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TOXICOLOGICAL REVIEW

OF

PENTABROMODIPHENYL ETHER
(BDE-99)

(CAS No. 60348-60-9)

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

December 2006

NOTICE

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U.S. Environmental Protection Agency
Washington, DC

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2,2',4,4',5-PENTABROMODIPHENYL ETHER (CAS No. 60348-60-9)**

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LIST OF ACRONYMS AND ABBREVIATIONS

Ah	aryl hydrocarbon
AIC	Akaike Information Criterion
BDE-99	2,2',4,4',5-pentabromodiphenyl ether
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
CB3	Coxsackie virus B3
cDNA	complementary DNA
CYP-450	cytochrome P-450
DHT	dihydrotestosterone
EC ₅₀	median effective concentration
ER	estrogen receptor
EROD	ethoxyresorufin O-dealkylase
FOB	functional observational battery
GD	gestational day
i.v.	intravenous
IC ₅₀	median inhibitory concentration
IGF	insulin-like growth factor
Ig	immunoglobulin
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
PAPS	3'-phosphoadenosine-5'-phosphosulfate
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofuran
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
s.c.	subcutaneous
T3	triiodothyronine
T4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TT4	total T4
TTR	transthyretin
UDPGA	uridine diphosphate glucuronic acid.
UDPGT	uridine diphosphoglucuronosyl transferase
UF	uncertainty factor

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to exposure to 2,2',4,4',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 relates to the pentabromodiphenyl congener 2,2',4,4',5-pentabromodiphenyl ether (CASRN 60348-60-9). Toxicological information related to other congeners in the pentabromodiphenyl ether homolog group (CASRN 32534-81-9) 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 Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

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

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

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of 2,2',4,4',5-pentabromodiphenyl ether (BDE-99). IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and less-than-lifetime exposure durations, and a carcinogenicity assessment.

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

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

Development of these hazard identification and dose-response assessments for 2,2',4,4',5-pentabromodiphenyl ether has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and technical panel reports that were

used in the development of this assessment include the following: *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2000a, 2005c), *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

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

2. CHEMICAL AND PHYSICAL INFORMATION

Pentabromodiphenyl ether (CASRN 32534-81-9) is one of the possible 10 homologs of polybrominated diphenyl ethers (PBDEs). Figure 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 1.

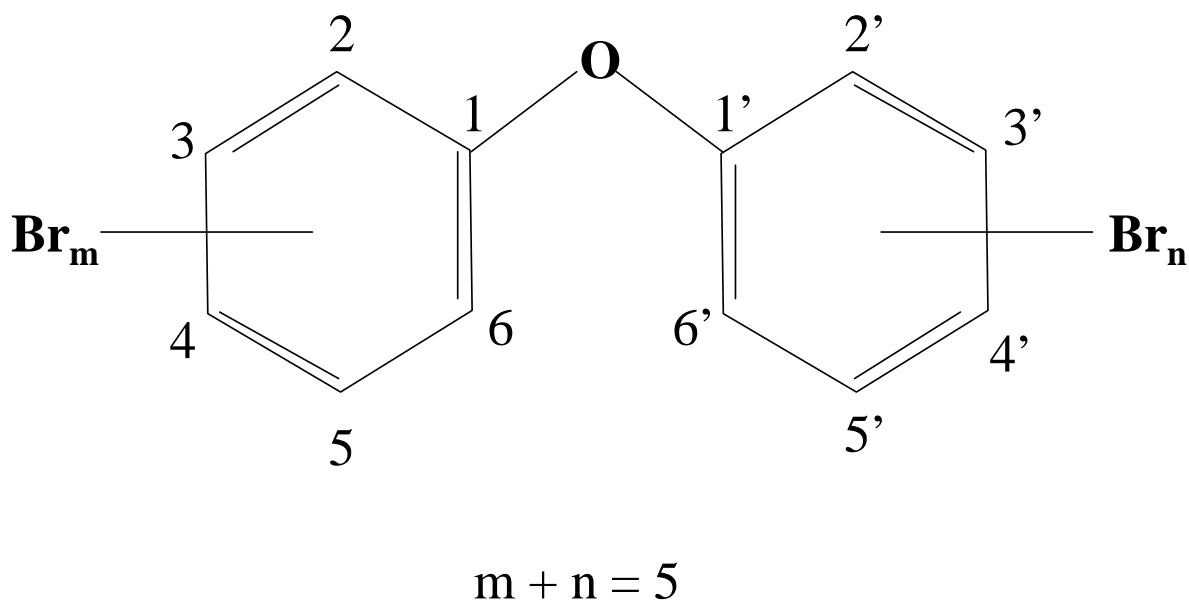


Figure 1. Chemical structure of pentabromodiphenyl ether.

Table 1. IUPAC number and bromine substitution pattern of some pentabromodiphenyl ether 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 pentabromodiphenyl ether, which is usually composed of a mixture of pentaBDE (50–62%), tetraBDE (24–38%) and hexaBDE (4–12%) (U.S. EPA, 2005d). The relative proportions by weight of various PBDE congeners in the commercial pentaBDE DE-71TM are approximately 43% (pentaBDE-99), 28% (tetraBDE-47), 8% (pentaBDE-100), 6% (hexaBDE-153), and 4% (hexaBDE-154). TriBDE-28 and -33 and tetraBDE-49 and -66 are present at about 1% or less in the formulation (Great Lakes Chemical Corporation, 2003).

The predominant 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.

Table 2. Physical and chemical properties of 2,2',4,4',5-pentabromodiphenyl ether

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 ₅ Br ₅ O	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 m ³ 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	Braekvelt et al. (2003); ATSDR (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)

3. TOXICOKINETICS

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), ¹⁴C-BDE-99 (>98% purity) in corn oil was given as a single oral dose of 8 mg/kg 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 between the rats, and estimates of absorption range from about 60% to 90%. The fecal excretion on day 1 was much higher in the bile cannulated rats (53% ± 27%) indicating that gastrointestinal emulsion by bile was a major factor governing absorption. The estimated amounts absorbed by the bile cannulated rats were also quite variable and ranged from 21% to 74%. There was excretion of BDE-99 in bile on day 1 after exposure (0.7 ± 0.8% of the dose). However, since it is low, it has little effect on the estimates of absorption.

Chen et al. (2006) estimated the absorption of BDE-99 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 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-pentabromodiphenyl ether) were studied in male Sprague-Dawley rats (Hakk et al., 2006). Doses of 4.1 mg/rat of C¹⁴-BDE-100 (purity >95%) in peanut oil, equivalent to about 15 mg/kg, were administered to groups of nine conventional or bile 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 cannulated rats was higher than in the

conventional rats ($16.8 \pm 25.7\%$).

Chen et al. (2006) found that the absorption of BDE-99 in male B6C3F1 mice administered 0.6 mg/kg (1 μ mol/kg) was comparable to that in F344 rats (85%) based on the comparison of fecal loss after oral ($27.1 \pm 5.3\%$) versus intravenous ($12.9 \pm 2.2\%$) exposures.

Darnerud and Risberg (2006) examined the tissue distribution of radiolabeled BDE-85 or BDE-99 in adult female C57BL mice after intravenous or gavage exposure to 20 μ mol/kg of the congener (about 10 mg/kg) in DMSO, using the whole-body autoradiography technique that gives qualitative results only. 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 intravenous 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%) in a fat emulsion on PND 3, 10, or 19 and the animals 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 in mice. In that same study, spontaneous motor behavior was affected to the greatest extent in mice exposed to BDE-99 on PND 10 and to a lesser extent in mice exposed on PND 3. Mice exposed on PND 19 were not affected. Differences in the amount of BDE-99 present in the neonatal brain at the different neonatal ages do not appear to explain the different behavioral effects seen in adult mice exposed on PND 3, 10, or 19 (see Section 4.3.1.5).

3.2. DISTRIBUTION

The high K_{ow} of BDE-99 suggests a strong potential for bioaccumulation 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 occurs. The data do not provide

information on the quantitative aspects of absorption or the kinetics of tissue distribution and retention. Monitoring data, described below, are available for human adipose tissue, liver, milk and blood samples and indicate a tendency for PBDEs to distribute to these tissues. However, distribution studies have not been conducted in humans, and therefore it is not known whether 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.

Adipose Tissue

Breast adipose samples were collected between 1996 and 1998 from 23 San Francisco Bay area women as part of a case-control study on organochlorine compounds and breast cancer (She et al., 2002). Women ranged from 28 to 62 years of age and were predominantly Caucasian and born in the United States. Pathology reports indicated 12 women had malignancies, 8 had benign tumors, and 3 had ductal carcinomas in situ, a condition considered by some as transitional to malignancy. Breast adipose samples were collected during biopsy or breast surgery and were analyzed for tetraBDE (BDE-47), pentaBDEs (BDE-99 and BDE-100), and hexaBDEs (BDE-153 and BDE-154). Mean and median concentrations of the sum of these PBDEs were 86 and 41 ng/g lipid weight (lw), respectively. Median concentrations of individual PBDE congeners are given in Table 3. 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 United States. There was an inverse relationship between the sum of the concentrations of these PBDEs in breast adipose tissue and age, with women younger than the median age of 48 years having significantly higher concentrations of PBDEs in adipose tissue than women older than 48. This may imply that different activities may expose different age groups more than others or that some PBDE congeners may accumulate differently with age. 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.

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 BDE-99. Median concentrations of individual PBDE congeners and the sum of these PBDEs are given in Table 3 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, ten 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 years 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).

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 years) were analyzed for nine tri- to hexaBDE congeners. PBDEs were found in all samples. BDE-47, BDE-99, and BDE-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 BDE-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 (Gruvenius et al., 2001).

Human Milk

In a study conducted in 2002 of levels of PBDEs in human milk in the United States, 47 samples from Caucasian, African-American, and Hispanic nursing mothers 20–41 years of age and living in Texas were analyzed for 13 PBDE congeners (Schechter et al., 2003). Mean and median total concentrations of tri- through decaBDEs were 74 and 34 ng/g lw, respectively. The maximum and mean concentrations of BDE-99 were 111 and 14 ng/g lw, respectively. Median

concentrations of individual PBDE congeners are given in Table 3. There was no correlation between age and level of PBDEs in human milk. Concentrations of PBDEs found in this study were substantially higher than those measured in human milk in Europe or Japan.

Breast milk was collected from 12 primiparous 24–33-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 BDE-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 of 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 BDE-100), and two hexaBDEs (BDE-153 and BDE-154) were determined in samples from 93 primiparous women, collected from 1996 to 1999 in Uppsala County, Sweden. The women ranged in age from 20 to 35 years. BDE-47 was the major congener (mean value 2.4 ng/g lw) and constituted 60% of the mean concentration of PBDEs of 4.0 ng/g lw, followed by BDE-99 and BDE-153 (0.6 ng/g lw each), BDE-100 (0.4 ng/g lw), and BDE-154 (0.07 ng/g lw). No significant relationship was found 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 BDE-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).

Blood

Levels of PBDEs in the blood are representative of either recent exposures or the slow release of PBDEs from tissue stores. Seven tetra- to decaBDEs were analyzed in serum samples collected in the United States in 1988 from male blood donors. The median serum concentration of the sum of tetra- to decaBDEs was 1.6 ng/g lw (Sjodin et al., 2001). In 2000–2002, the sum of the median concentrations of six tetra- to hexaBDEs in serum pools collected in the United States 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 (Sjodin et al., 2004).

In Norway, pooled serum samples collected in 1998 from eight population groups of different age (0 to >60 years) and gender 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 0–4-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 0–4-year-olds who seem to experience elevated exposure, there was a lack of an age-related trend of PBDEs body burden. This may be explained by the fact that PBDEs are relatively new contaminants in the environment; the time period for human exposure is therefore relatively short, and different age groups (except the 0–4 years group) may thus have experienced a similar exposure period (Thomsen et al., 2002). The high level of PBDEs in the serum of the 0–4-year-olds could be due to higher exposure from human milk and/or certain behavioral activity, such as crawling or sucking on flame-retarded materials.

Concentrations of BDE-47, hexaBDEs (BDE-153 and BDE-154), heptaBDE (BDE-183), and decaBDE (BDE-209) were determined in blood serum from groups of 19–20 Swedish male and female subjects in the following occupational groups: hospital workers (control), clerks working full-time at computer screens and personnel at an electronic-dismantling plant (Sjodin et al., 1999). Commercial PBDEs used as flame retardants in the electronic industry are usually decaBDE and to a lesser extent 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.

Table 3. Median PBDE congener concentrations in human biological media in the United States, ng/g lipid weight

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

^aYear = Year of Sampling;

^bN = Number of donors

^c 7 serum pools with number of donors in each serum pool ranging from 40 to 200.

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 Table 3, and in Table 4 for comparison with a Swedish study (Gruenewald et al., 2003) described below. TetraBDE-47 was the most abundant congener followed by BDE-99 and BDE-100. PBDEs

concentrations were highly correlated between mother and fetal sera, indicating that PBDEs cross the placenta into the fetal circulation. In addition, the results indicate that all tetra- through hepta-substituted congeners have approximately the same potential to cross the placenta. There was a decreasing trend in concentration of PBDE congeners in maternal and fetal sera with increasing degree of bromination.

Samples of maternal and cord blood plasma were collected during 2000–2001 from 15 Swedish mothers (Guvenius et al., 2003). BDE-47 was the most abundant of all congeners and comparable median concentrations were found in maternal and cord blood plasma (Table 4). The levels of the higher brominated congeners, BDE-99 to BDE-183, were higher in maternal blood than in cord blood, indicating that the higher brominated PBDEs do not pass through the placenta to the same extent as the lower brominated congener BDE-47. This trend was not apparent in the Mazdai et al. (2003) study where comparable levels were found in maternal and fetal sera for all PBDE congeners studied.

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

Table 4. Median PBDE congener concentrations (ng/g lipid weight) in maternal and fetal sera in the United States and Sweden

PBDE congener	Maternal serum		Fetal serum	
	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

^aUnited States: year of sampling 2001; number of donors 12.

^bSweden: year of sampling 2000–2001; number of donors 15.

In summary, median concentrations of PBDE congeners in the United States are available for human adipose tissue (Johnson-Restrepo et al., 2005; She et al., 2002), human milk (Schechter

et al., 2003), and serum (Sjodin et al., 2004; Mazdai et al., 2003). The concentration profiles in the United States of PBDEs in adipose tissue, serum, and human milk are similar, although these studies were conducted in different regions of the United States (Table 3). The predominant congener found in adipose tissue, human milk, and blood samples in the United States is tetraBDE-47, followed by pentaBDE-99 and pentaBDE-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 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 the conventional rats (2% in the carcass, 1.5% in the gastrointestinal tract, and 0.8% in adipose tissue).

The remaining carcass from conventional rats was 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 a 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 for 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 intravenous injection of 1 mg/kg BDE-99 to C57BL/6 mice, Staskal et al. (2006) found that the percentage in muscle was greater than that in skin five 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 that 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 BDE-99 in male F344 rats 24 hours after a single exposure to 0.6 mg/kg or 6 mg/kg to the tissue levels 24 hours after 10 days of exposure to 0.6 mg/kg-day (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 ten single 0.6 mg/kg-day doses. In adipose tissues, the level of label 24 hours after the last of the ten 0.6 mg/kg-day single doses was greater than that after the single 6 mg/kg dose illustrating the potential for bioaccumulation 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 remained in the body of conventional rats after 72 hours and was found in the adipose tissue, GI 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. NMRI male mice (five/group) were administered 8 mg/kg of ¹⁴C-BDE-99 (purity >98%) in a fat emulsion on postnatal day (PND) 3, 10, or 19 and the animals 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, ¹⁴C-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 to 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 ¹⁴C-labeled pentaBDE-85 and -99 was studied in C57BL mice using whole-body autoradiography (Darnerud and Risberg, 2006). ¹⁴C-BDE-85 or -99 (>95% purity) was administered to male and female C57BL mice by intravenous (i.v.) injection or by gavage at 20 μmol/kg of 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 ¹⁴C 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 from that in 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 shows that the gastrointestinal uptake is effective. 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.

The qualitative distribution of ¹⁴C-BDE-85 and -99 was also studied in pregnant mice sacrificed 1 day after i.v. administration of 11 mg/kg of ¹⁴C-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 the 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 that for surrounding tissues. Faint radiolabeling was observed in the fetal brain.

Darnerud and Risberg (2006) also studied the partition of ¹⁴C-BDE-85 and ¹⁴C-BDE-99 to maternal milk in lactating C57BL mice. The two pentaBDE congeners were injected

intravenously at 2.0 $\mu\text{mol/kg}$ (1 mg/kg) to lactating dams at 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 dam, 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 two to four times higher plasma concentration was found in offsprings.

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 at day 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 four days after maternal administration of a single dose of radioactive pentaBDEs. 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 subcutaneous (s.c.) injection (1 or 10 mg/kg-day) for 6 consecutive days 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 1500 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 at 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 activity of P450-mediated ethoxyresorufin *O*-dealkylase (EROD) and pentoxyresorufin *O*-dealkylase

(PROD) enzyme activities 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.

3.3. METABOLISM

BDE-99 and BDE-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 urinary excretions in mice and 80% of the fecal matter were present as metabolites following intravenous 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 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 BDE-100. This can be followed by debromination in some cases and possibly by conjugation of the hydroxylated derivatives with glutathione, 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 glutathione, glucuronate, and/or sulfate are more variable.

Chen et al. (2006) have proposed a metabolic pathway for BDE-99 (Figure 2) which 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 BDE-100) and in different species.

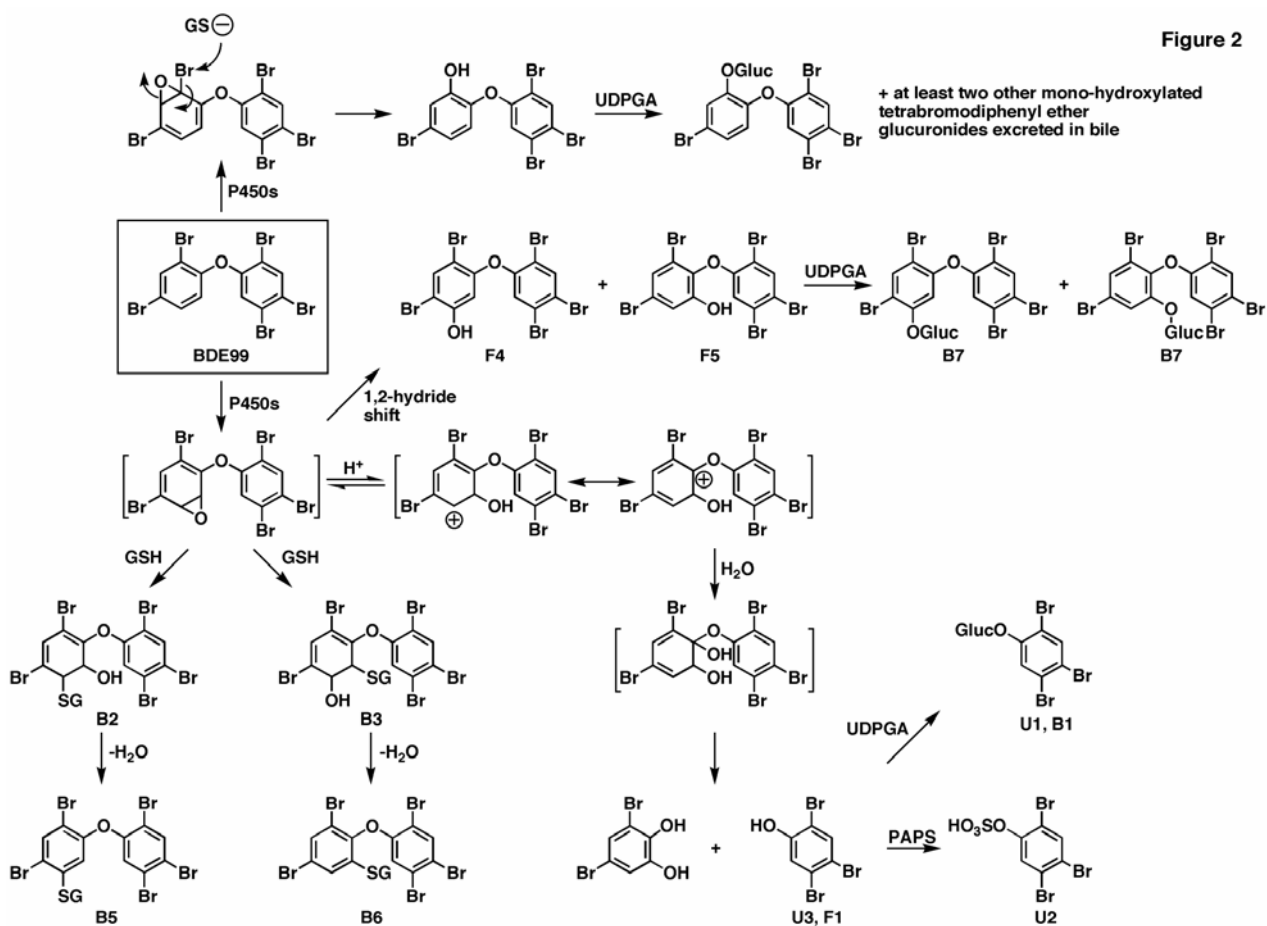


Figure 3-1. Proposed metabolic pathway for BDE-99 in male rats.

PAPS = 3'-phosphoadenosine-5'-phosphosulfate (PAPS).

UDPGA = uridine diphosphate glucuronic acid.

Source: Chen et al. (2006).

According to the proposed metabolic pathway, BDE-99 can undergo two primary reactions (Figure 2). Both involve cytochrome P-450 (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 2): (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 glutathione.

The 1,2 hydride shift generates monohydroxylated pentabromodiphenyl ethers 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 2). Chen et al. (2006) tentatively identified 2,4,5-tribromophenol in rat feces and its sulfate and glucuronate conjugates in urine.

Conjugates of metabolites with glutathione 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 2). Chen et al. (2006) proposed that this would lead to formation of tetrabromohydroxylated metabolites. Any brominated carbon with a neighboring nonbrominated carbon is liable to this reaction, permitting generation of several tetrabromohydroxylated derivatives. Tetrabromohydroxylated metabolites have been identified in rats for both BDE-99 and BDE-100 by Hakk et al. (2002a, 2006) 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 the 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 P-450 isozymes that participate in the epoxidation of the BDE-99 or -100, but involvement of the 1A1/2 and 2B isozymes has been investigated in vitro with BDE-99 because of their link to the activation of the aryl hydrocarbon receptor (Sanders et al., 2005; Chen and Bunce, 2003; Chen et al., 2001). The activity of P450 1A1/2 and 2B is generally evaluated through analysis of the Phase I enzymes EROD for 1A1/2

activity and PROD for 2B activity. In the study by Darnerud et al. (2005) of the effects of a viral infection on the distribution of ¹⁴C-BDE-99 (see Section 3.2.2), both EROD and PROD were active in the noninfected control adult female Balb/c mice treated with ¹⁴C-BDE-99, EROD to a greater extent than PROD. On day 3, in the infected mice treated with ¹⁴C-BDE-99, the enzyme activities of EROD and PROD were about 17% and 31% of those in the noninfected ¹⁴C-BDE-99 treated mice, respectively.

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 RNA (mRNA) was isolated from a portion of the right medial lobe of the liver and converted to its complementary DNA (cDNA) by using real-time polymerase chain reaction (PCR). Target gene amplification was evaluated using specific probes for CYP-1A1, CYP-2B, and CYP-3A. These analyses indicated that CYP-1A1 expression was significantly up-regulated (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 for the 5.7 and 57 mg/kg doses in one of two assays and at the highest dose for the other assay. At the highest dose, the 2B mRNA levels were 14 to 25 times those for the corn oil controls. These data conflict with the data from the control mice from Darnerud et al. (2005). The expression of CYP-3A was up-regulated (four- to fivefold) with the highest dose.

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 for both enzymes; the values for the pentaBDE hydroxy congener fell between the values for tested tri- and tetraBDE hydroxy congeners. In the case of the estrogen sulfotransferase, sulfate conjugation of 13% of 4-OH-3,5,2',4',6'-pentaBDE was observed, while the phenol sulfotransferase was able to conjugate sulfate with 0.7%. Studies of excreted pentaBDE metabolites do not indicate that sulfate conjugation is a major metabolic process.

3.4. ELIMINATION

Elimination of BDE-99 and BDE-100 occurs by way of the fecal matter and urine. Urinary excretion appears to be more substantial in mice than rats. Both parent and metabolites are identified in the excreta, with the amounts of each varying among studies. Radiolabel in fecal matter represent 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 ¹⁴C-BDE-99 was given to conventional and bile-duct-cannulated male Sprague-Dawley rats. Parent BDE-99 compounds 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 days 1–2 from 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 was found in the feces within 3 days. In both conventional and bile-duct-cannulated rats, the fecal radioactivity was highest on the first day (22% and 53%, respectively) and then declined steadily thereafter, suggesting that enterohepatic circulation of BDE-99, if it occurs, plays a minor role in the male rat.

The Hakk et al. (2006) study of 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% to 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 amount in feces increased to 56% on 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 also appears 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 intravenous administration of 10 mg/kg BDE-99 or BDE-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 ¹⁴C 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. 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 at day 1 from exposure indicated that 61% of the biliary ^{14}C was unbound, decreasing steadily to 43% by day 3 from exposure (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% to 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. Metabolites 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 is 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 major urinary protein (MUP) (Staskal et al., 2006c). These proteins are synthesized in the liver, secreted into serum, and eliminated in urine. Male mice secrete more protein than females. Analysis of pooled urine samples from BDE-99 and BDE-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.

Half-life Determinations

In the study by Hakk et al. (2002a), BDE-99 preferentially deposited in the most lipophilic tissues. BDE-99 was only slowly mobilized from skin and fat deposits. In the conventional rat, an estimated 21% of the dose deposited in the skin at 3 days from 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, 14% and 10% for the 6- and 12-day conventional rat, respectively, remained in adipose tissue. 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.

Elimination half-life of individual tetra, penta, and hexa components of commercial pentaBDE (Bromkal 70) was investigated in groups of male and female Wistar rats given a single oral dose of 300 mg/kg of Bromkal 70 dissolved in peanut oil (von Meyerinck et al., 1990). Groups of three animals of either sex were sacrificed on days 1, 2, 3, 4, or 7 and then once a week for 10 weeks. Perirenal fat was collected and analyzed. The half-lives of two unspecified pentaBDE congeners were 25 and 47 days for female rats and 25 and 37 days for male rats. The difference in half-lives between sexes was not significant. Half-lives of pentaBDEs in this study were substantially higher than the half-life of 6 days for BDE-99 in the Hakk et al. (2002a) study. However, as von Meyerinck et al. (1990) indicated, the metabolic rate and therefore the elimination of PBDEs may be affected by the high dose given to the animals, and half-lives of PBDEs as determined in this study may not be representative of cases where the exposure doses are lower.

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 using physiologically based pharmacokinetic models is not possible at this time.

4. HAZARD IDENTIFICATION

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

To assess whether PBDEs may be detrimental to neurodevelopment, Mazdai et al. (2003) determined concentrations of PBDEs and total and free serum thyroxine (T_4) and triiodothyronine (T_3) in human fetal and maternal sera (see also Section 3.2.1). Twelve paired maternal and cord blood samples were obtained from women 18 to 37 years old, presenting in labor at an Indiana hospital. The PBDE congeners and their concentrations measured in fetal and maternal serum samples are given in Table 4. There was no relationship between infant birth weight and PBDE concentrations. No birth defects were documented. Thyroid hormones were assayed in 9 of the 12 sample pairs. There was no correlation between total PBDEs and T_3 or T_4 concentrations (total or free). The authors cautioned that the sample size may have been too small to detect an association between serum concentrations of PBDEs and thyroid hormone levels.

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

In summary, 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. LESS-THAN-LIFETIME AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

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

4.2.1. Acute, 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: 37% BDE-99 and 35% tetraBDE-47) or with a total dose of 0.8 mmol/kg Aroclor 1254 (260 mg/kg). 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.

Dams 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 T₄ 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 T₄ 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 CYP1A1 activity. Hepatic EROD activity in pregnant dams sampled on GD 17 and in postweaning dams sampled on PND 20 were 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.

The hepatic enzyme uridine diphosphoglucuronosyl transferase (UDPGT) activity was studied in offspring on PND 11 and PND 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 T₄ 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 T₄ levels in offspring on PND 11. One of the components of Bromkal is tetraBDE-47 which has been shown

to cause a decrease in T₄ 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 T₄ (TT₄) plasma levels in young adult male Sprague-Dawley rats. A single oral dose of 8 mg/kg of ¹⁴C-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 T₄ concentration (bound and free) was 1.7 µg/dL in the control rats. In the treated conventional rats, the average TT₄ levels increased approximately twofold to 3.2 µg/dL at 3 days from exposure, remained elevated at 3.0 µg/dL 6 days from exposure, but by day 12 the levels returned to control levels at 1.9 µg/dL. TT₄ levels in plasma of the bile-duct-cannulated rats were not significantly different from control levels. TetraBDE-47, which has been shown to cause a decrease in T₄ levels in mice and rats (Hallgren and Darnerud, 2002; Hallgren et al., 2001), may be the component responsible for the reduction of T₄ levels seen with commercial pentaBDE mixtures (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. 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. Male NMRI mice received by gavage on PND 10 a single dose of BDE-99 (>98% purity) 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 3 to 4 different litters, were subjected to spontaneous behavior testing (locomotion, rearing, and total activity) for a 60-minute period, 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 nicotine base/kg and immediately tested again for spontaneous motor behavior during another 60-minute period. 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 PND10 can affect the cholinergic system, seen as changes in the adult mice response to the cholinergic agent nicotine.

4.3.1.2. *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. Spontaneous motor behavior was tested at ages 2, 5, and 8 months in eight 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 vs. 8-month-old mice), data from the spontaneous motor behavior tests were used. The habituation ratio was calculated between the performance period 40–60 minutes and 0–20 minutes for two of the spontaneous motor behavior variables, locomotion and rearing. 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.

There were no clinical signs of toxicity, effects on body-weight gain or body weight at any of the dose groups. Control mice showed habituation (i.e., a decrease in locomotion, rearing, and total activity in response to the diminishing novelty of the test chamber) over the three 20-minute test periods. 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. The habituation capability for the locomotion and rearing variables were significantly decreased in the 2- and 8-month-old male and female mice at 0.8 mg/kg and above. Reduced habituation capability was more pronounced in the 8-month-old compared with the 2-month-old mice. The habituation ratio for rearing (ratio between the performance period 40–60 minutes and 0–20 minutes for rearing) which provided a good fit in the BMD modeling (see Section 5.1.2), was 0.39, 0.49, 8.11, 70.8, 104, and 263 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 0.36, 0.30, 11.9, 90.4, 95.9, and 511, 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 lowest-observed-adverse-effect level (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.

4.3.1.3. *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 4.4.1.3 for the discussion of the results of the receptor binding.) 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 behavior tests used measured locomotion, rearing, and total activity. There were no clinical signs of toxicity nor significant difference 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 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 BDE-99 at 0.2 or 0.4 mg/kg 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.

4.3.1.4. *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 of BDE-99 (>98% purity) in a 20% fat emulsion (1:10 egg lecithin to peanut oil) 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, the ratio between performance in spontaneous motor behavior period 40–60 minutes and period 0–20 minutes for the three different variables, was used to analyze alteration in habituation to a novel environment in 2-month-old and 4-month-old mice. 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-response-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 LOAEL in this study was 0.8 mg/kg for effects on spontaneous motor behavior and decreased habituation capability.

4.3.1.5. 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. Male and female NMRI mice were given by gavage on PND 3, 10, or 19 a single oral dose of 0 or 8 mg BDE-99/kg in a 20% fat emulsion (1:10 egg lecithin to peanut oil). There were no effects on body weights or body-weight gains 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 at age 3, 10, or 19 days 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 ¹⁴C-BDE-99 on PND 3, 10, or 19 and the animals 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, ¹⁴C-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 different behavioral effects seen in adult mice exposed to BDE-99 on PND 3, 10, or 19.

4.3.1.6. *Branchi et al. (2002)*

The neurobehavioral effect of perinatal exposure to BDE-99 was investigated in mice. BDE-99 dissolved in corn oil was administered by gavage at 0, 0.6, 6, or 30 mg/kg-day to groups of CD-1 Swiss female mice (four/group) from GD 6 through PND 21, at which time the pups were weaned. 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 gains of pups from birth to weaning were not affected by treatment with BDE-99.

Male and female pups (n = 6 to 8) from each litter of each treatment group were used in a series of tests to assess somatic and neurobehavioral development from PND 2 to 20. These tests were carried out every two 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 immediately 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 to 20, BDE-99 treatment did not affect somatic development (hair growth and day of eyelid and ear opening and of incisor eruption). There was a statistically significant two-day delayed appearance of screen climbing response in

the high-dose group (30 mg/kg-day); all other responses based on neuromotor coordination from PND 2 to 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 one 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, exhibiting statistically significant increases in distance traveled and 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. Thigmotactic response, considered an index of emotionality, was not affected at any dose. On PND 60, mice in the 0.6 and the 6 mg/kg-day groups, but not at 30 mg/kg-day, 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), only the 0.6 mg/kg-day group displayed a significantly lower level of locomotion 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 these results show that prenatal and postnatal exposure of mice to BDE-99 produces a transient hyperactivity, characterized by an inverted dose-response relationship, that ends around 4 months of age.

The inverted dose-response curve in this study does not permit clear identification of the NOAEL/LOAEL for alteration in behavioral or activity parameters. 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.

4.3.1.7. *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 drink spontaneously 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 nor 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, $n = 7$ to 9 mice/group) were tested in an open-field apparatus that measured locomotion (horizontal movement), rearing (vertical movement) and thigmotaxis (time and distance traveled immediately 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 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 controls and BDE-99 gavage and self-administered groups were sacrificed and BDE-99 levels determined in the brain. No effect of administration route was found on level of BDE-99 in the brain. Mean BDE-99 levels in treated animals were 640 $\mu\text{g}/\text{kg}$ compared to 5 $\mu\text{g}/\text{kg}$ for the controls.

Serum total and free T_4 levels were also measured in BDE-99 treated male mice ($n = 8$ to 9 per 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.

4.3.1.8. 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 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 animals received by gavage 8 mg/kg BDE-99 in 20% fat emulsion or 10 mL/kg of 20% fat emulsion. Ten to eight mice were tested at the age of 5 months 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, animals that received nicotine on PND 10 and BDE-99 as adults showed a lack of habituation. These animals displayed hypoactive behavior in the beginning of the spontaneous behavior test period (0–20 minutes), but became hyperactive toward the end of the test period (40–60 minutes), indicating that the neonatal nicotine exposure has affected the susceptibility to BDE-99 in the adult animals. 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 worsens with age. Overall, this study indicates that neonatal nicotine exposure has affected the susceptibility to BDE-99 in the adult animal.

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 to 20 Wistar rats were given by gavage single doses of 0, 0.06, or 0.3 mg/kg of BDE-99 (98% purity) 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 to 21 with 0.5% (about 0.9 mg/kg-day) of the goitrogen 6-*n*-propyl-2-thiouracil (PTU) in drinking water.

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 all pups, males and females (n = 163 to 218). The eruption of incisors was significantly delayed in the PTU-treated group and in the 0.3 mg/kg BDE-99 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, 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 count per active phase (an active phase is defined when the animal begins to move until a pause), 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 offsprings 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 male reproductive system of adult male offspring (PND 140) was 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 examined, and testosterone and luteinizing hormone (LH) levels were measured. No effects were seen in BDE-99 treated animals on body weight or absolute and relative liver, thymus, seminal vesicle, or prostate weights. 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; 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) were 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 offsprings approximately 150 days old (n = 15–19/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 (n = 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% of the males, respectively, 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 offsprings 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), can also be induced in another species, namely the rat. Results from the receptor assay are reported in Section 4.4.1.

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 at the age of 2 months in nine 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 nor significant difference in body-weight gain or adult weight between controls and rats treated with BDE-99, at any time during the experimental period. 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 but 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 two-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 two-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 F1 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 F1 females were not statistically different from controls at both doses of BDE-99. The only 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 group. Pregnancy rate was also significantly lower than that in controls in the PTU-treated group.

histologic evaluation by electron microscopy of the ovary, uterus, and vagina was performed in the F1 female offspring on PND 90. Ultrastructural changes in the ovaries and hyperplastic vacuolar degeneration of the vaginal epithelium were observed in the F1 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 F2 generation from two different litters following exposure of the F0 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. The histologic changes in the ovaries and vaginal epithelium were not associated with any effect on fertility.

4.3.2.4. *Lilienthal et al. (2005)*

BDE-99 was administered by s.c. injections to pregnant Long-Evans rats from GDs 10 to 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 organs' 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 at PND 240. In addition, activity in the open field was studied in male offspring on PNDs 30, 90, and 400.

In male offspring at PND 160, a slight but significant reduction in anogenital distance,

a marker of sexual development, was reported in adult males (n = 8/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 (n = 10 to 12/group). Locomotor activity of male rats in the open field was not changed by BDE-99 or Aroclor treatment on PND 30, 90, or 400. In contrast, on the catalepsy test, all exposed groups exhibited, in comparison with the control group, 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.

4.3.2.5. *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 to 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 A1254. The number of implantations per litter, the number of pups per litter, and the percentage of male pups were not different from controls. At weaning, there were no significant differences between BDE-99-exposed and control rats in weights of brain, thymus, testis, ventral prostate, and uterus. 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 after exposure to 1 and 10 mg/kg-day BDE-99 compared with controls.

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 (n = 8/group) at PND 21 and PND 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 by exposure 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 (n = 10 to 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. A marked effect was the decrease in thyroid weights in adult offspring, which was more pronounced in the high-dose group.

4.3.2.6. 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 the uteri of the exposed pups as adults. Groups of 6–9 time pregnant Long-Evans dams were given daily s.c. injections of 0, 1, or 10 mg/kg BDE-99 from GDs 10 to 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. At birth 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; 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 and absolute and relative liver, uterine and ovarian weights were measured. There were no significant differences between groups except for a slight but significant increase in absolute and relative ovarian weights in the animals exposed to 10 mg/kg-day prenatally. Analysis of plasma and adipose tissue for the presence of BDE-99, 120 days after exposure ceased, identified small amounts in the brain and plasma for both dose groups and substantially higher levels in the adipose tissues.

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- α (ER- α) and estrogen receptor- β (ER- β) were measured after amplification by using real-time PCR and targeting of the resultant cDNA using appropriate probes. There was a dose-dependant decrease in PR at both

doses. 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 to controls with 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 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-17 β . The rats for this component of the study were ovariectomized at 10 weeks. The purpose of the ovariectomies was to reduce the exposures 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 for the low-dose BDE-99 treated ovariectomized animals were significantly higher than those for the ovariectomized controls for PR. In the high-dose ovariectomized BDE-99 treated animals, the IGF-1 and ER- β levels were significantly higher than the 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 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. OTHER STUDIES

4.4.1. Receptor Site Interactions

There is considerable evidence from studies of PCBs, polychlorinated dibenzo-*p*-dioxins

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

4.4.1.1. Aryl Hydrocarbon Receptors

The transcription of the genes for cytochrome CYP-450, 1A1, 1A2, and 1B1 are linked to a signal transduction cascade that is initiated by activation of the Ah receptor by an appropriate ligand. The CYP1 family of enzymes is highly conserved in mammals and is responsible for the oxidative metabolism of a variety of planar and near-planar compounds (Lewis et al., 1998). The CYP1 family of enzymes metabolically activates and metabolizes polycyclic aromatic hydrocarbons and aromatic amines as well as PBDEs. Many substrates for the CYP1 family enzymes are also Ah receptor ligands. Differences in Ah receptor affinity are correlated to variations in CYP1 inducibility. Receptor site affinity has been shown to reflect potency and the potential for a xenobiotic to cause adverse health effects.

Chen et al. (2001) studied the affinity of several PBDE congeners for rat hepatic Ah receptor through competitive binding assays and determined their ability to induce hepatic CYP-450 enzymes by means of EROD assays (a biomarker for CYP1A1/2 induction) in chick and rat hepatocytes, in liver cell lines from rainbow trout, and in rat and human tumor cell lines. 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 is 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 10^{-3} to 10^{-4} that 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 BDE-100 as well as other PBDEs on the Ah receptor in cultured liver cells 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 co-exposures of TCDD and the PBDE were tested as evidenced by a decrease in the activation caused by TCDD alone. The impact of the PBDEs was receptor-localized rather than through inhibition of the enzyme since no EROD inhibition occurred if TCDD exposure preceded the PBDE exposure. Environmentally relevant concentrations of PBDEs (1 to 10 μ M) were evaluated. There was variability in the response of the four monkeys, likely reflecting individual differences in the animals.

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 CYP1A1. These issues are environmentally relevant because of the strong rank-order correlation among strength of Ah receptor binding, CYP1A induction, and toxicity for many halogenated aromatic compounds.

There were four components to this study (Chen and Bunce, 2003): (1) the binding of the PBDE congener to the Ah receptor, (2) the binding of the receptor/PBDE complex to an oligonucleotide segment of the dioxin response element, (3) the induction of EROD, and (4) the production of CYP1A mRNA and CYP1A protein. The 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 CYP1A1 mRNA and CYP1A1 protein equivalent to the maximal response of TCDD in primary Sprague-Dawley rat hepatocytes, although at concentrations three to five orders of magnitude greater than TCDD. 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 BDE-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 to 500 ng/mL. Luminescence was measured and compared to the maximum response observed with a 1500 picomolar TCDD standard (%-TCDD-max). A positive response was defined as any response that was greater than three standard deviations above the mean value for the control. BDE-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 three days with BDE-99 (96% pure) in corn oil at 0, 1, 10, or 100 $\mu\text{mol/kg-day}$. The animals were sacrificed 24 hours after receiving the last dose. The liver was removed, and RNA from a 100 g liver sample was isolated, converted to its cDNA, and amplified by using the PCR. The resultant DNA samples were then analyzed to determine the expression of the CYP-4501A1, a protein linked to Ah receptor activation.

BDE-99 had a significant effect on the level of CYP1A1 (8.1 times the vehicle-treated controls) only at 100 $\mu\text{mol/kg-day}$ (57 mg/kg-day), making it a weak activator of the Ah receptor. When the 1A1 expression from BDE-99 was compared to that for tetraBDE-47 and hexaBDE-153, the impact on the Ah receptor seemed to be correlated to the levels of polybrominated dibenzofurans in each congener, which in turn correlated with increased bromine content of the congeners.

The results from this study confirm *in vitro* data, suggesting that PBDEs are, at best, weak activators of the Ah receptor. These results also raise the possibility that brominated

dibenzofuran impurities identified in the congeners studied may, in some cases, have confounded the results from other studies.

4.4.1.2. Estrogen Receptors

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

The in vitro estrogenic and antiestrogenic potencies of seventeen PBDEs, including BDE-85, -99, -100, and -119 and three hydroxylated PBDEs, were investigated in a human T47-breast-cancer cell line based on ER-dependent luciferase reporter gene expression. The modified T47D cells, which contained ER- α and ER- β receptors, 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 incubated. The luciferase activity was measured with a luminometer. BDE-100 and BDE-119 showed estrogenic potencies in the assay with concentrations leading to 50% induction (median effective concentration [EC₅₀]) of 2.5 and 3.9 μ M, respectively, in comparison to an EC₅₀ value of 1.0×10^{-5} μ M for estradiol. These pentaBDEs were thus, respectively, 250,000 and 390,000 less potent than estradiol. BDE-85 and BDE-99 did not show any estrogenic activity in the ER-CALUX assay (Meerts et al., 2001).

Several hydroxylated derivatives of PBDEs ($\geq 99\%$ purity) were also evaluated in the CALUX assay described above. 2,4,6,3',5'-Pentabromo-4'-hydroxyBDE (2,6-dibromo-4-(2,4,6-tribromophenoxy)-phenol), a T₄-like hydroxylated-BDE, demonstrated no estrogenic activity up to concentrations of 10 μ M. 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 T₄-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 ^3H -steroids from carp serum proteins which 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 using concentrations of zero to 1000 nM. All three compounds tested acted as inhibitors of the enzyme. The pentaBDE hydroxy congener (4-OH-3,5, 2',4',6'-BDE) 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 competition 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 receptor indicate that the activity of the pentaBDEs are much lower than the activities of dioxin and PCBs. Receptor-site mediated activity via the estrogen receptor site appears to be minimal for the pentaBDEs.

4.4.1.3. Androgen Receptors

DE-71, a commercial pentaPBDE mixture, was found by Stoker et al. (2004) to delay puberty and suppress the growth of androgen-dependent tissues in male Wistar rats exposed to doses of 30 or 60 mg/kg during the peri-pubertal period but not to doses of 0 or 3 mg/kg. In order to examine which components of the mixture might be responsible for the observed effects, androgen receptor binding by several of the individual congeners found in DE-71 was examined in vitro (Stoker et al., 2005). The assays of the individual congeners examined competitive binding of BDE-99 (98% pure) and BDE-100 (100% pure) in the presence of a tritium-labeled androgen agonist (R1881) by using ventral prostate cytosolic extracts along with an assay in an MDA-kb2 cell line containing the human androgen receptor and a transfected luciferase reporter

element.

In the assay with the ventral prostate extract, 0.001, 1.6, 3.3, 16.7, or 33 μM concentrations of BDE-99 and BDE-100 were incubated in the presence of 1.0 nM R1881 and 10 μM triamcinolone acetonide, an agent that blocks the progesterone and glucocorticoid receptors. Both congeners acted as competitive inhibitors for the binding of R1881, but the activity of BDE-100 was more potent than 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 BDE-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-dependant 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.

4.4.1.4. *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 muscles and neurons. The muscarinic receptors are found in smooth muscles, 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. The study demonstrated that there appears to be a critical window of vulnerability to nicotine exposure during postnatal development. Cholinergic receptor changes during the window of vulnerability are irreversible and cause a hypoactive response to exposure to cholinergic stimulants in adulthood.

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 PNDs 19–23. The hippocampal tissues from the nicotine-exposed animals were evaluated 24 hours after nicotine 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 postnatally on days 10–14 prior to their adult exposure had receptor levels as adults that were significantly lower than the 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 the 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 tritium-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 [³H]-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 an irreversible 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.

4.4.1.5. Other Receptors

The study of CYP-450 mRNA expression in rat liver by Sanders et al. (2005) (See Section 3.3) found that expression of CYP2B was up-regulated by BDE-99 in F344 rats to a greater extent than CYP1A1, a biomarker for the activation of the Ah receptor. Up-regulation of CYP3A was also observed. CYP2B and CYP3A are respective biomarkers for activation of the constitutive androstane receptor (CAR) and pregnane X receptor (PXR). In the case of BDE-99, the effect on CAR was greater than that on PXR. The CAR and PXR receptors are classified as orphan receptors. They are both involved in the metabolism of xenobiotics and are stimulated by phenobarbital. The CAR receptor is also involved with steroid metabolism. The impact of BDE-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.

4.4.2. Thyroid Effects

Because PBDEs have some structural similarity to the thyroid hormone T₄, it has been suggested that they may interfere with thyroid hormone transport by competitively binding with transthyretin (TTR), one of the thyroid hormone-binding transport proteins in plasma of vertebrate species. The possible interference of several pentaBDEs with T₄-TTR binding was investigated in an in vitro competitive binding assay, using human TTR and ¹²⁵I-labeled T₄ as the displaceable radioligand. The four pentaBDE congeners evaluated (BDE-85, -99, -100, and -119) did not compete with T₄-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 T₄ from TTR. The pentaBDEs were individually incubated with liver microsomes

prepared in the presence of phenobarbital (a CYP2B inducer), β -naphthoflavone (a CYP1A inducer), or clofibrate (a CYP4A3 inducer). Incubation of the pentaBDEs with CYP2B-enriched rat liver microsomes resulted in the formation of metabolites that were able to displace $^{125}\text{I-T}_4$ from TTR. The metabolites of BDE-100 and BDE-119 were able to displace more than 60% of the $^{125}\text{I-T}_4$ from TTR. BDE-85 and BDE-99 showed a lower ability to displace $^{125}\text{I-T}_4$ from TTR (20-60%). No T_4 -TTR displacement by pentaBDEs occurred after incubation with liver microsomes enriched with CYP1A or CYP4A3. 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. Thyroxine-binding globulin, rather than TTR is the major thyroxine-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, serum 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.

4.4.3. Neurotoxicity

The effects of BDE-99 on the developing brain were investigated by Alm et al. (2006). Neonatal NMRI mice pups (10 randomly selected from three–four litters) were given a single 12 mg/kg dose of emulsified BDE-99 or vehicle (egg lecithin and peanut oil) by gavage on PND 10, during the rapid brain growth spurt. Both groups were sacrificed 24 hours after dosing, and the brain was excised. Striatal and hippocampal tissues were removed from the brains of three mice at a time, homogenized, and cleaned to remove lipids and nucleic acids. There were four pooled tissue samples from the control brains and four from the exposed brains. One control and one exposed sample were replicates. Samples were analyzed to determine total protein content. The samples were labeled using cyanine dye, and the proteins were separated by using two-dimensional fluorescence difference gel electrophoresis. Separation in the first dimension employed isoelectric focusing. Separation in the second dimension was done using 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 protein spots, 40 differentially expressed striatal proteins and 56 from the hippocampus were selected for further analysis.

The gel spots selected for further analysis were removed, the protein extracted and

subjected to trypsin digestion. The resultant peptides were analyzed using time-of-flight mass spectrometry and identified using 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. Mortalin, a heat-shock protein, (down-regulated in the striatum and up-regulated 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, an enzyme that functions in neuronal growth, learning, and memory. In the hippocampus, two γ -enolases, α -enolase, ATP synthase, a mitochondrial hydrogen ion transporter, and isocitrate dehydrogenase were all up-regulated. Several of these proteins participate in protein kinase C 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 identify the mode of action for the neurodevelopmental effects of BDE-99. It does indicate that there are differences in brain development that occur during the brain growth spurt within the hippocampus and striatum. Several of the differentially expressed proteins are linked to the protein kinase C 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.

4.4.4. Immunotoxicity

Mitogen-induced DNA synthesis and immunoglobulin (Ig)G synthesis by human lymphocytes were examined after exposure to BDE-85 ($\geq 98\%$ 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 anti-human 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.

4.4.5. 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 cleaves MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) 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 using a hemocytometer. BDE-99 and Aroclor 1254 caused comparable concentration-dependent inhibition of MTT. Aroclor 1254 caused significant release of LDH at the two highest concentrations (50 and 100 μ M), 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).

Activation of protein kinase C (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.

4.4.6. Genotoxicity

Evandri et al. (2003) studied the genotoxicity of BDE-99 using *Salmonella typhimurium* strains TA98 and TA100, *Escherichia coli* WP2 *uvrA* and *Allium cepa* chromosome aberration test. BDE-99 was nontoxic in bacteria at the highest dose tested of 0.305 mg/plate. This dose caused a comparable number of revertant colonies as in the solvent control group, with and without S9. BDE-99 was also not cytotoxic in the *A. cepa* test at concentrations up to 100 μ M (56 mg/L). The number of structural chromosome aberrations induced by BDE-99 was not significantly different from that of the control. Results from the bacterial reverse mutation assay indicate that BDE-99 does not appear to covalently bind to DNA.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

4.5.1. Oral

Alterations of behavioral parameters, namely 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 seen on spontaneous motor behavior were not species, gender, or strain specific and occurred 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). 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 offsprings 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 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 any effect on fertility (mean implantation sites per dam, live fetuses per dam, resorption rate).

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 United States 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

A growing body of data suggest that the observed neurodevelopmental responses seen in several studies may be a consequence of impaired development of the acetylcholine receptor

network in the brain during the “brain growth spurt” period (Viberg et al., 2005, 2004b; Ankarberg, 2003). In rats and mice this 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. Postnatal days 10 to 14 appear to be a period of maximum vulnerability for the developing cholinergic system and coincides with the most pronounced neurodevelopmental effects from BDE-99 exposure. Proteomic studies of brains (striatum and hippocampus) of mice exposed to BDE-99 on PND 10 show that there are some distinct differences in the proteins expressed in the exposed brain compared with those in control brains (Alm et al., 2006). Several of the proteins that seemed to be biomarkers for the BDE-99 exposure are linked to the protein kinase C signaling cascade that plays a role in neuron development, memory, and learning.

Exposure of rats to BDE-99 resulted in an increase of total T_4 plasma levels 3–6 days from exposure, but returned to normal levels by 12 days from exposure (Hakk et al., 2002a). Serum total and free T_4 levels of male mice offsprings of dams treated with BDE-99 from GD6 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 on 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 normal brain development in humans 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 (Skarman et al., 2005; Hakk et al., 2002a; Branchi et al., 2005) do not seem to indicate that BDE-99 interferes with thyroid hormone homeostasis. However, thyroid hormone levels and behavioral activity were not co-measured in any of the developmental toxicity studies in mice or rats.

Hydroxylated pentaBDE metabolites have been shown in vitro to compete with thyroxine 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. Thyroxine-binding globulin, rather than TTR is the major thyroxine-binding protein in humans.

Two studies have found subtle effects on the female reproductive systems in rats exposed to BDE-99 prenatally (Ceccatelli et al., 2006; Talsness et al., 2005). The Talsness et al. (2005)

study observed changes in ovarian and vaginal histopathology of female offspring of Wistar dams treated with 0.06 or 0.3 mg/kg BDE-99 on GD 6. The Ceccatelli et al. (2006) study evaluated the adult female offspring of Long-Evans rats exposed to either 1 or 10 mg/kg daily on GDs 10 to 18. The only standard developmental parameter that seemed to be affected was a slight but significant increase in absolute and relative ovary weight in the animals exposed to 10 mg/kg BDE-99. Histopathologic examinations of the tissues were not conducted. In an extension of the Ceccatelli et al. (2006) study, ovariectomized adult pups treated with BDE-99 prenatally showed a dose-related decrease in the amount of change in the up-regulated progesterone receptor mRNA response to estradiol-17 β exposure and an increase in the amount of change in down-regulated response of estrogen receptor- β mRNA. The animals were ovariectomized to eliminate the ovaries as a source of endogenous estrogen. It is not clear whether the observed hormonal changes are linked to the histologic vaginal and ovarian changes observed by Talsness et al. (2005).

No effects on pokeweed mitogen-induced DNA proliferation or IgG synthesis were observed 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 aryl hydrocarbon and estrogen 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).

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

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.

There is “inadequate information to assess the carcinogenic potential” of BDE-99 (U.S. EPA, 2005a,b).

4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.7.1. Possible Childhood Susceptibility

A population subgroup is susceptible if exposure occurs during a period of sensitivity, as

observed in 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 of development. The animal model indicates a potential for concern for early lifetime exposure (i.e., fetal or infant exposure) to the chemical. The identification of BDE-99 in human maternal and cord serum, milk, and children's serum (Mazdai et al., 2003; Schechter et al., 2003; Thomsen et al., 2002) implies humans are exposed to BDE-99 during a period of rapid development of the brain, a critical window of development, indicating a potential for susceptibility. Whether such exposure constitutes a health risk for adverse neurodevelopmental effects in children is not known at this time because of the limited toxicological data base 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 (Viberg et al., 2004a; Kuriyama et al., 2005), 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 experimental animals.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

The short-term study (Section 4.2.1) in mice (Skarman et al., 2005) and acute study in rats (Hakk et al., 2002) measured hepatic mixed function oxidase system enzyme activities and/or plasma thyroid hormone levels following exposure to BDE-99. Changes in the activity of the mixed function oxidase system enzymes often accompany exposure to xenobiotic compounds and are not suitable endpoints for dose-response assessment. Enzyme activities returned to normal levels during the postexposure period. The transient increase in T₄ levels seen in the single dose study by Hakk et al. (2002) was not confirmed in the longer duration study of Skarman et al. (2005). Thus both studies are not good candidates for the dose-response assessment.

Table 5 summarizes oral reproductive and developmental toxicity studies that were considered as candidates for the derivation of an RfD for BDE-99.

Table 5. 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 behavior. 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)

Species (strain) sex	Duration (purity)	Dose levels (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Observed effects	Reference
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, characterized by an inverted dose-response relationship, that ends around 4 months of age.	Branchi et al. (2002)
Rat (Wistar), M & F	Single dose on GD 6 (98%)	0, 0.06, or 0.3	Not identified	0.06	Neurobehavioral developmental effects. Effects on locomotion (hyperactivity), increasing with increasing age.	Kuriyama et al. (2005)
			Not identified	0.06	Decreased daily sperm production, spermatid count and relative epididymis weight in offspring. No effect on fertility at any dose.	
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	0.06	Qualitative histologic changes in ovaries and vaginal epithelium in offspring.	Talsness et al. (2005)
			0.3	Not identified	No effect on fertility at any dose.	

The Viberg et al. (2004a) study was selected as the principal study, and the neurobehavioral developmental effect was 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 their amenability to BMD modeling (see Section 5.1.2). The primary reasons for selecting the Viberg et al. (2004a) study were that several doses were examined, quantitative dose-response data were available to conduct BMD modeling, a good fit was obtained in the BMD modeling, a clear NOAEL was identified, and the study is supported

by several other studies in mice. In this study, 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 in this study was 0.4 mg/kg. Adverse effects noted in 2-, 5-, and 8-month-old mice at 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 disturbances becoming more pronounced with increasing age.

Administration of BDE-99 on PND 10 was shown to be a period of maximum vulnerability of the developing mouse brain (Eriksson et al., 2002). Gavage administration of BDE-99 is not expected to have caused any stress in mice tested at the age of 2 months and older (Branchi et al., 2005).

There are several concerns regarding the design of the Viberg et al. (2004a) study. The protocol was unique and did not conform to health effects test guidelines for neurotoxicity screening battery or developmental neurotoxicity studies (U.S. EPA, 1998b). The dosing regimen did not include gestation and lactation exposure (U.S. EPA, 1998b); only single doses were given. In some respects the observation that effects occurred with such limited dosing argues for the importance of this study. While the study design appears to have been conducted during a developmental window of susceptibility, it is not adequate to determine the effect of longer dosing. Translating the implications of these data to more traditional dosing regimens is problematic, particularly with regard to evaluating the implications of in utero and postnatal exposure. Another concern is that, based on the data provided in the published report, more than one pup per litter was used for the behavioral testing (eight mice were randomly selected from three to five different litters in each treatment group). Increasing the number of samples from each litter may bias the analyses towards false positives and the observed neurobehavioral effects may be attributable to non treatment-related differences in pups born to a single dam¹. Another concern regarding the study design was the limited number of neurobehavioral parameters that were assessed. The absence of a full functional observational battery (FOB) limits the ability to correlate the reported effects with other FOB parameters. This would be helpful in gauging the reliability of the limited parameters that were measured. As indicated in the *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), it is assumed that an agent that produces

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

detectable adverse neurotoxic effects in experimental animal studies 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 limitations cloud the utility of this study, several additional considerations support the use of these data. Supporting data that exposure occurred during the period of maximum vulnerability of the developing mouse brain come from the study of BDE-99 that demonstrated that vulnerability of adult mice to the neurodevelopmental effects occurs during a narrow phase of neonatal brain development (Eriksson et al., 2002). Acute exposure to a highly lipophilic and long half-life chemical, such as BDE-99, will result in exposure that lasts much longer than just acutely. In addition, there are a wide variety of brain structures that have very limited critical windows during development. These short critical windows translate to a susceptible period of exposure that can be very short. Therefore, even chronic exposures may lead to developmental neurotoxicity via disruption of developmental events that take place during a short critical window of development (Rice and Barone, 2000). The concept that exposure during critical periods of development can induce functional neurological effects later in development has been demonstrated with structurally-related PBDE congeners, including tetra-, hexa-, and decaBDEs (Kuriyama et al., 2005; Viberg et al., 2004ab, 2003a,b; Branchi et al., 2002; Eriksson et al., 2001). Therefore, the observed neurobehavioral effects are biologically plausible and exposure to BDE-99 may pose a potential hazard to humans (U.S. EPA, 1998a). Taken together, these considerations support the use of the Viberg et al. (2004a) study for deriving the RfD for BDE-99.

Supporting studies for neurobehavioral effects in mice include the study by 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 in this study was 0.4 mg/kg and the LOAEL 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 the study of Branchi et al. (2002), transient hyperactivity characterized by an inverted 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.

Supporting studies in rats for the neurobehavioral developmental toxicity of BDE-99 include the study of 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 8.0 mg/kg for dose-related changes in spontaneous motor behavior in adult rats.

In the study of Kuriyama et al. (2005), Wistar dams were exposed to 0, 0.06, or 0.3 mg/kg PBDE-99 on GD 6. Male and female offspring showed significant increases in locomotor activity on PND 36 and PND 71 at both doses. The LOAEL for hyperactivity was 0.06 mg/kg.

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

The effects of BDE-99 on the female reproductive system were evaluated in rats (Talsness et al., 2005). Qualitative histologic changes in the ovaries and vaginal epithelium were seen at both doses tested, 0.06 and 0.6 mg/kg BDE-99, but were not associated with any effect on fertility (mean implantation sites per dam, live fetuses per dam, resorption rate).

5.1.2. Methods of Analysis

The RfD for BDE-99 was derived by using the benchmark dose (BMD) approach for continuous data. In the case of motor activity, there is no specific change that is generally regarded as indicative of an adverse response. In the absence of guidelines for the level of response to consider adverse for the motor activity endpoint, the benchmark response (BMR) selected was for a change in the mean equal to one control standard deviation from the control mean. However, a change in mean response equal to 0.5 and 1.5 estimated control standard deviations (0.5 and 1.5 SD, respectively) was applied to provide perspective on the variability in the response.

BMD modeling output results providing good data fit are summarized in Table 6 and given in detail in Appendix A.

Table 6. Summary of BMD modeling output results with good data fit

Reference	Endpoint	Model	AIC ^a	BMD _{1SD}	BMDL _{1SD} ^b
Viberg et al. (2004a)	Rearing habituation in 2-month-old male mice	Hill	96	0.63	0.48
Viberg et al. (2004a)	Rearing habituation in 2-month-old female mice	Hill	122	0.71	0.50
Viberg et al. (2004a)	Rearing habituation in 8-month-old female mice	Hill	110	0.42	0.32
Kuriyama et al. (2005)	Locomotor activity duration per day	poly-nomial	194	0.28	0.22
Kuriyama et al. (2005)	Percent of animals with < 2 ejaculations	Log-logistic	79	0.04	0.02

^aAIC = Akaike Information Criterion

^bBMDL = 95% lower bound on the BMD

The studies of Viberg et al. in mice (2004b) and rats (2005) were not amenable to BMD approach because the data needed for the use of a BMD approach are not available in the published studies. Experimental data points for locomotion, rearing, and total activity and their standard deviations are displayed graphically only, and such values cannot be read with any accuracy from the graphs. The study of Talsness et al. (2005) also could not be used for BMD modeling, because the histologic changes in the ovaries and vaginal epithelium of rats are qualitatively described and in pictorial form. There was no effect on fertility in this study, and the NOAEL was the highest dose tested.

Several BMD analyses were conducted by using all relevant endpoints in the Viberg et al (2004a), Eriksson et al. (2001), and Kuriyama et al. (2005) studies. The data set used and details of the BMD modeling results are presented in Appendix A. No satisfactory data fits were obtained for habituation ratios for locomotion in male and female mice in the Viberg et al. (2004a) study nor for any of the endpoints in the Eriksson et al. (2001) study.

For the Viberg et al. (2004a) study, the most successful model was the Hill, model applied to the data on rearing habituation in 8-month-old female mice. The BMD modeling of this endpoint resulted in a BMD_{1SD} of 0.42 and a BMDL_{1SD} of 0.32 mg/kg. The BMDs and BMDLs corresponding to 0.5 SD and 1.5 SD were also calculated in order to evaluate the impact of BMR selection on model-derived BMDs and BMDLs. The BMD_{0.5SD} and BMDL_{0.5SD} were 0.30 and 0.22 mg/kg, respectively, and the BMD_{1.5SD} and BMDL_{1.5SD} were 0.51 and 0.40 mg/kg,

respectively.

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. From additional data not available in the published study of Viberg and coworkers (2004a), spontaneous behaviors (locomotion, rearing, and total activity) were quantified in terms of a fractional response defined as the cumulative response after 20 minutes, divided by the cumulative response produced over the whole one-hour test period. The fractional response contains information about the time-response profile (which differs between the treatment groups) and was found to have appropriate statistical characteristics. In the analysis, male and female mice could be characterized by a common dose-response model (i.e., they responded equally to the exposure to BDE-99). By using the Hill model and spontaneous motor behavior data observed in 2-month-old male and female mice, the BMDs and BMDLs corresponding to 10% BMR levels were calculated. Total activity was found to be the most sensitive endpoint. The BMD_{10} and $BMDL_{10}$ for this endpoint were 0.61 and 0.42 mg/kg, respectively. These values are similar to the BMD_{1SD} of 0.42 and a $BMDL_{1SD}$ of 0.32 mg/kg obtained from the published data in Viberg et al. (2004a) on rearing habituation in 8-month-old female mice. Values of the $BMD_{0.5}$ and $BMDL_{0.5}$ corresponding to 5% benchmark response levels were also calculated by Sand et al. (2004) and were 0.33 and 0.21 mg/kg, respectively.

For the Kuriyama et al. (2005) study, additional information on data points and standard deviations for the means for locomotor activity on PND 36 and PND 71 were obtained from the study authors (email from Ibrahim Chahoud, Charite University Medical School Berlin to Mary Manibusan, U.S. EPA, dated 11/14/04). The continuous effect modeled was locomotor activity at PND 36. BMD analysis of locomotor activity on PND 71 was not amenable to modeling. The best data fit was obtained with the polynomial model for duration of activity per day measured on PND 36, giving a BMD_{1SD} of 0.28 mg/kg and a $BMDL_{1SD}$ of 0.22 mg/kg.

The $BMDL_{1SD}$ of 0.32 mg/kg in mice (Viberg et al., 2004a) and the $BMDL_{1SD}$ of 0.22 mg/kg in rats (Kuriyama et al., 2005) are therefore very similar and indicate that rats and mice are 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. The study in mice of Viberg et al., 2004a is used in the derivation of the RfD because it examined five doses, a good fit was obtained in the BMD modeling, a clear NOAEL was identified for the critical effect, and it is supported by several other studies in mice. 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 in the Kuriyama et al., 2005 study, no satisfactory data fits were obtained for spermatid, sperm numbers, or daily sperm production. The only effect that could be modeled was the percent of adult rats with less than two ejaculations (see Table 6 and Appendix A). The biological significance of this effect is uncertain, and therefore the result of the BMD modeling was not used in the health assessment.

5.1.3. RfD Derivation

By using benchmark dose modeling, the $BMDL_{1SD}$ of 0.32 mg/kg for decreased 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 3000 was applied: 10 for extrapolating animal data to humans (UF_A interspecies variability), 10 for susceptible human subpopulation (UF_H interhuman variability), 3 for extrapolating from subchronic to chronic exposure (UF_S)s and 10 to account for a deficient database (UF_D). The rationale for application of the UFs is described below.

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

A default UF_H of 10 was applied to account for variations in susceptibility within the human population (intraspecies or interhuman variability). This factor accounts for humans who may be more sensitive than the general population to exposure to BDE-99.

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

A UF_D of 10 was used to account for database uncertainty. The available oral database for BDE-99 lacks prenatal developmental neurotoxicity studies and multigeneration reproductive toxicity studies.

Application of a total UF of 3000 to the $BMDL_{1SD}$ of 0.32 mg/kg results in a reference dose for BDE-99 of 1×10^{-4} mg/kg-day or 0.1 μ g/kg-day.

For a NOAEL/LOAEL approach to the derivation of the RfD, a total UF of 3000 is applied to the NOAEL of 0.4 mg/kg for neurodevelopmental effects identified in the Viberg et al. (2004a) study, giving a reference dose for BDE-99 of 1.3×10^{-4} mg/kg-day or 0.1 μ g/kg-day.

The Kuriyama et al., 2005 study is not amenable to the derivation of the RfD using a LOAEL approach (0.06 mg/kg for decreased spermatid count and daily sperm production), because the total UF would amount to 30,000 (10 for interspecies variability, 10 for intraspecies variability, 3 for extrapolating from subchronic to chronic exposure, 10 for database deficiency, and 10 for extrapolating from a LOAEL to a NOAEL). The magnitude of the net UF of 30,000 and the fact that there is uncertainty in five areas indicate that this study is unsuitable for RfD derivation by using the LOAEL approach.

5.1.4. Previous RfD Assessment

An IRIS health assessment of commercial grade pentabromodiphenyl ether (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 μ g/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) and by using a UF of 1000. The UF of 1000 reflects 10 for both intraspecies and interspecies variability to the toxicity of this chemical in lieu of specific data and 10 for extrapolation of a subchronic effect level to its chronic equivalent. 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 a reference concentration for BDE-99.

5.3. CANCER ASSESSMENT

Data are not available to assess the carcinogenic potential of BDE-99.

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 the commercial pentabromodiphenyl ether flame retardant. BDE-99 has been found in human milk, adipose tissue, and blood. As a result, fetuses and infants are exposed to BDE-99. These data do not elucidate the effects of BDE-99 on the human populations but do demonstrate that exposure is occurring among the human population. The presence of BDE-99 in breast milk allows transfer from a mother's body stores to her infant through breast feeding.

No data are available regarding the potential toxicity of BDE-99 in exposed humans 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 milk, such exposure may constitute a health risk for 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, and LH levels or the ability to sire offsprings. Histologic changes in the ovaries and vaginal epithelium were seen in rats exposed to BDE-99. However, these changes were not associated with any effect on fertility indices (Talsness et al., 2005).

There are no studies of 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.

6.2. DOSE RESPONSE

The RfD of 0.1 µg/kg-day was calculated from a BMDL_{1SD} of 0.32 mg/kg-day for effects on spontaneous motor behavior in mice (Viberg et al., 2004a). A total UF of 3000 was used: 10 for interspecies variability, 10 for interindividual variability, 3 for extrapolation from single to lifetime exposure, and 10 for database deficiencies.

No data are available regarding the potential toxicity of BDE-99 in exposed humans via the oral route, and no suitable toxicokinetic or toxicodynamic models have been developed to reduce uncertainty in extrapolating from mice to humans.

The extent of variability in susceptibility to BDE-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 are fetuses and children. Chronic studies relevant to BDE-99 toxicity have not been performed in experimental animals.

The principal study used in the derivation of the RfD (Viberg et al., 2004a) examined a number of behavioral parameters in a limited number of male and female mice at ages 2, 5, and 8 months, exposed to BDE-99 on PND 10, and tested five oral dose levels. Supporting studies for neurobehavioral developmental effects 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 uterus 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 database for BDE-99 is sparse for the derivation of an RfD: there are no standard reproductive, developmental, subchronic, or chronic studies in rats or mice nor a much needed developmental neurotoxicity study. In addition, there are several concerns regarding the experimental design of the Viberg et al. (2004a) study used in proposing an RfD (see Section 5.1.1). The overall confidence in the RfD assessment is low.

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APPENDIX A. BENCHMARK DOSE MODELING FOR BDE-99

I. METHODS

All dose-response modeling was conducted using the EPA's BMD software (BMDS). Two versions of BMDS were used in the benchmark dose (BMD) modeling. BMDS version 1.3.2 was used to run the power and Hill models, and BMDS version 1.4 beta was used for linear and polynomial models. This was done due to some errors in these two versions of the BMDS. All continuous models (including linear, polynomial, power, and Hill) in version 1.3.2 of BMDS calculate incorrect degrees of freedom for p -value determination when the estimated parameter(s) hit the boundary, and this problem appears to be corrected in version 1.4 beta of BMDS. However, preliminary analyses revealed that the power model and Hill model in version 1.4 beta of BMDS occasionally failed to optimize model fitting where version 1.3.2 succeeded. Therefore, version 1.4 beta was used as the best available software for linear and polynomial models, while the version 1.3.2 was used to conduct Power model and Hill model fitting. The degrees of freedom for p -value in the power and Hill models with the Version 1.3.2 were manually corrected. A benchmark response (BMR) equal to one estimated control standard deviation (1.0 SD) was used.

A. Viberg et al. (2004a)

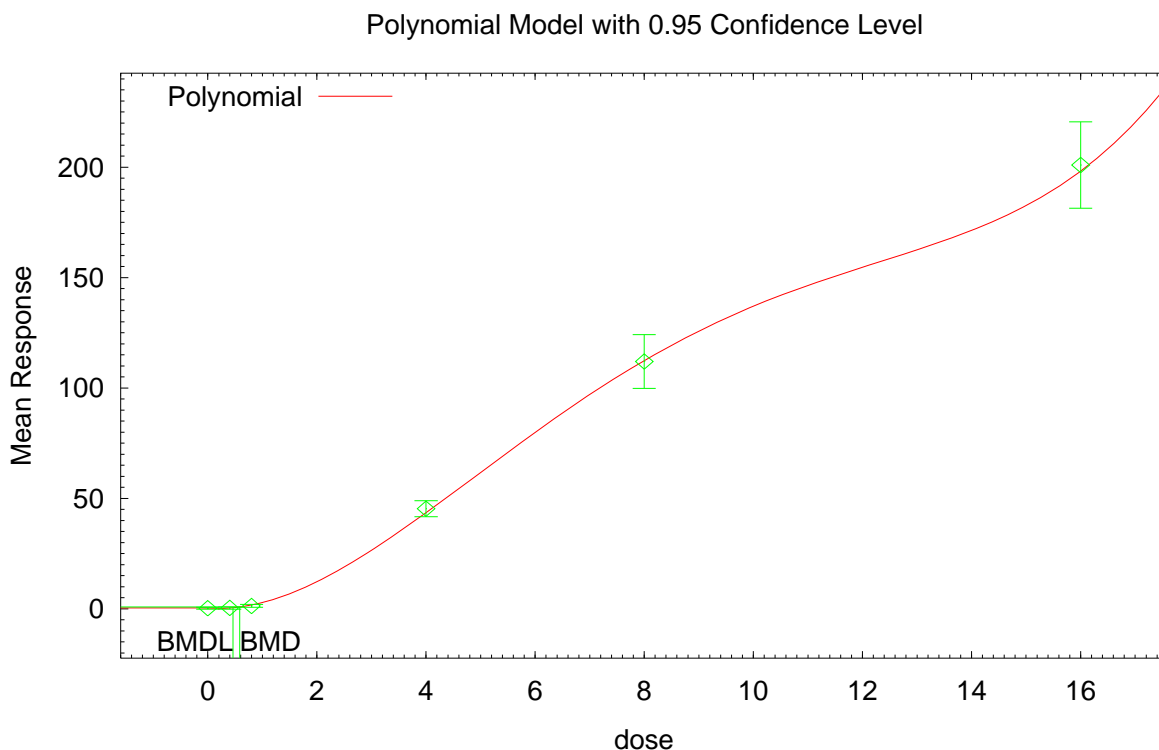
The locomotion and rearing habituation response data in male and female mice were modeled separately by using the linear, polynomial, power, and Hill continuous dose-response models. The test for equal variances across dose groups failed for all data sets modeled, and so the standard deviation was modeled as a power function of the mean. The variance model failed to adequately predict the observed variances in some cases, but no alternative variance model is available in the latest version of BMDS. Since the variance modeled as a power function of the mean provides much better estimate of the variance upon which the BMR is based, all the modeling runs were conducted with modeled variance.

The sample size used in the Viberg et al. (2004a) study was not explicitly stated in the published paper. It is stated that a total of eight mice were randomly selected from each treatment group, and we assume that there were eight males and eight females exposed to each level. The most reasonable alternative interpretation is that there were a total of eight, counting males and females together, but then there is no way to know how many were males and how many were females if they were randomly chosen. It seems more likely that the investigators had

eight male mice and eight female mice in each dose group, since they did not provide the sample size explicitly in the tabulation of results.

For both locomotion and rearing habituation response, when all six dose groups were included in the modeling, none of the continuous models provided an adequate (i.e., goodness-of-fit p -value > 0.10) fit to the mean responses for any endpoint when appropriate restrictions were applied to the models. However, satisfactory goodness-of-fit p -values were obtained for some endpoints by using an unrestricted polynomial model (e.g., Figure A-1, but this model frequently yielded unreasonable behavior at low doses in the region of the BMD as shown in Figure A-2). Figure A-2 is a plot of the unrestricted polynomial on the small scale at the low dose range that is of interest. There is an initial decrease in the dose-response function that allows the model to obtain good agreement with the observations, but the decrease is not consistent with biological knowledge about responses to PBDE exposure. Therefore, we used parameter restriction in all our model runs to prevent regions of decreasing dose response.

Figure A-1. Unrestricted fourth order polynomial fit to rearing habituation



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in 2-month-old male mice.

In most cases, when all six dose groups were included in the modeling, the dose response

trend at the highest doses is different from that in the low dose range. To obtain a better data fit at low doses in the range of the BMR, the highest dose, 16 mg/kg body weight, was dropped. This approach is reasonable because the LOAEL and estimated BMD are close to 0.8 mg/kg in this study, making it less important for the model to agree with the response at 16 mg/kg. Among the four continuous models available, only the Hill model provided an adequate fit to these truncated data sets, and adequate goodness-of-fit *p*-values were obtained for rearing in 2-month-old male mice and rearing in 2- and 8-month-old female mice (Tables 1, 3, and 4). However, adequate fits were not obtained for rearing in 8-month-old male mice with models other than the Hill model, so the 8 mg/kg dose group was dropped as well, and the continuous models except the Hill model were fit to the remaining four dose groups. This is reasonable because the 4 mg/kg dose groups are retained, and this dose is still above the region of the BMR, the range of interest. Again, adequate fits to the truncated rearing data were obtained from some of the continuous models.

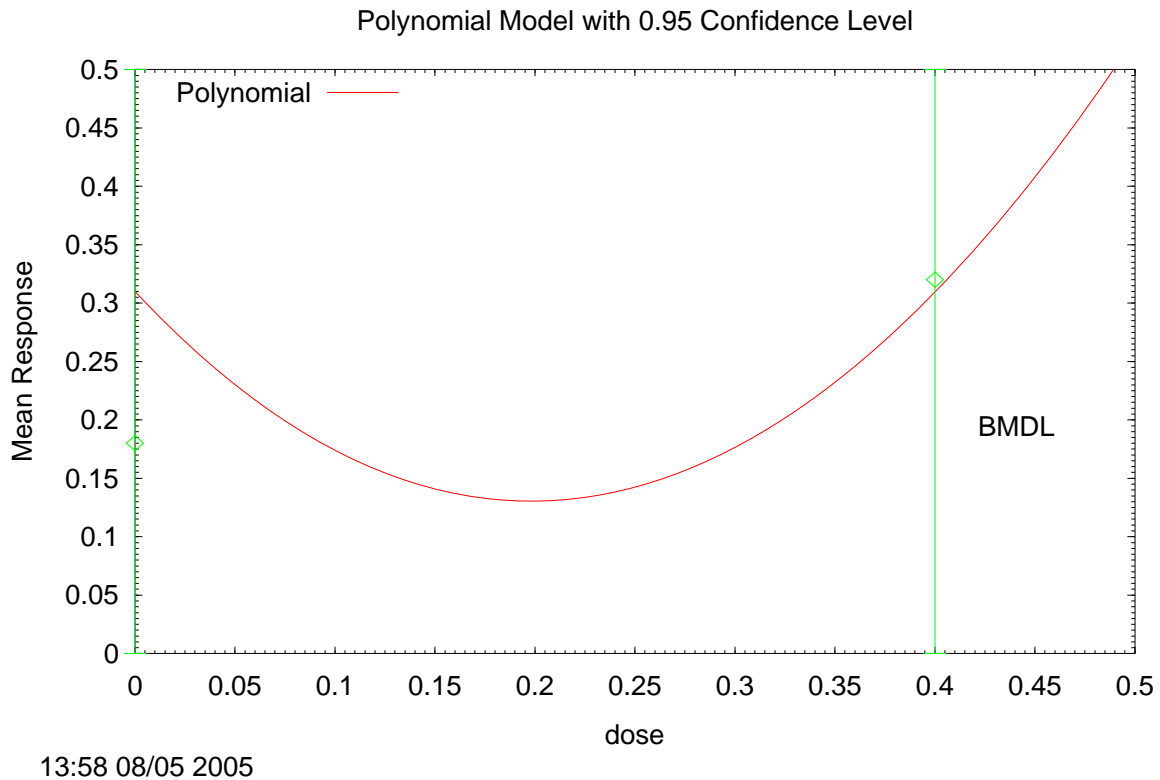


Figure A-2. Low-dose behavior of unrestricted 4th-order polynomial model.

The results are summarized in Tables A-1, A-2, A-3, and A-4. The Hill model was not used for the four-group truncated data sets because this model requires at least five data points to compute goodness-of-fit p -values. Adequate fits were not obtained for locomotion with either full or truncated data sets. The Akaike Information Criterion (AIC) scores cannot be used to compare the quality of fit between the Hill model and the remaining continuous models. This is because the Hill model is fit to five data points, while there are only four data points for the other models. The AIC is a function of the log-likelihood and the number of parameters in the model, and each data point contributes something to the log-likelihood. This means that models must be run on the same data sets in order for quality of fit comparisons to be made by using the AIC. However, the AIC can be used to make comparisons among the linear, polynomial, and power models when the same data sets are used in the modeling (see Tables A-1–A-4).

Most of the low dose (0.4 mg/kg) data show no increase above the mean of the control group response and have significantly smaller standard deviations than high dose groups. As a result, the monotonic increasing dose-response models could not precisely match the low dose mean and resulted in poor global fitting statistics (goodness-of-fit p -value <0.1) because of the very small standard deviation at the 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, the BMD results with goodness-of-fit $p < 0.1$ were not considered when estimating BMD and BMDL in these cases because the nonresponse at the 0.4 mg/kg dose is critical to determining the shape of the curve in the low-dose region.

Results

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

The original BMD modeling output results for this endpoint in 2-month-old male mice are summarized in Table A-1. Based on the results from four continuous models, the Hill and power models provided the best data fit because these models provide good global data fit, with goodness-of-fit p -values of 0.84 and 0.71, respectively. The estimated BMD and BMDL obtained from the Hill model were considered the best estimated values. Thus, for rearing habituation in 2-month-old male mice, the estimated $BMD_{1.0SD}$ is 0.63 mg/kg and $BMDL_{1.0SD}$ is 0.48 mg/kg.

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

Model	# Groups	AIC	<i>p</i> -Value	BMD 1 SD	BMDL 1 SD
Hill	5	96	0.84	0.63	0.48
Linear	4	86	<0.01	4.2	1.7
Polynomial	4	44	0.029	0.39	0.33
Power	4	39	0.71	0.59	0.44

Source: Viberg et al. (2004a).

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

The original BMD modeling output results for this endpoint in 8-month-old male mice are summarized in Table A-2. Based on the results from four continuous models, none of the models provide satisfactory data fits because all the goodness-of-fit *p*-values are smaller than the minimum requirement ($p > 0.1$). There is a possibility that this is due to the response in the 0.4 mg/kg dose group which was reported mean response of zero with a standard deviation of zero. The published manuscript did not indicate the basis for the zero value at 0.4 mg/kg (e.g., whether the calculated values were very small and reported as zero or whether no data were obtained for this dose group). In the absence of this information modeling was not conducted with these values dropped from the data set. Therefore, no BMD or BMDL could be used for this endpoint.

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

Model	# Groups	AIC	<i>p</i> -Value	BMD 1 SD	BMDL 1 SD
Hill	5	104	<0.01	0.57	0.45
Linear	4	160	<0.01	0.055	0.019
Polynomial	4	62	<0.01	0.42	0.36
Power	4	60	<0.01	0.56	0.42

Source: Viberg et al. (2004a).

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

The original BMD modeling output results for this endpoint in 2-month-old female mice are summarized in Table A-3. Based on the results from four continuous models, the Hill, polynomial and power models provide good data fit with goodness-of-fit *p*-values of 0.53, 0.12, and 0.62, respectively. Among these three models, the Hill model used the most data (five dose groups) and achieved a similar fit in the low-dose region of the curve to the power model, which had the lowest AIC from the models that used four dose groups. Thus, for rearing habituation in

2-month-old female mice, the estimated $BMD_{1.0SD}$ is 0.71 mg/kg and $BMDL_{1.0SD}$ is 0.50 mg/kg.

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

Model	# Groups	AIC	<i>p</i> -Value	BMD 1 SD	BMDL 1 SD
Hill	5	122	0.53	0.71	0.50
Linear	4	130	<0.01	0.065	0.034
Polynomial	4	73	0.12	0.45	0.38
Power	4	71	0.62	0.70	0.47

Source: Viberg et al. (2004a).

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

The original BMD modeling output results for this endpoint in 8-month-old female mice are summarized in Table A-4. Based on the results from four continuous models, the Hill and Power models provide good data fit with goodness-of-fit *p*-values of 0.21 and 0.16, respectively. The Hill model utilizes more of the data than the Power model while achieving a slightly better fit. Therefore, the estimated BMD and BMDL obtained from the Hill model were considered the best estimated values. Thus, for rearing habituation in 8-month-old female mice, the estimated $BMD_{1.0SD}$ is 0.42 mg/kg, and $BMDL_{1.0SD}$ is 0.32 mg/kg.

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

Model	# Groups	AIC	<i>p</i> -Value	BMD 1 SD	BMDL 1 SD
Hill	5	110	0.21	0.42	0.32
Linear	4	100	<0.01	1.9	0.56
Polynomial	4	95	<0.01	0.34	0.093
Power	4	63	0.16	0.41	0.29

Source: Viberg et al. (2004a).

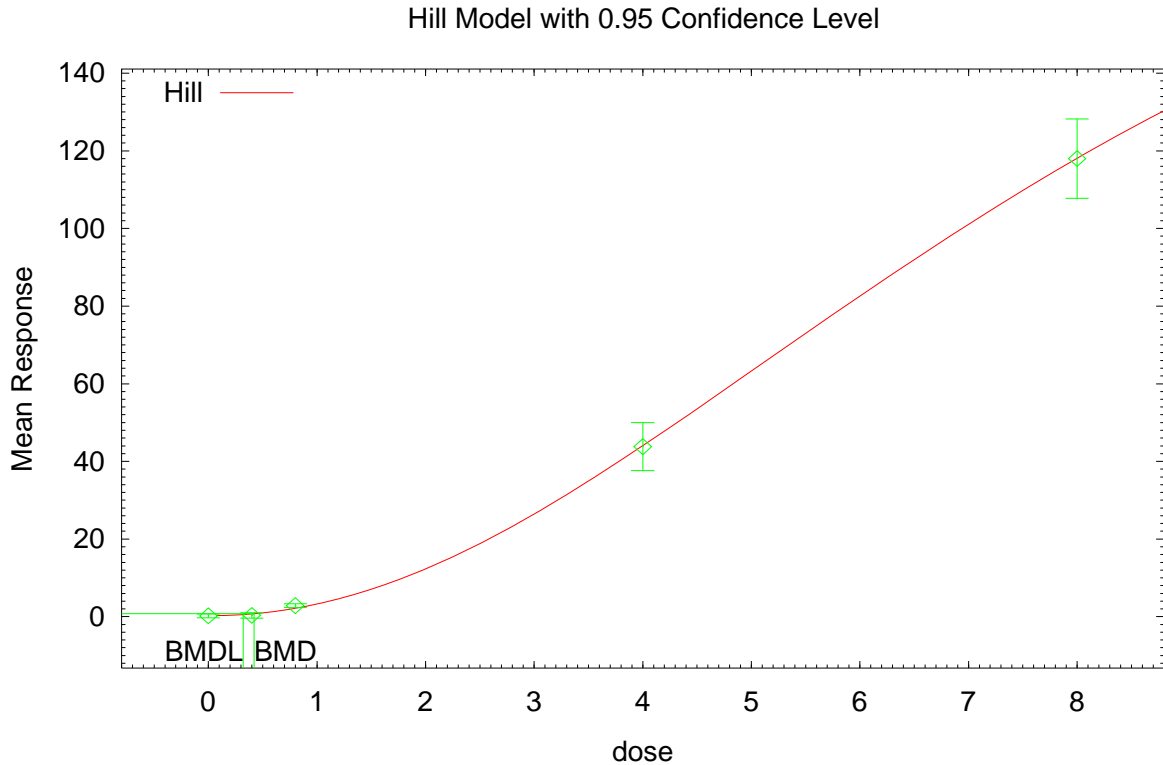
The BMD results are summarized in Table A-5. Based on these BMD results, the lowest BMD_{1SD} and $BMDL_{1SD}$ were from rearing habituation response in 8-month-old female mice, and the corresponding values were 0.42 and 0.32 mg/kg. The Hill model of five dose groups provided the best fit to the response in the 8-month-old female mice. Figure A-3 shows the Hill model fit to the rearing habituation data in the female mice. Detailed BMD_{10} model output file is presented below.

Table A-5. Summary of BMD and BMDL estimated for the responses reported in Viberg et al. (2004a)

			BMD 1 SD	BMDL 1 SD
Rearing	2-month-old	male mice	0.63	0.48
Rearing	8-month-old	male mice	---	---
Rearing	2-month-old	female mice	0.70	0.47
Rearing	8-month-old	female mice	0.42	0.32

Source: Viberg et al. (2004a).

Figure A-3. Rearing habituation in 8-month-old female mice.



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Omitting 16 mg/kg group

BMR = 1.0 SD

```

=====
Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
Input Data File: S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\VFR.(d)
Gnuplot Plotting File: S:\PROJECT FILES\EPA DECABDE\DBDE2\TETRA PENTA
BMD\VFR.plt

```

Fri Aug 05 10:26:50 2005

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=====
BMDS MODEL RUN
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```

The form of the response function is:

$$Y[\text{dose}] = \text{intercept} + v \cdot \text{dose}^n / (k^n + \text{dose}^n)$$

Dependent variable = MEAN

Independent variable = mg/kg

Power parameter restricted to be greater than 1

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

Total number of dose groups = 5

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      =    0.959908
rho        =    0.960063
intercept  =    0.22
v          =   117.78
n          =    1.34964
k          =    1.1747

```

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	intercept	v	n	k
alpha	1	-0.67	-0.6	-0.092	0.14	-0.1
rho	-0.67	1	0.53	-0.0029	0.0015	0.0049
intercept	-0.6	0.53	1	-0.098	0.15	-0.098

v	-0.092	-0.0029	-0.098	1	-0.85	0.99
n	0.14	0.0015	0.15	-0.85	1	-0.89
k	-0.1	0.0049	-0.098	0.99	-0.89	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.931131	0.322884
rho	1.01435	0.105187
intercept	0.276832	0.123538
v	249.72	58.7711
n	2.06997	0.15741
k	8.45509	1.75311

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	0.277	0.503	-0.113
0.4	8	0.33	0.83	0.727	0.821	-0.484
0.8	8	2.83	0.56	2.16	1.43	0.471
4	8	43.8	7.4	44	6.58	-0.034
8	8	118	12.2	118	10.8	0.000482

Model Descriptions for likelihoods calculated

Model A1: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma^2$

Model A2: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \alpha * (\mu(i))^\rho$

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-91.598335	6	195.196671
A2	-43.269145	10	106.538289
A3	-49.119530	7	112.239060
fitted	-49.904695	6	111.809390
R	-173.638912	2	351.277825

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)	Test df	p-value
Test 1	260.74	8	<.0001
Test 2	96.6584	4	<.0001
Test 3	11.7008	3	0.008482
Test 4	1.57033	1	0.2102

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 = 0.421873

BMDL = 0.321204

5. Locomotion habituation in treated male and female mice

No satisfactory data fits were obtained for locomotion data in either male or female mice (i.e., all the goodness-of-fit p -values are smaller than 0.1) when all six dose groups or truncated dose groups were used in BMD modeling with model parameter restriction. Thus, BMD results for this endpoint were not summarized.

B. Eriksson et al. (2001)

Locomotion, rearing, and total activity habituation in male mice were modeled using the linear, polynomial, and power continuous dose-response models. The Hill model was not used to model these data because this model requires at least five data points and Eriksson et al. (2001) reported only three dose groups.

Similar to the Viberg et al. (2004a) data modeling, the test for equal variances across dose groups failed for all data sets modeled, and so the standard deviation was modeled as a power function of the mean. The variance model failed to adequately predict the observed variances in some cases, but no alternative variance model is available in the latest version of BMDS. Since the variance modeled as a power function of the mean provides a much better estimate of the control variance upon which the BMR is based, all the modeling runs were conducted with modeled variance.

As mentioned for Viberg et al. (2004a) data modeling, restrictions were placed on the model parameters to avoid biologically unreasonable predictions. The coefficients of the polynomial model were restricted to be nonnegative, and the power in the power model was restricted to be at least 1. These restrictions were applied to the parameter estimates to prevent a wavy curve appearance for polynomial model or supralinear curves with very sharp initial increases that gradually leveled for the power model. The supralinear dose-response shape was not considered to be plausible, given the responses observed in the Viberg et al. (2004a) study that implemented tighter dose spacing (i.e., more data points in the low dose range).

Results

All of the continuous models failed to provide an adequate fit to the mean responses for any endpoint in the Eriksson et al. (2001) study. The linear model did not provide adequate data fit for any of the endpoints. Although the plots of the model fits to the 2-month rearing data appear good, using the polynomial and power models, there are too many parameters in these models to compute a p -value for goodness-of-fit to three data points. Also note that the Viberg et al. (2004a) study observed very little or no response above control at 0.4 mg/kg, but the Eriksson

et al. (2001) study did not test 0.4 mg/kg, while it showed a response at 0.8 and 12 mg/kg. The Eriksson et al. (2001) study likely missed the initial flat portion of the dose-response curve for this endpoint that was observed by Viberg et al. (2004a), leading to nearly linear fits through the data points using the polynomial and power models. Because no adequate data fit was achieved with available continuous models in the BMDS, no BMD or BMDL was estimated for the responses reported in this study.

C. Kuriyama et al. (2005)

Kuriyama et al. (2005) exposed rat dams to a single dose of polybrominated diphenyl ethers (PBDE) on gestational day 6 (at dose level of 0, 60 $\mu\text{g}/\text{kg}$, or 300 $\mu\text{g}/\text{kg}$) and observed the response in the male offspring. Though this design is nested, the data are not presented in a form amenable to nested modeling. Therefore, the data were modeled by using the continuous and quantal models in BMDS.

The continuous effects modeled were locomotor activity at postnatal day (PND) 36 and 71 (i.e., light beam interruption [LBI] counts per day, duration of activity [hours] per day, LBI counts per active phase, and duration of activity [minutes] per active phase), and spermatid, daily sperm production, and sperm number at PND 140. Other endpoints assessed in the study were not evaluated using BMD modeling because inspection of the data revealed no clear dose response.

For continuous data BMD modeling, the test for equal variances across dose groups failed for the locomotor activity data, and so the variance was modeled as a power function of the mean. The variance model failed to adequately predict the observed variances in some cases, but no alternative variance model is available in latest version of BMDS. Since the variance modeled as a power function of the mean provides much better estimate of the control variance upon which the BMR is based, all the modeling runs were conducted with modeled variance. A homogeneous variance model was appropriate for sperm measurement data; therefore, a constant variance was used in the modeling.

Similar to the BMD modeling for Viberg et al. (2004a) data, restrictions were placed on the continuous model parameters to avoid biologically unreasonable predictions. The coefficients of the polynomial model was restricted to be nonnegative, and the power in the power model was restricted to be at least 1. These restrictions were applied to the parameter estimates to prevent decreasing curves at low dose, which were not considered to be plausible for locomotion response for the polynomial model and supralinear curve for the power model.

The quantal data were observations of the percent of adult animals with two or more ejaculations. The quantal data had to be converted such that increased responses were adverse for

modeling with the quantal BMDS models (i.e., the percent of adult animals with less than two ejaculations was modeled).

Results

1. Continuous locomotion and reproductive effects

The continuous effects modeled were locomotor activity at PND 36 and 71 (i.e., LBI counts per day, duration per day, LBI counts per phase, and duration per phase [these data were presented in the published manuscript in graphical format, but raw data were obtained from the authors]) and spermatid, daily sperm production, and sperm number at PND 140. Among all the continuous endpoints, only duration per day and LBI counts per phase on PND 36 were adequately modeled with linear and polynomial models, and the results are summarized in Table A-6. None of the continuous models provided an adequate fit to the mean responses for any other continuous endpoints.

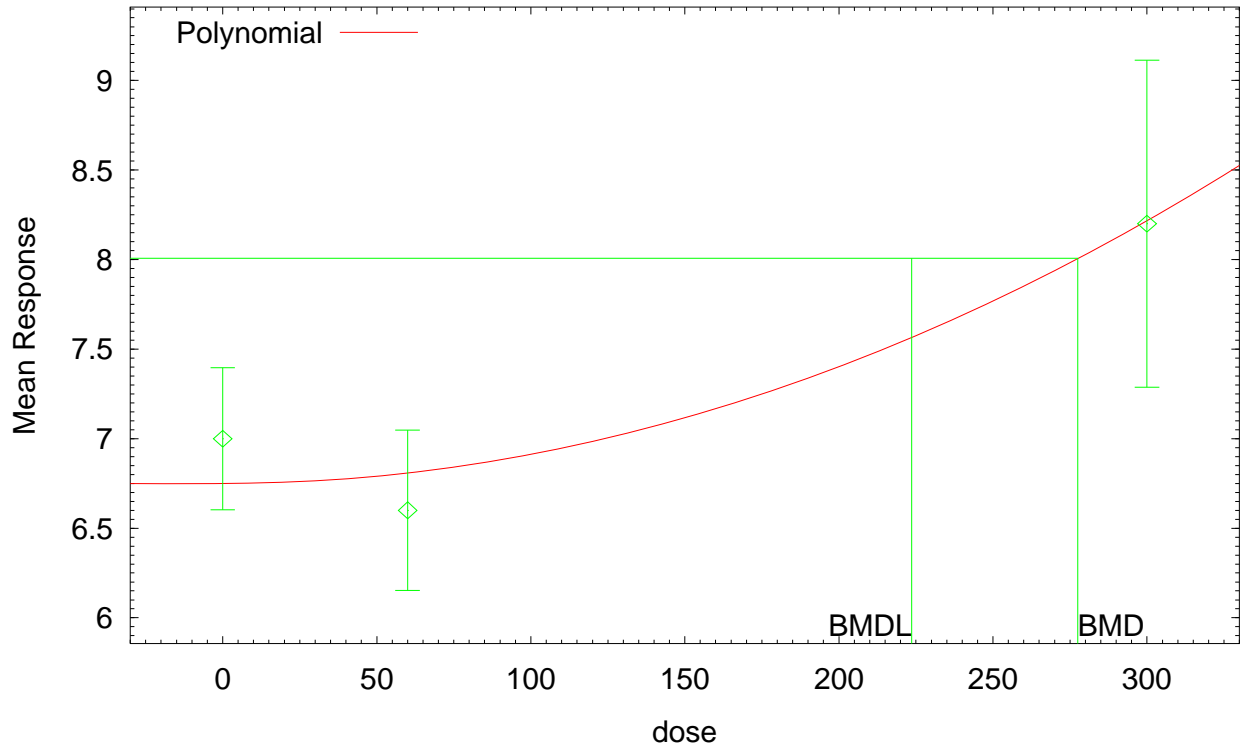
For duration per day, the Polynomial model provided much better data fit; therefore, the BMD estimates from this model were used. The estimated BMD_{1SD} and $BMDL_{1SD}$ for duration per day are 0.28 and 0.22 mg/kg, respectively. For LBI counts per phase, only the linear model provided satisfactory data fit. The estimated BMD_{1SD} and $BMDL_{1SD}$ for this endpoint are 0.16 and 0.11 mg/kg, respectively.

Table A-6. Duration per day and LBI counts per phase on PND 36

Endpoint	Model	# Groups	AIC	p-Value	BMD 1 SD	BMDL 1 SD
Duration per day	Linear	3	196	0.15	0.25	0.17
Duration per day	Polynomial	3	194	0.67	0.28	0.22
LBI counts per phase	Linear	3	415	0.92	0.16	0.11

Source: Kuriyama et al. (2005).

Polynomial Model with 0.95 Confidence Level



09:32 08/08 2005

Figure A-4. Light beam interruption counts per active phase on PND 36.

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:

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

Dependent variable = MEAN

Independent variable = dose

The polynomial coefficients are restricted to be positive

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

Total number of dose groups = 3

Total number of records with missing values = 0

Maximum number of iterations = 250

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

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

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

Model A2:  $Y_{ij} = \mu(i) + e(ij)$   
 $\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3:  $Y_{ij} = \mu(i) + e(ij)$   
 $\text{Var}\{e(ij)\} = \alpha * (\mu(i))^\rho$

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

Likelihoods of Interest

| Model  | Log(likelihood) | d.f. | AIC        |
|--------|-----------------|------|------------|
| A1     | -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   | $-2 * \log(\text{Likelihood Ratio})$ | 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

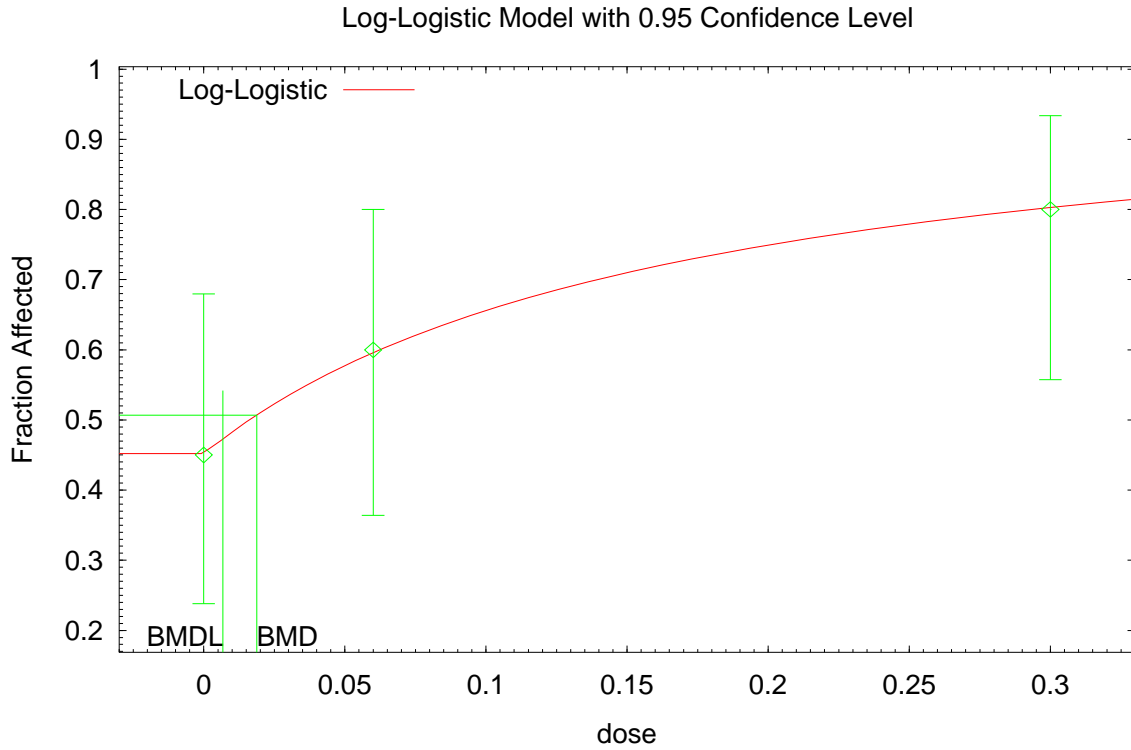
BMDL = 223.634

## 2. Quantal reproductive effects

The percent of adult animals with less than two ejaculations was modeled with all the available dichotomous models available in the current BMDS (1.3.2) with a BMR of 10% extra risk. All the doses were converted from  $\mu\text{g}/\text{kg}$  to  $\text{mg}/\text{kg}$ . Table A-7 summarizes the BMD results for percent of adult offspring with less than two ejaculations. All of the dichotomous models provided a satisfactory fit to the data ( $p > 0.4$ ). The best-fitting model is the log-logistic model with the highest  $p$ -value of 0.96, indicating a good global data fit and the lowest AIC (see Figure A-5). In addition, this model also produced a lowest chi-square residual at 0.06  $\text{mg}/\text{kg}$ , indicating the best fit in the region of the BMR. Therefore, based on results from this model, the estimated BMD is 0.019  $\text{mg}/\text{kg}$  and the BMDL is 0.0067  $\text{mg}/\text{kg}$ . (Note that this corresponds to the dose administered to the dam).

**Table A-7. Summary of dose-response modeling of the percent of animals with less than two ejaculations**

| Model               | AIC         | $p$ -Value  | Residual     | BMD          | BMDL          |
|---------------------|-------------|-------------|--------------|--------------|---------------|
| Gamma               | 78.6        | 0.72        | 0.28         | 0.031        | 0.016         |
| <b>Log-logistic</b> | <b>78.5</b> | <b>0.96</b> | <b>0.041</b> | <b>0.019</b> | <b>0.0067</b> |
| Logistic            | 78.7        | 0.63        | 0.38         | 0.041        | 0.025         |
| Log-probit          | 78.9        | 0.52        | 0.50         | 0.058        | 0.027         |
| Probit              | 78.7        | 0.61        | 0.39         | 0.043        | 0.027         |
| Multistage          | 78.6        | 0.72        | 0.13         | 0.031        | 0.016         |
| Quantal linear      | 78.6        | 0.72        | 0.28         | 0.031        | 0.016         |
| Quantal quadratic   | 79.2        | 0.40        | 0.62         | 0.10         | 0.071         |
| Weibull             | 78.6        | 0.72        | 0.28         | 0.031        | 0.016         |



**Figure A-5. Log-logistic model fit to the percent of animals with less than two ejaculations.**