provided the best fit based on its lower AIC value (Figure B-6); therefore, the

BMD and BMDL estimates of 0.28 and 0.22 mg/kg, respectively, were selected based on this endpoint. For the endpoint LBI counts per phase, only the linear model provided a satisfactory fit to the data. The BMD_{1SD} and $BMDL_{1SD}$ estimates based on this endpoint are 0.16 and 0.11 mg/kg, respectively.

Table B-6. BMD modeling results for the endpoints duration per day and LBI counts per phase on PND 36 for male rats exposed to BDE-99 in utero on GD 6

Endpoint	Model	No. groups	*p* Value	AIC	$BMD_{1.0SD}$	$BMDL_{1.0SD}$
Duration per day	Linear	3	0.15	196	0.25	0.17
Duration per day	Polynomial	3	0.67	194	**0.28**	**0.22**
LBI counts per phase	Linear	3	0.92	415	**0.16**	**0.11**

Source: Kuriyama et al. (2005).

Figure B-6 displays the polynomial model fit to the duration per day data in male rats exposed to BDE-99 in utero on GD 6 (Kuriyama et al., 2005). Following this graph, the standard BMDS output from the fitting of the polynomial model to these data is presented.

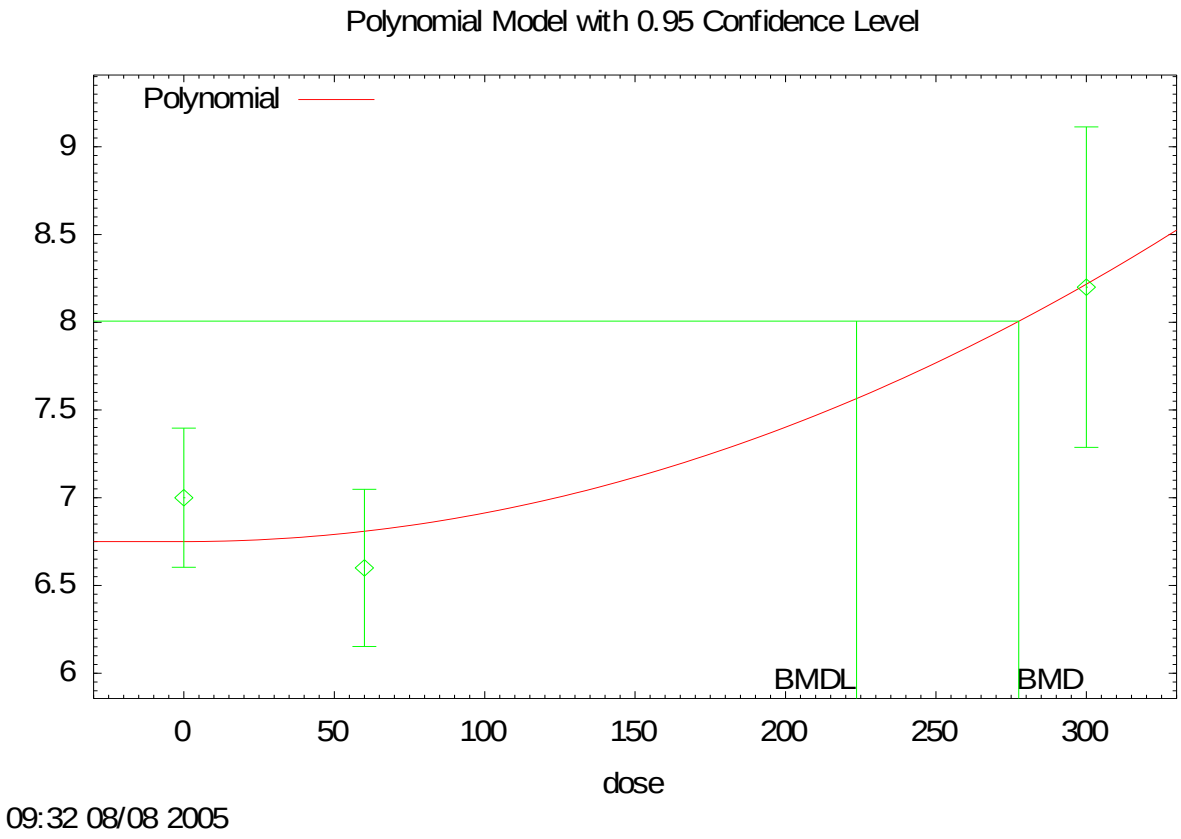


Figure B-6. Dose-response relationship based on activity duration per day on PND 36 in male rats exposed to BDE-99 in utero on GD 6.

Data source: Kuriyama et al. (2005).

```
      BMR = 1.0 SD
 ================================================================
        Polynomial Model.  (Version: 2.3;  Date: 6/21/2005)
        Input Data File: S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\KURIYAMAPND36.(d)
        Gnuplot Plotting File:  S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\KURIYAMAPND36.plt
                                          Mon Aug 08 09:32:55 2005
 ================================================================
 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = MEAN
Independent variable = dose
The polynomial coefficients are restricted to be positive
The variance is to be modeled as Var(i) = alpha*mean(i)^rho

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
           alpha =     2.80847
           rho   =     0
           beta_0      =     7
           beta_1      =     0
           beta_2      =     0

!!! Warning:  optimum may not have been found.  !!!
!!! Bad completion code in maximum likelihood optimization routine  !!!
!!! You may want to try choosing different initial values.  !!!

      Parameter Estimates
```

95.0% Wald Confidence Interval

Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
alpha	9.41543e-006	3.00252e-005	-4.94328e-005	6.82637e-005
rho	6.30301	1.61672	3.13429	9.47173
beta_0	6.74761	0.154227	6.44533	7.04989
beta_1	1.91982e-041	NA		
beta_2	1.63437e-005	5.22144e-006	6.10987e-006	2.65775e-005

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	beta_0	beta_2
alpha	1	-1	-0.016	0.005
rho	-1	1	0.016	-0.0041
beta_0	-0.016	0.016	1	-0.38
beta_2	0.005	-0.0041	-0.38	1

The following parameter(s) have been estimated at a boundary point or have been specified. Correlations are not computed:

beta_1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	32	7	1.1	6.75	1.26	1.13
60	40	6.6	1.4	6.81	1.29	-1.01
300	29	8.2	2.4	8.22	2.34	-0.0426

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	d.f.	AIC
A1	-101.125573	4	210.251146
A2	-90.874249	6	193.748498
A3	-92.783358	5	195.566717
fitted	-92.871731	4	193.743462
R	-108.721847	2	221.443695

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	35.6952	4	<.0001
Test 2	20.5026	2	<.0001
Test 3	3.81822	1	0.0507
Test 4	0.176745	1	0.6742

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

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

The p-value for Test 3 is less than .1. You may want to consider a different variance model

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

Benchmark Dose Computation

```
 Specified effect =     1
Risk Type         =     Estimated standard deviations from the control mean
Confidence level  =     0.95
BMD          =     277.537
BMDL         =     223.634
```

2. Effects modeled as quantal variables

The percent (or proportion) of adult animals with less than two ejaculations was modeled using the dichotomous models currently available in BMDS. A BMR of 10% extra risk was chosen, consistent with the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b). Prior to modeling, all doses were converted from μg/kg to mg/kg. Table B-7 summarizes the BMD modeling results based on this endpoint. None of the dichotomous models exhibited a significant lack of fit ($p > 0.1$). Of the models fit to the data, the best-fitting model is the log-logistic, as it has the lowest AIC, although the AICs for all of the models in Table B-7 are not significantly different from each other. The fit of the log-logistic model to the data is shown in Figure B-7, followed by the standard BMDS output generated by this model. Based on this model, the estimated BMD is 0.019 mg/kg and its corresponding BMDL is 0.0067 mg/kg.

Table B-7. Summary of the quantal dose-response modeling results based on the percent of animals with less than two ejaculations in male rats exposed to BDE-99 in utero on GD 6

Model	Chi^2 Res	*p* Value	AIC	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)
Gamma	0.28	0.72	78.6	0.031	0.016
Log-logistic	**0.041**	**0.96**	**78.5**	**0.019**	**0.0067**
Logistic	0.38	0.63	78.7	0.041	0.025
Log-probit	0.50	0.52	78.9	0.058	0.027
Probit	0.39	0.61	78.7	0.043	0.027
Multistage	0.13	0.72	78.6	0.031	0.016
Quantal linear	0.28	0.72	78.6	0.031	0.016
Quantal quadratic	0.62	0.40	79.2	0.10	0.071
Weibull	0.28	0.72	78.6	0.031	0.016

Data source: Kuriyama et al. (2005).

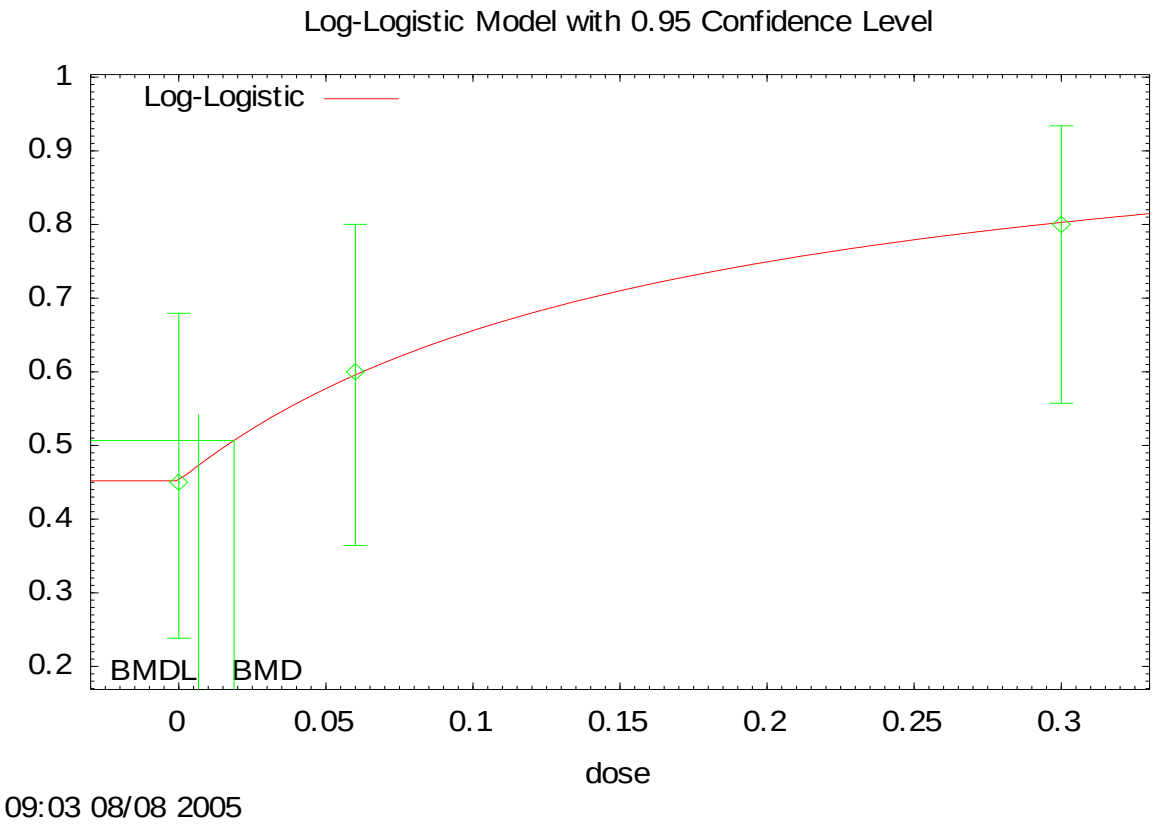


Figure B-7. Log-logistic model fit based on percent of animals with less than two ejaculations in male rats exposed to BDE-99 in utero on GD 6.

Data source: Kuriyama et al. (2005).

```
 ================================================================
        Logistic Model $Revision: 2.3 $ $Date: 2003/09/03 11:53:20 $
        Input Data File: C:\MY DOCUMENTS\BMD\LL1.4.(d)
        Gnuplot Plotting File:  C:\MY DOCUMENTS\BMD\LL1.4.plt
                                          Sun Aug 07 21:25:09 2005
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Column2
   Independent variable = COLUMN1
   Slope parameter is restricted as slope >= 1

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =         0.45
                     intercept =       1.7933
                         slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been
specified by the user,
                 and do not appear in the correlation matrix )

             background    intercept
background            1         -0.6

 intercept         -0.6            1
                              Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     background         0.452005         0.104855            0.246492
0.657517
      intercept          1.77733         0.730579            0.345417
3.244``````````````````````````````````````````````````14556+`+0924
          slope                1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.
```

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test d.f.	P-value
Full model	-37.2311			
Fitted model	-37.2324	0.002753	1	0.9582
Reduced model	-39.9403	5.41857	2	0.06658

AIC: 78.4649

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.4520	9.040	9	20	-0.01801
0.0600	0.5955	11.911	12	20	0.04075
0.3000	0.8025	16.049	16	20	-0.02772

Chi^2 = 0.002753 d.f. = 1 P-value = 0.9582

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0187877

BMDL = 0.00670333