EPA/635/R-07/008F
www.epa.gov/iris

TOXICOLOGICAL REVIEW

OF

DECABROMODIPHENYL ETHER (BDE-209)

(CAS No. 1163-19-5)

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

June 2008

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

CONTENTS—TOXICOLOGICAL REVIEW OF

2,2',3,3',4,4',5,5',6,6'-DECABROMODIPHENYL ETHER (CAS No. 1163-19-5)

LIST OF TABLES v
LIST OF FIGURES vi
LIST OF ACRONYMS vii
FOREWORD ix
AUTHORS, CONTRIBUTORS, AND REVIEWERS x

1. INTRODUCTION 1

2. CHEMICAL AND PHYSICAL INFORMATION 3

3. TOXICOKINETICS 4
   3.1. ABSORPTION 4
      3.1.1. Studies in Humans 4
      3.1.2. Studies in Animals 4
   3.2. DISTRIBUTION 6
      3.2.1. Studies in Humans 6
         3.2.1.1 Data in Human Milk 7
         3.2.1.2. Data in Human Blood 7
      3.2.2. Studies in Animals 9
   3.3. METABOLISM 12
   3.4. ELIMINATION 15
      3.4.1. Half-life Estimates 17
   3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS 18

4. HAZARD IDENTIFICATION 19
   4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL TRIALS 19
   4.2. SHORT-TERM, SUBCHRONIC, AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION 19
      4.2.1. Short-term and Subchronic Studies 19
         4.2.1.1. Studies in Rats 19
         4.2.1.2. Studies in Mice 22
      4.2.2. Chronic Studies and Cancer Bioassays 22
         4.2.2.1. Study in Rats 23
         4.2.2.2. Study in Mice 26
   4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION 28
      4.3.1. Studies in Rats 28
      4.3.2. Studies in Mice 31
   4.4. OTHER ENDPOINT-SPECIFIC STUDIES 35
      4.4.1. Receptor Site Interactions 35
         4.4.1.1. Aryl Hydrocarbon Receptor Studies 35
         4.4.1.2. Estrogen Receptor Studies 37
         4.4.1.3. Other Receptors 37

4.4.2. Immunological Studies ... 37
4.4.3. Genotoxicity ... 38
4.5. SYNTHESIS OF MAJOR NONCANCER EFFECTS ... 39
4.5.1. Oral ... 39
4.5.2. Inhalation ... 40
4.5.3. Mode-of-Action Information ... 40
4.6. EVALUATION OF CARCINOGENICITY ... 43
4.6.1. Summary of Overall Weight of Evidence ... 43
4.6.2. Synthesis of Human, Animal, and Other Supporting Evidence ... 44
4.6.3. Mode-of-Action Information ... 44
4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES ... 48
4.7.1. Possible Childhood Susceptibility ... 48
4.7.2. Possible Gender Differences ... 48

5. DOSE-RESPONSE ASSESSMENTS ... 50
5.1. ORAL REFERENCE DOSE (RfD) ... 50
5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification ... 50
5.1.2. Methods of Analysis ... 54
5.1.2.1. Benchmark Dose Modeling ... 54
5.1.3. RfD Derivation ... 55
5.1.4. Previous RfD Assessment ... 58
5.2. INHALATION REFERENCE CONCENTRATION (RfC) ... 58
5.3. CANCER ASSESSMENT ... 58
5.3.1. Choice of Study/Data—with Rationale and Justification ... 58
5.3.2. Dose Adjustments ... 59
5.3.3. Extrapolation Method(s) ... 60
5.3.4. Oral Slope Factor ... 62
5.3.5. Previous Cancer Assessment ... 63

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE ... 64
6.1. HUMAN HAZARD POTENTIAL ... 64
6.2. DOSE RESPONSE ... 65
6.2.1. Noncancer/Oral ... 65
6.2.2. Cancer/Oral ... 66

7. REFERENCES ... 67

APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION ... A-1

APPENDIX B: BENCHMARK DOSE ANALYSIS OF NONCANCER ENDPOINTS ... B-1

APPENDIX C: BENCHMARK DOSE ANALYSIS OF CANCER ENDPOINTS ... C-1

LIST OF TABLES

Table 2-1. Physical properties and chemical identity of decaBDE 3

Table 4-1. Oral short-term and subchronic toxicity studies for decaBDE in laboratory animals 20

Table 4-2. Oral chronic toxicity studies for decaBDE in laboratory animals 24

Table 4-3. Oral developmental and reproductive studies for decaBDE in laboratory animals 29

Table 4-4. Incidence of liver neoplastic nodules and carcinomas in rats fed decaBDE for 2 years 46

Table 4-5. Incidence of nonneoplastic or neoplastic lesions in the liver and thyroid gland of mice fed decaBDE for 2 years 47

Table 5-1. BMDs for potential critical effect from chronic rat and mouse studies 55

Table 5-2. Cancer slope factors derived from BMDs for neoplastic effect from chronic rat and mouse studies using the multistage model 61

Table B-1. Summary of BMD modeling results for thrombosis in the liver of male rats B-2

Table B-2. Summary of BMD modeling results for degeneration in the liver of male rats B-2

Table B-3. Summary of BMD modeling results for fibrosis in the spleen of male rats B-6

Table B-4. Summary of BMD modeling results for lymphoid hyperplasia in male rats B-7

Table B-5. Summary of BMD modeling results for centrilobular hypertrophy in the liver of male mice B-7

Table B-6. Summary of BMD modeling results for follicular cell hyperplasia in the thyroid of male mice B-8

Table C-1. Summary of BMD modeling results for increase in neoplastic nodules in the liver of male rats C-1

Table C-2. Summary of BMD modeling results for increases in neoplastic nodules or carcinomas (combined) in the liver of male rats C-4

Table C-3. Summary of BMD modeling results for increases in neoplastic nodules in the liver of female rats C-7

Table C-4. Summary of BMD modeling results for increases in neoplastic nodules

and carcinomas (combined) in the liver of female rats C-10

Table C-5. Summary of BMD modeling results for increases in thyroid follicular cell hyperplasia as a key event of thyroid tumor in male mice C-13

Table C-6. Summary of BMD modeling results for increases in combined hepatocellular adenomas or carcinomas in male mice C-16

LIST OF FIGURES

Figure 3-1. Proposed metabolic pathway for BDE-209. 13

LIST OF ACRONYMS

AhR	aryl hydrocarbon receptor
AIC	Akaike Information Criterion
ALH	amplitude of the lateral head displacement
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BDE-209	decabromodiphenyl ether
bDNA	branched deoxyribonucleic acid
BMD	benchmark dose
BMDL	BMD 95% lower bound
BMDS	benchmark dose software
BMR	benchmark response
CALUX	Chemical-Activated LUciferase eXpression
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service Registry Number
CD25	interleukin-2-receptor α chain
cDNA	complementary DNA
$\mathbf{C_{max}}$	maximum concentration
CYP-450	cytochrome P-450
decaBDE	decabromodiphenyl ether
DRE	dioxin-responsive element
E2	17β-estradiol
ED	effective dose
ER	estrogen receptor
EROD	ethoxyresorufin O-deethylase
FOB	functional observational battery
fw	fresh weight
GC/MS	gas chromatography/mass spectrometry
HED	human equivalent dose
hexaBDE	hexabromodiphenyl ether
i.v.	intravenous
IRIS	Integrated Risk Information System
LED	95% lower confidence limit for the effective dose
LOAEL	lowest-observed-adverse-effect level
lw	lipid weight
MMP	mitochondrial membrane potential
MRL	minimal risk level
mRNA	messenger ribonucleic acid
NAS	National Academy of Science
NOAEL	no-observed-adverse-effect level
nonaBDE	nonabromodiphenyl ether
NTP	National Toxicology Program

octaBDE	octabromodiphenyl ether
PBDE	polybrominated diphenyl ether
PBPK	physiologically based pharmacokinetic
PCB	polychlorinated biphenyl
pentaBDE	pentabromodiphenyl ether
PND	postnatal day
PROD	pentoxyresorufin O-deethylase
PXR	pregnane X receptor
RfC	reference concentration
RfD	reference dose
RSD	risk-specific dose
SXR	steroid X receptor
T_3	triiodothyronine
T_4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
tetraBDE	tetrabromodiphenyl ether
triBDE	tribromodiphenyl ether
TSH	thyroid stimulating hormone
UDPGT	uridine diphosphate glucuronyl transferase
UF	uncertainty factor

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to decabromodiphenyl ether. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of decabromodiphenyl ether (BDE-209).

This health assessment deals with BDE-209 of relatively high purity (≥94%) and does not deal with earlier commercial decabromodiphenyl ether mixtures containing lower proportions of decabromodiphenyl ether (e.g., 75% purity). In addition to BDE-209, IRIS health assessments have also been prepared for three other polybrominated diphenyl ether congeners: tetraBDE-47, pentaBDE-99, and hexaBDE-153. These four congeners are those for which toxicological studies suitable for dose-response assessments were available and are the ones most commonly found in the environment and human biological media.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclulsions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

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

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

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

AUTHORS

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

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

Qiyu (Jay) Zhao, Ph.D.
Toxicology Excellence for Risk Assessment
Cincinnati, OH

Bernard Gadagbui, Ph.D.
Toxicology Excellence for Risk Assessment
Cincinnati, OH

Andrew Maier, Ph.D., CIH, DABT
Toxicology Excellence for Risk Assessment
Cincinnati, OH

Toxicology Excellence for Risk Assessment staff performed work under Purchase Order 4W-1550-NATX.

OFFICE OF RESEARCH AND DEVELOPMENT CO-LEAD

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

REVIEWERS

This document has been reviewed by EPA scientists, interagency reviewers from other federal agencies, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the Toxicological Review of 2,2',3,3',4,4',5,5',6,6'-Decabromodiphenyl ether (BDE-209).

INTERNAL EPA REVIEWERS

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

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

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

EXTERNAL PEER REVIEWERS

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

Richard J. Bull, Ph.D.
MoBull Consulting

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

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

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

1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE-209). IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

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

Development of these hazard identification and dose-response assessments for BDE-209 has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures (U.S. EPA, 1986a), Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity*

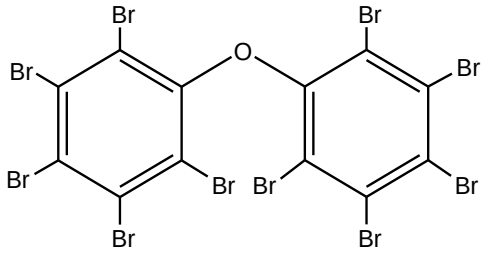
Risk Assessment (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through November 2007.

2. CHEMICAL AND PHYSICAL INFORMATION

Decabromodiphenyl ether (decaBDE or BDE-209) is a fully brominated diphenyl ether compound (i.e., 10 bromine atoms) used as a flame retardant. The composition of commercial decaBDE is almost exclusively the deca-substituted BDE-209 (typically ≥97%), the remainder being nonabromodiphenyl ether (nonaBDE); trace amounts of octabromodiphenyl ether (octaBDE) may be present (Schecter et al., 2003; American Chemistry Council, 2002; Hardy, 2002). The composition of older decaBDE formulations, which are no longer commercially produced in the U.S., was approximately 77% decaBDE, 22% nonaBDE, and 1% octaBDE (Kociba et al., 1975). Physical and chemical properties of decaBDE (≥97% purity) are listed in Table 2-1.

Table 2-1. Physical properties and chemical identity of decaBDE

	Physical property/chemical identity	Reference
CASRN	1163-19-5	U.S. EPA (2004)
Synonyms	2,2',3,3',4,4',5,5',6,6'-decaBDE; BDE-209; decaBDE; benzene, 1,1'-oxybis[2,3,4,5,6,-pentabromo]-; decabromodiphenyl oxide; decabromodiphenyl ether; decabromobiphenyl ether; ether, bis(pentabromophenyl)	U.S. EPA (2004); ATSDR[a] (2004)
Physical state	Solid	Hardy (2002)
Melting point, °C	300–310	ECB[a] (2003)
Boiling point, °C	decomposes at >320°C	ECB (2003)
Vapor pressure at 21°C, Pa	4.63×10^{-6}	Hardy (2002)
Henry's law constant: atm m^3/mol (Pa m^3 mol^{-1}) at 25°C	 1.93×10^{-8} 0.04	Hardy (2002); Cetin and Odabasi (2005)
Density, g/cm^3	3.0	NAS[a] (2000)
Water solubility at 25°C, µg/L	<0.1	Hardy (2002)
Log K_{ow}	6.3–12.6	Hardy (2002)
Log K_{oc}	6.3	Hardy (2002)
Molecular weight	959.17	U.S. EPA (2004)
Chemical formula	$C_{12}Br_{10}O$	U.S. EPA (2004)
Chemical structure		

[a]ATSDR = Agency for Toxic Substances and Disease Registry; ECB = European Chemicals Bureau; NAS = National Academy of Science.

3. TOXICOKINETICS

Data on the toxicokinetics of decaBDE in humans are limited to findings on the levels in serum and maternal milk that demonstrate it is absorbed from the environment and distributed to tissues. Several studies have been conducted to evaluate the absorption, distribution, metabolism, and elimination of decaBDE after oral or intravenous (i.v.) dosing in rats and mice. Absorption is low (7–26%) after oral exposure to rats. Following absorption, the highest levels are found in the liver, muscle, and skin. This differs from studies of the lower brominated congeners, where the highest levels are generally found in the adipose tissues. The available metabolic data apply exclusively to rats, where both debrominated and hydroxylated-debrominated species have been identified in plasma. In one study, the metabolites in the feces exceeded those that could be accounted for from bile, raising the possibility that some conversion of the parent compound may be mediated by the intestinal epithelium or microflora. This phenomenon was not reported for the lower brominated BDE congeners. Urinary excretion of decaBDE-209 is minimal in rats; no data are available for mice. In rats, some of the urinary radiolabel (18%) was bound to albumin.

3.1. ABSORPTION

3.1.1. Studies in Humans

There are no direct studies of decaBDE absorption in humans. The data that demonstrate human absorption come from measurements of decaBDE in human biological media after anthropogenic exposures but do not provide information on the quantitative aspects of absorption or the kinetics of tissue distribution and retention.

3.1.2. Studies in Animals

Studies on BDE-209 absorption following oral dosing demonstrate its absorption potential. However, there were few direct measurements of absorbed dose, and absorption estimates are based on a combination of concentrations in blood and data on excretion. Among the several oral dosing studies, the percentage of an administered dose absorbed across the gastrointestinal tract ranged from approximately 7–26%. In some cases it was difficult to derive an accurate estimate of absorption because of the high proportion of the dose found in the feces (>90%) and the high percentage that was present as metabolites in feces.

Sandholm et al. (2003) evaluated the bioavailability of unlabeled decaBDE in male Sprague-Dawley rats. One group of rats (n = 18) was dosed by gavage with unlabeled decaBDE (>98% purity) in dimethylamide/polyethylene glycol/water vehicle at 2 µmol/kg (1.9 mg/kg). Another group of rats (n = 18) was dosed intravenously via the tail vein with unlabeled decaBDE

at the same dose. At specific time intervals, blood samples from three rats per group were collected according to the following schedule: group 1 was sampled 1 hour and 24 hours after dosing; group 2 after 3 hours and 48 hours; group 3 after 6 hours and 72 hours; group 4 after 96 hours; group 5 after 120 hours; and group 6 after 144 hours. Plasma samples were extracted, and decaBDE and its metabolites were quantified. Based on comparison to plasma levels (area under the curve [AUC]) following i.v. injections, the oral bioavailability (the percent of the dose reaching systemic circulation) was calculated to be 26% in the rat. The mean maximum plasma concentration (C_{max}) following oral dosing was 264 pmol/mL in the 6-hour sample.

One of the limitations of the absorption data for decaBDE is a lack of knowledge about the mechanism of gastrointestinal absorption. Passive diffusion across the lipid membrane appears to be restricted to lipophilic compounds with low molecular weight rather than high molecular weight compounds such as decaBDE. Accordingly, facilitated transport and/or uptake with dietary lipids by way of the chylomicrons may provide routes for absorption.

In a gavage study by Morck et al. (2003), male Sprague-Dawley rats (n = 8) received 3 µmol/kg (2.9 mg/kg) ^{14}C-labeled decaBDE diluted with unlabeled compound (>98% purity, 15 Ci/mol), prepared by dissolving the decaBDE labeled/unlabeled mixture in toluene, followed by sonication, suspension in Lutrol F127/soy phospholipone/water vehicle, and evaporation of the toluene by nitrogen flow. Four rats were sacrificed after 3 days and the other four rats after 7 days. Urine and feces were collected at 24-hour intervals for 3 and 7 days, respectively, and assayed for radioactivity. Two additional male Sprague-Dawley rats were bile-duct-cannulated, treated similarly with ^{14}C-labeled decaBDE, and sacrificed 3 days later. Bile from these rats was collected and assayed for radioactivity.

Results indicated that in the conventional rats, about 90% of the dose was excreted in the feces within 3 days after a single oral dose of ^{14}C-labeled decaBDE, and the majority of this radioactivity (65%) represented decaBDE metabolites. Radioactivity in bile collected from the cannulated rats accounted for 10% of the fecal excretion. Almost all of the excreted radioactivity in the bile represented metabolites, indicating that at least 10% of the decaBDE dose had been absorbed. It is also possible that greater than 10% of the oral dose may have been absorbed since 65% of the radioactivity excreted in the feces was in the form of metabolites. However, interpretation of the data is difficult. The relatively large amount of metabolites excreted in the feces could be due to a combination of biliary excretion of metabolites, metabolism of decaBDE by the microflora in the gastrointestinal tract, metabolism in the intestinal epithelium, and/or nonbiliary systemic secretion into the gut.

Hughes et al. (2001) conducted a study to evaluate the in vitro dermal absorption of decaBDE in mice. In this study, the dorsal skin of female hairless (Crl:SKH1-hr–BR) mice was removed, cut to a thickness of 255 µm, and exposed in a flow-through diffusion cell system to carrier-free ^{14}C-decaBDE (>98% purity; specific activity not provided) at doses of 6, 30, or

60 nmol. The percent dose absorbed as measured by the amount of material in the collecting chamber was determined at 6, 12, 18, and 24 hours. Very little compound passed through the skin sections; the percentages of dose reaching the receptor fluid in the collecting chamber after 24 hours ranged from 0.07–0.34% and were inversely related to dose.

For all three doses used in the experiment, the largest portion of the dose was taken up during the first 6 hours: 0.17%, 0.04%, and 0.03% of the applied 6, 30, and 60 nmol decaBDE doses, respectively. Results also showed that the 24-hour cumulative percent of the dose absorbed decreased with increasing applied dose, whereas the mass of chemical absorbed increased with increasing applied dose. This result suggests saturation of uptake at the higher doses. The authors calculated the total for the dose retained in the skin sections and transported to the receptor fluid as 20.5%, 3.3%, and 1.9% of the applied dose for 6, 30, and 60 nmol decaBDE, respectively. Most of the compound taken up in the 24-hour period was retained in the skin. The authors acknowledged that the in vitro results observed in this study using mouse skin may overestimate the amount of decaBDE that would be absorbed by human skin, given that the mouse skin is more permeable to several chemicals, at least in vitro, than rat, pig, or human skin.

3.2. DISTRIBUTION

BDE-209 has very low water solubility and a relatively high K_{ow}. Accordingly, high distribution to adipose tissue might be expected. However, as indicated by the data that follow, that is not the case. Systemic distribution of hydrophilic metabolites, as well as molecular mass and favored conformation, may play a role in the limited uptake by adipocytes. The low uptake of decaBDE into tissue lipids makes it different from the less highly substituted, lower molecular weight polybrominated diphenyl ethers (PBDEs).

3.2.1. Studies in Humans

The human data described below come from monitoring of PBDEs in human populations rather than from measured dosing studies. The data demonstrate that humans are exposed to PBDEs and that absorption and distribution to some tissues occur. The data do not provide information on the quantitative aspects of absorption or the kinetics of tissue retention. Limited data are available on the occurrence of BDE-209 in human biological media. Data, described below, are available for human milk and blood samples and indicate a tendency for BDE-209 to distribute to these tissues. However, thorough distribution studies have not been conducted in humans, and therefore it is not known whether BDE-209 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.

3.2.1.1 *Data in Human Milk*

In a study conducted in 2002 of levels of PBDEs in human milk in the U.S., 47 samples from Caucasian, African-American, and Hispanic nursing mothers 20–41 years of age and living in Texas were analyzed for 13 PBDE congeners (Schecter et al., 2003). Mean and median total concentrations of tribromodiphenyl ether (triBDE) through decaBDE were 74 and 34 ng/g lipid weight (lw). DecaBDE was present in these samples, with a mean concentration of 0.9 ng/g lw (1.2% of total PBDEs in the milk; median concentration not available), suggesting that some decaBDE is absorbed, distributed to mammary tissue, and secreted in human milk during lactation.

Milk samples were collected in 2003 from 40 first-time mothers with 8-week-old infants and residing in urban areas in the Pacific Northwest of the U.S. (Montana, Oregon, and Washington State) and British Columbia, Canada (She et al., 2007). Mean and median total concentrations of 12 tri- through decaBDE congeners were 96 and 50 ng/g lw, respectively. These values are substantially higher than the values reported in the study of Schecter et al. (2003) and could be due to the fact that the mothers in the later study had been nursing for longer periods of time. BDE-47 was found at the highest level, followed by hexabromodiphenyl ether (hexaBDE)-153 and pentabromodiphenyl ether (pentaBDE)-99 and -100. DecaBDE-209 with mean and median concentrations of 0.8 and 0.4 ng/g lw, respectively, was a minor congener in breast milk.

3.2.1.2. *Data in Human Blood*

Sjodin et al. (2001a) reported a finding of BDE-209 in serum samples collected from 12 U.S. blood donors in 1988. The median concentration was <1 pmol/g lw with a range of <1–35 pmol/g lw (approximately <1–35 ng/g lw). These concentrations of BDE-209 were comparable to blood levels collected 10 years later, in 1997, from non-occupationally exposed Swedish female cleaners (Sjodin et al., 1999). There is no demographic or questionnaire information on the donor; therefore, no information is available for assessing exposure sources.

Concentrations of tetrabromodiphenyl ether (tetraBDE) and penta-, hexa-, and decaBDE congeners were measured in serum samples collected during 2004 from a family residing in Berkeley, California (35- and 36-year-old father and mother, respectively, 5-year-old daughter, and 18-month-old son) (Fischer et al., 2006). The 18-month-old was exclusively breast-fed for 6 months and was breast-feeding during the study period. PBDE levels for the sum of the five lower brominated congeners BDE-47, -99, -100, -153, and -154 were much higher in the infant (418 ng/g lw) and child (247 ng/g lw) than in their parents (mother 106 ng/g lw, father 64 ng/g lw). BDE-47 was the predominant congener for all ages, followed by hexaBDE-153, pentaBDE-100, pentaBDE-99, and hexaBDE-154. Levels of BDE-209 in the infant (233 ng/g lw) and child (143 ng/g lw) were unusually high compared with those in the parents (mother

14 ng/g lw, father 23 ng/g lw). The authors suspected house dust and breast milk to contribute appreciably to the child and infant exposures; however, no firm conclusions can be drawn from this study, given the small number of subjects investigated.

Sjodin et al. (1999) investigated the exposure to PBDEs of Swedish workers by comparing blood PBDE concentrations collected in 1997 from personnel at an electronics dismantling plant, clerks working full time in front of computer screens, and a control group of hospital cleaning workers. Electronics dismantling involved grinding plastic goods in a shredder. This process releases airborne particulate matter from plastic parts containing brominated flame retardants. The investigators found decaBDE in the serum of individuals from all three groups (19–20 male and female subjects per group). The median BDE-209 concentration in hospital cleaners was <0.7 pmol/g lw (<9.7 ng/g), with a range of ≤0.3–3.9 pmol/g lw (≤0.3–3.7 ng/g), while the median BDE-209 concentration in computer clerks was <0.7 pmol/g lw (<0.7 ng/g), with a range of ≤0.3–8.0 pmol/g lw (≤0.3–7.8 ng/g). Plasma levels of BDE-209 were significantly higher in the electronics dismantling workers than in the other two groups with a median BDE-209 concentration of 5.0 pmol/g lw (4.8 ng/g, range <0.3–9.9 pmol/g lw [<0.3–9.5 ng/g]). The higher BDE-209 concentration in the serum of the electronics dismantling workers was attributed to the relatively high total BDE-209 concentration in the air of the dismantling hall (mean 36 ng/m^3) and at the shredder (175 ng/m^3) (Sjodin et al., 2001b). The presence of BDE-209 in the blood samples from these three groups of workers qualitatively indicated the bioavailability of BDE-209 in humans, even though this compound is not expected to be greatly bioavailable based on its high molecular mass. There was no correlation between plasma levels of BDE-209 with age or fish consumption (the only food evaluated in the study). The serum concentrations of all PBDE congeners decreased in electronic-dismantling workers after vacation. The median decreases, standardized to 30 days of leave, were 14% for BDE-47, -153, and -154, 30% for BDE-183, and 66% for BDE-209. These results indicate shorter half-lives of the more highly brominated diphenyl ethers. However, serum half-lives were not estimated by the authors.

In another Swedish study, increased BDE-209 in the serum was also found in computer technicians who repair or partially dismantle computers (Jakobsson et al., 2002). The median BDE-209 concentration in groups of 19–20 subjects was 1.6 pmol/g lw (1.5 ng/g) in computer technicians, while it was <0.7 pmol/g lw (<0.7 ng/g) in hospital cleaners and computer clerks. A possible source of exposure is airborne dust particulate matter to which PBDEs strongly adsorb (Sjodin et al., 1999).

Thuresson et al. (2005) assessed the exposure to PBDEs in Swedish workers engaged in manufacturing decaBDE flame-retarded rubber goods or electric cables. A referent group, abattoir (slaughterhouse) workers with no occupational exposure to PBDEs, was also investigated. The commercial decaBDE used was Saytex 102E, consisting mainly of BDE-209

with traces of nonaBDEs (BDE-206, -207, and -208) and unknown octaBDE congeners. Consumption of fatty fish was low in all three groups (median 0.5 meals/month), age ranged between 24 and 60 years, with a median of 40 years in all three groups, and each group consisted of approximately 20 subjects. The concentration of 12 PBDE congeners, ranging from tetra- to decaBDE, was measured in the serum samples of all individuals participating in the study. Elevated serum concentrations of octa-, nona-, and decaBDE were present in serum of workers handling decaBDE flame-retarded rubber. Serum concentrations of BDE-209 were up to 50- to 100-fold higher than those of the referent group. In contrast, the serum concentrations of tetra- to heptaBDEs were similar among rubber workers and referents.

3.2.2. Studies in Animals

No studies were identified regarding distribution of decaBDE in animals after inhalation exposure. Several rodent oral dosing studies were identified and are described below. Sandholm et al. (2003) evaluated the distribution of unlabeled decaBDE in male Sprague-Dawley rats. A group of rats (n = 18) was dosed by gavage with unlabeled decaBDE (>98% purity) in dimethylamide/polyethylene glycol 400/water vehicle at 2 µmol/kg (1.9 mg/kg). Another group of rats (n = 18) was dosed intravenously with the same dose of decaBDE. At specific time intervals, blood samples from three rats per group were collected for up to 144 hours. Plasma samples were extracted, and decaBDE and its metabolites were quantified. Oral results indicated that the mean plasma C_{max} was 264 pmol/mL (253 ng/g) at 6 hours after dosing and AUC was 12 nmol × hour/mL (11.5 µg × hour/mL). The results from the i.v. study indicated that the clearance was 0.60 mg/minute-kg and the apparent volume of distribution at steady state was 1.4 L/kg. Concentrations in other tissues were not measured; therefore, relative tissue distribution could not be determined. However, the identification of decaBDE in the plasma supports other studies, suggesting wide tissue distribution.

Morck et al. (2003) evaluated the distribution of decaBDE (>98% purity) in male Sprague-Dawley rats after oral administration. Four rats were sacrificed 3 or 7 days after administration of 3 µmol/kg (2.9 mg/kg) of ^{14}C-labeled and unlabeled decaBDE combined (15 Ci/mol). Liver, adipose tissue, lung, kidney, adrenal glands, skin, muscle, spleen, testis, thymus, heart, plasma, colon wall and contents, and small intestine wall and contents were collected. Radioactivity was determined in all tissues. Analysis of tissues and organs for radioactivity indicated that approximately 9% of the dose remained in the body at 3 and 7 days. On a fresh weight (fw) adjusted basis, the concentrations at 3 and 7 days were highest in the adrenal glands (1.25 and 0.41 nmol/g fw [1.2 and 0.4 µg/g]), liver (0.55 and 0.20 nmol/g fw [0.7 and 0.2 µg/g]), kidney (0.17 and 0.07 nmol/g fw [0.16 and 0.07 µg/g]), and heart (0.14 and 0.05 nmol/g fw [0.13 and 0.05 µg/g]), respectively. On an lw basis, the plasma and liver had the highest concentrations of radiolabel (22 and 14.9 nmol/g lw [21 and 14 µg/g] on day 3 and

8.8 and 5.3 nmol/g lw [8.4 and 5.1 µg/g] on day 7, respectively), whereas adipose tissue, testis, thymus, spleen, small intestine wall, skin muscle, lung, kidney, adrenal, and heart had low concentrations. These results indicate that decaBDE was not readily distributed to lipid-rich tissues but rather was found in plasma and blood-rich tissues, including liver, kidney, heart, and intestinal wall.

Huwe and Smith (2007) conducted a repeat dosing study of BDE-209 during which a group of 18 male Sprague-Dawley rats were given 0.36 µg/day BDE-209 in an oil-based supplement added to their diets for 21 days. Eight control animals received the same diet and oil-based supplement. At the end of dosing, groups of three animals were sacrificed on days 0, 3, 7, 10, 14, and 21 of a follow-up period that began 24 hours after the last feeding of the treated diet. There was no collection of excreta during the feeding period. Twenty-four hours after the last feeding, the levels of BDE-209 in liver, plasma, gastrointestinal tract, and carcass were measured. The highest levels were found in the liver (48.2 ± 8.9 µg/g wet weight) and gastrointestinal tract (35.9 ± 8.5 µg/g wet weight). The amounts in plasma (3.6 ± 0.9 µg/g wet weight) and carcass (14.0 ± 4.0 µg/g wet weight) were lower. After the 21-day exposure, the tissues of the exposed rats contained levels of BDE-209 that were 10–20 times greater than those in control rats. In addition, hepta-, octa-, and nonaBDEs were isolated in tissues and were apparently formed via reductive debromination of BDE-209. Two of the octaBDE congeners (BDE-201 and -197) bioconcentrated in the tissues to a greater extent than the parent and other debrominated metabolites. Huwe and Smith (2007) made no attempt to determine if hydroxylated metabolites had been formed. These results differ from those for the tetra-, penta-, and hexaBDE congeners, where there is no evidence for debromination in the absence of hydroxylation.

Viberg et al. (2003a) studied the distribution of decaBDE in mice. NMRI male mice (n = 6–8) received a single oral dose (2.22 mg/kg) of ^{14}C-decaBDE (40.5 µCi/kg) by gavage on postnatal day (PND) 3, 10, or 19. The animals were sacrificed 1 and 7 days after administration of the radiolabeled decaBDE, and the label in the brain, heart, and liver was measured. The highest concentrations as a percentage of the administered dose were seen in the liver, followed by the brain and heart. The authors did not state whether the radioactivity was that of the parent compound or its metabolites.

One day after administration, the mean radioactivity in the liver of mice treated with radiolabeled decaBDE on PND 3, 10, or 19 was 12.6%, 9.4%, or 5.8% of the administered dose, respectively, whereas 7 days after administration, the radioactivity decreased significantly to 4.8%, 4.6%, or 0.3%, respectively. The distribution to and retention by the liver was greater for the dosing on PNDs 3 and 10 than for the dosing on PND 19. One day after administration, mice treated on PNDs 3 and 10 had 0.5 and 0.4% of total activity administered, respectively, in the brain, and the radioactivity increased to 0.7 and 1.1%, respectively, 7 days after the dosing. In

contrast, mice treated with ^{14}C-decaBDE on PND 19 had only 0.06% of the total radioactivity administered in the brain 1 and 7 days after administration. Significant age-dependent differences in distribution to the liver and developing brain were noted. On the other hand, the amount of radioactivity in the heart was minimally affected by age of the animal 24 hours after administration (0.28, 0.31, and 0.22% of the total dose was measured in the heart 24 hours after administration on PNDs 3, 10, and 19, respectively). After 7 days of administration, the 3-day-old and 10-day-old animals did not have significant changes in the amounts of radioactivity in the heart (0.34 and 0.32% of the total administered dose). The amount of radioactivity detected in the hearts of 19-day-old mice had decreased significantly to 0.08%.

In a study by el Dareer et al. (1987) the liver of rats fed two diets containing low (0.0277%; 25 mg/kg) or high (4.8%; 4,400 mg/kg) amounts of ^{14}C-labeled decaBDE contained 0.45, 0.21, and 0.11% of the administered dose on days 1, 2, and 3 after administration of the low dose and 0.007, 0.007, and 0.016% after administration of the high dose, respectively. These data are also included in Appendix O of the National Toxicology Program (NTP) (1986) report. These results suggest saturation in uptake or distribution. High-pressure liquid chromatography and ultraviolet spectral analysis indicated that 81% of the radioactivity in the liver was decaBDE rather than metabolites. The maximum percent of dose in the organs and tissues obtained from the rats fed the lower dose of decaBDE (0.0277%) was in the following order: liver > skin > muscle > fat > blood > gut tissue > plasma > kidneys > lungs > spleen > brain.

Hakk et al. (2002) investigated the disposition of decaBDE in rats. Groups of four male Sprague-Dawley rats (conventional rats) and bile-duct-cannulated rats (cannulated rats) were administered ^{14}C-labeled decaBDE (>97% purity; 15 mCi/mol) in Lutrol F127/soya phospholipid/water vehicle as a single oral dose of 3 µmol/kg (2.9 mg/kg). Gastrointestinal mucosa, kidneys, liver, and lungs were collected from the animals 72 hours following dosing. Radioactivity content was assayed in tissue supernatants and protein-bound extracts. Radioactivity was also quantified from pellets obtained after centrifugation of homogenized tissues. In addition, binding of radiolabeled decaBDE and/or its metabolites to liver proteins was determined. Analysis of liver, lung, intestinal cells, and kidney tissues of noncannulated rats 72 hours following decaBDE administration indicated that the majority (>45–80%) of the decaBDE-derived radioactivity was associated with the membrane fraction, whereas 0–29% and 4–24% were associated with microsomal and soluble fractions, respectively.

Collectively, these studies suggest wide tissue distribution, although relative distribution among various tissues differed across studies in adult rodents. Significant age-dependent differences in distribution to the undeveloped brain were noted.

3.3. METABOLISM

Oral and i.v. studies suggest that decaBDE is metabolized through oxidative dehalogenation reactions to form phenolic metabolites and debrominated to form a variety of nona-, octa-, and heptaBDE congeners. This conclusion is based on the extensive presence of metabolites in the fecal matter and in blood. However, the sites of metabolism have not been identified. At least a portion is likely to occur in the liver. Extrahepatic metabolism may occur in the epithelium of the gastrointestinal tract or through preabsorption metabolism of decaBDE by the intestinal microflora. Figure 3-1 provides a summary of the proposed metabolic pathway as derived from the metabolites that have been identified in plasma, tissues, and fecal material.

It should be noted that some metabolites may be formed by intestinal microbes. The number of bromines removed will depend on whether the starting material is the parent or a partially debrominated metabolite. If absorption occurs with lipids via the chylomicrons, other tissues may also participate in metabolism. The identification of the feces as the major excretory pathway and the high percentage of metabolites present in the feces that cannot be accounted for through biliary excretion provide support for the hypothesis that the liver may not be the only site for metabolic conversions.

Morck et al. (2003) studied decaBDE metabolism in rats given a single oral dose by gavage. Results indicated that in noncannulated rats about 90% of the dose was excreted in the feces within 3 days after a single oral dose of ^{14}C-labeled decaBDE and the majority of this radioactivity (65%) represented decaBDE metabolites. Measurement of bile radioactivity indicated that close to 10% of the total dose was excreted in the bile during the same period, with almost all of the excreted dose in bile in the form of metabolites.

Analysis of radiolabeled materials from tissues of these rats at day 3 after decaBDE administration revealed that 42% of the radioactivity in the liver represented solvent extractable lipid-bound metabolites and 30% was in the form of extractable unconjugated metabolites (4 and 26% of which were hydroxylated and neutral metabolites, respectively). Twenty-seven percent of the radiolabel could not be extracted and was tissue bound. Only 1% of the extractable material was water soluble. A larger percentage (61%) of the radioactivity in the small intestine wall was tissue bound. Lipid-bound metabolites accounted for 7% of the label, water-soluble compounds for 11%, and unbound parent or metabolites for 20%. The percentage of the radiolabel found as water-soluble compounds in the intestinal wall was 10 times greater than the water-soluble metabolites in the liver, providing some support for the hypothesis that oxidative metabolism can occur in the intestinal mucosa. Most of the radioactivity (71–80%) in the lung, adipose tissue, and kidney was unbound parent or metabolites; 15–21% represented lipid-bound metabolites, and 1.5–8% was tissue bound. Formation of adducts was indicative of covalent and/or noncovalent interactions with cellular macromolecules.

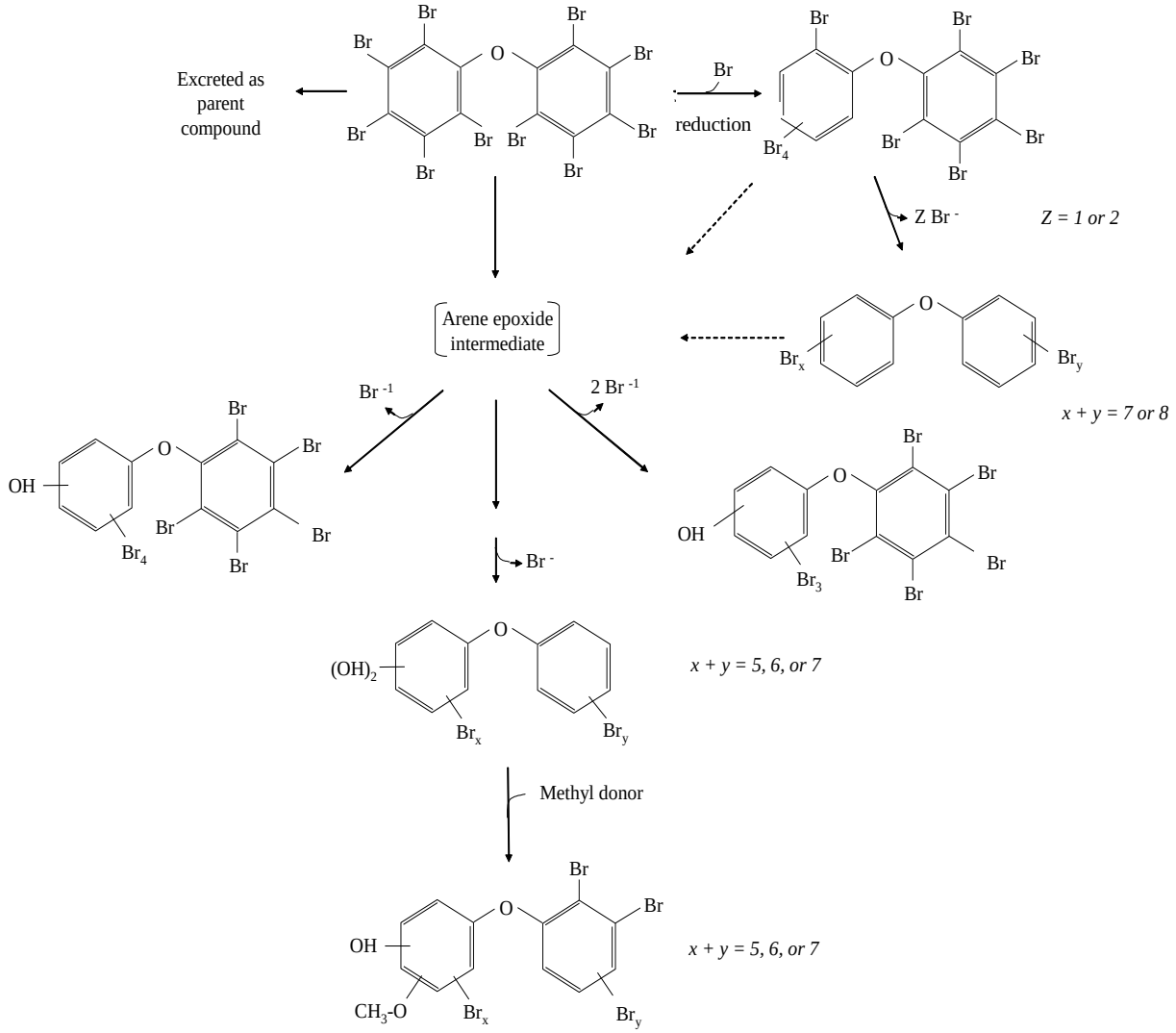


Figure 3-1. Proposed metabolic pathway for BDE-209.

Source: Derived from Huwe and Smith (2007), Sandholm et al. (2003), and Morck et al. (2003).

Sandholm et al. (2003) evaluated the metabolism of unlabeled decaBDE in male rats after gavage or i.v. injection. Blood samples were collected at specific intervals for up to 6 days. Pooled plasma samples from all 6 days were extracted and decaBDE and its metabolites were quantified. Analysis of the pooled samples indicated that the major neutral compound in the plasma was unmodified decaBDE with trace amounts of three nonaBDEs. Thirteen phenolic metabolites were determined in the plasma of both the orally and i.v. dosed rats, but only three phenolic metabolites were present in sufficiently high concentration for further analysis. These metabolites were characterized as a hydroxy-octaBDE, an hydroxy-nonaBDE, and an hydroxy/methoxy hexaBDE (Figure 3-1). The relative amount of each metabolite recovered was

not reported, but the concentration of phenolic radioactivity in the plasma collected 3 and 7 days after oral gavage was four times higher than that of the neutral compounds (i.e., the parent or debrominated decaBDE). The authors indicated that reductive debromination may be the first step in the metabolic pathway of decaBDE, followed by oxidation to form phenolic metabolites. It was also suggested that the hydroxy/methoxy metabolites were probably formed via an arene oxide hydrolyzed to a dihydrodiol and further rearomatized followed by a methylation reaction (Figure 3-1).

In the study conducted by Morck et al. (2003), the unconjugated phenolic fractions isolated from the feces were examined by gas chromatography/mass spectrometry (GC/MS). Methoxyhydroxylated penta- to heptabrominated diphenyl ethers were identified. As was the case in the Sandholm et al. (2003) study, the methoxy and hydroxy substituents were on the same phenyl ring when both were present. In addition, trace amounts of debrominated metabolites and nonaBDEs were also found in the feces and bile (see Figure 3-1), indicating debromination may have been the first step in decaBDE metabolism. A small proportion of monohydroxylated metabolites was found in tissues and feces, indicating a role for reductive dehalogenation followed by an oxidation step or direct oxidative dehalogenation reactions.

The plasma samples collected during the Morck et al. (2003) study were analyzed and reported by Sandholm et al. (2003). As was the case with the plasma samples collected by Sandholm et al. (2003), the neutral fraction was almost all BDE-209 with a small fraction of three nonaBDEs. Neutral compounds represented 19% of the plasma total in the samples drawn at 3 days and 21% in the samples collected at 7 days. The phenolic compounds in the plasma could not be purified enough to allow GC/MS analysis.

Huwe and Smith (2007) identified nonhydroxylated metabolites found in feces and tissues after 21 days dosing with 0.36 μg/day BDE-209 in an oil-based supplement added to their diets. Only 5% of the parent dose was present in the body after 21 days of dosing. Three nonaBDEs (206, 207, and 208), four octaBDEs (196, 197, 201, and 203), and one heptaBDE (183) were identified in the body tissues. No attempt was made to determine if hydroxylated metabolites were present. The results are indicative of considerable fecal excretion during the dosing period.

Intravenous administration of 1.07 mg/kg decaBDE to rats (el Dareer et al., 1987; NTP, 1986) resulted in the major part of the dose (74%) being excreted as metabolites in feces. About 63% of the excreted material in the feces was metabolites, and the remaining 37% was unchanged decaBDE. In these rats, 1% of the radioactivity in the bile was intact decaBDE, while 99% of the radioactivity excreted in the bile was in the form of metabolites.

Animal studies indicated that decaBDE is a weak inducer of the phase I or phase II xenobiotic metabolizing enzymes responsible for oxidation and conjugation of many xenobiotic compounds (Darnerud et al., 2001). Zhou et al. (2001) conducted a study in weanling rats to

investigate the mechanism(s) by which decaBDE interferes with thyroid hormone homeostasis. In this study, Long-Evans female rats (eight animals/dose group) were orally administered decaBDE (>98% purity) in corn oil at doses of 0, 0.3, 1, 3, 10, 30, 60, or 100 mg/kg-day for 4 consecutive days. Hepatic enzyme activities (ethoxyresorufin O-deethylase [EROD], a marker for cytochrome P-450 1A1 [CYP-1A1]; pentoxyresorufin O-deethylase [PROD], a marker for CYP-2B1) were measured. PROD activity increased only at 1 and 30 mg/kg-day. The lack of dose dependence was also found for EROD activity, where maximal induction was only 1.8-fold in the 1 mg/kg-day group, indicating that there were no significant or dose-related effects on CYP-1A1 or CYP-2B1. However, the presence of active EROD and PROD suggests that there may be some oxidative debromination of the decaBDE during metabolism, through the activity of CYP-1A1 and/or CYP-2B1.

Pacyniak et al. (2007) evaluated the ability of BDE-209 to induce CYP-3A11, -2B10, and -1A1/2. Six C57BL/mice (10 weeks of age) were injected with doses of 10 or 100 μmol/kg (10 or 100 mg/kg) in corn oil for 4 days. The livers were removed 24 hours after the last dose and the levels of messenger ribonucleic acid (mRNA) measured by Northern blot and branched deoxyribonucleic acid (bDNA) analyses. The bDNA was considered to be the more accurate of the two assay systems. Northern blot analysis indicated that the levels of CYP-2B10 were induced three- and fivefold, respectively, at the two doses tested, while the bDNA results indicated 3.6-fold and sevenfold inductions. CYP-3A11 did not show a difference with respect to the dose administered but was induced 4-fold by the Northern blot analysis and 1.7-fold by the bDNA analysis. The two doses of PBDE-209 induced CYP 1A1/2 9- and 6-fold, respectively, by Northern blot analysis and 0.3- and 0.8-fold by bDNA analysis.

3.4. ELIMINATION

As has been mentioned previously, the excretion of decaBDE and its metabolites in rats is almost exclusively through the fecal and biliary routes. In toxicokinetic studies (Morck et al., 2003; Hakk et al., 2002; el Dareer et al., 1987), the amounts excreted in urine have consistently been less than 1% of the dose (about 18% bound to albumin); biliary excretion has accounted for about 10% of the label found in the feces. There have been no studies that examined the excretion of BDE-209 in mice. Studies by Staskal et al. (2006) using BDE-47, -99, and -153 found that urinary excretion in male mice involves binding with one or two major urinary excretory proteins.

In one of the early toxicokinetic studies (el Dareer et al., 1987; NTP, 1986), the fate of decaBDE in male F344 rats was investigated. The study consisted of four substudies. In substudy one, groups of 2-month-old male F344 rats (three animals/group) were fed a standard diet containing unlabeled decaBDE (92% purity) on days 1–7 and 9–11 and the test diet containing ^{14}C-labeled decaBDE (>98% purity) on day 8. The diets contained 0, 0.0250, 0.0509,

0.250, 0.487, 2.49, or 4.99% decaBDE. Based on the rat body weight information reported in el Dareer et al. (1987) and the average feed intake from the NTP (1986) report, the corresponding daily doses were 0, 27, 55, 272, 512, 2396, or 4577 mg/kg. Radioactivity was determined in feces collected daily on study days 9–12.

In substudy two, rats were fed two diets containing low (0.0277% or 25 mg/kg) or high (4.80% or 4400 mg/kg) amounts of decaBDE or similar amounts of ^{14}C-labeled decaBDE, using the same protocol as in the first substudy, except that groups of three rats were killed on study days 10, 11, or 12. Urine and feces were collected from each rat and analyzed for radioactivity.

In substudy three, a group of rats (n = 3) was injected intravenously via the tail vein with 1.07 mg/kg of ^{14}C-labeled decaBDE; urine and feces were collected daily for 3 days and analyzed for radioactivity 72 hours after dosing. In the fourth substudy, bile-duct-cannulated rats (n = 5) were injected intravenously with 0.947 mg/kg of ^{14}C-labeled decaBDE. Bile was collected over a 4-hour period. Radioactivity was determined in each pooled bile sample.

The recovery of radioactivity in the feces ranged from 91–101% of the ingested dose in the first feeding study and 83–86% in the second feeding study, indicating that the recovery was not related to the dose of decaBDE or to the time of sacrifice (24, 48, or 72 hours) after consumption of ^{14}C-labeled decaBDE within a specific substudy. (Note: no reasons were given for the differences in the radioactivity recovered in these two studies.) Greater than 99% of the radioactivity was recovered in the feces and gut contents.

After i.v. dosing of ^{14}C-labeled decaBDE (substudy three), 74% of the radioactivity was recovered in the feces and gut contents at 72 hours. About 63% of the excreted material in the feces was metabolites and the remaining 37% was unchanged decaBDE. In these rats, only traces of radioactivity were noted in the urine. In bile-duct-cannulated rats, 7.2% of the radioactivity intravenously administered appeared in the bile in 4 hours, 1% of which was intact decaBDE (i.e., 99% of the radioactivity excreted in the bile was in the form of metabolites). Together, these results show that the vast majority of an oral dose of decaBDE is excreted in the feces, mostly as unabsorbed material.

Morck et al. (2003) administered orally ^{14}C-radiolabeled decaBDE as a single 3 µmol/kg (2.9 mg/kg) dose in soy phospholipid/Lutrol F127/water vehicle to male Sprague-Dawley rats (i.e., conventional rats). An average of 90 and 91% of the radioactivity was excreted in the feces at 3 and 7 days, respectively. About 71% of the total dose was excreted in the first 24 hours, another 17% between 24 and 48 hours, and a further 2% between 48 and 72 hours. In bile-duct-cannulated rats subjected to the same oral treatment, an average of 88% of the dose was excreted in the feces and 9.5% in the bile within 3 days. Less than 0.1% of the radioactivity was excreted in the urine. In the cannulated rats, 66% of the total dose was excreted in the feces in the first 24 hours and another 19% during 24–48 hours. In the first 12 hours, 4.4% of the total dose was recovered in the bile, whereas 1.6, 2, and 0.4% of the total dose were excreted at 12–24, 24–48,

and 48–72 hour intervals, respectively. These results indicate that excretion of decaBDE via feces was the dominant route after the oral dose, and biliary excretion plays a major role in the systemic elimination of absorbed decaBDE. The urinary excretion of radioactivity was insignificant.

In the Hakk et al. (2002) study described earlier, ≤0.02% of the administered decaBDE dose was excreted daily via the urine in both conventional and cannulated rats, with a total of 0.033 and 0.047%, respectively, of the administered dose excreted over the 72-hour period. About 9% of the administered dose was excreted in the bile by 72 hours, 6% of which was eliminated within the first 24 hours, indicating that excretion via the bile was favored over that of urine.

About 20% of the decaBDE-derived radioactivity in urine from noncannulated rats was protein bound at 72 hours (compared to >73% not associated with protein). Eighteen percent of the bound material was bound to albumin, a serum protein with the ability to bind (nonspecific protein binding) short chain fatty acids. In cannulated rats, 18.2% of the decaBDE-derived radioactivity was unbound, and all the remaining 68.3% was associated with albumin. Two polar metabolites were noted but not identified. Under the assumption that the bound materials are less polar than the unbound materials, this observation supports the concept that a substantial portion, but not all, of the metabolites in the fecal matter originate from the bile.

About 90% of the biliary radiolabel was associated with an unidentified 79-kDa protein, with the percent of bound label decreasing from 94 to 87% in bile samples pooled at 1–24 hours, 24–48 hours, and 48–72 hours. Approximately 17% of the protein-bound biliary radioactivity collected over the first 24 hours was parent compound (and the remainder was unidentified metabolites); no parent compound was detected at 48 and 72 hours. The percent of total bound label in the bile samples also declined over time. None of the label in the bile was found to be unbound.

3.4.1. Half-life Estimates

In computer technicians, the half-life of BDE-209 is estimated to be in the range of a week (Sjodin et al., 2003; Jakobsson et al., 2002); however, the data to support this estimate are limited. Sjodin et al. (1999) also found that the levels of BDE-209 in the blood of occupationally exposed Swedish workers decreased by 66% over a 30-day absence from the workplace. These authors did not present any half-life estimate in their publication.

The half-lives of hepta- to decaBDE in human serum were estimated by using data from occupationally exposed workers sampled before, during, and after a vacation period (Thuresson et al., 2006). The half-lives were found to decrease with increasing bromination. The half-life of heptaBDE-183 was 94 days, while that for BDE-209 was 15 days.

Sandholm et al. (2003) evaluated the kinetics of unlabeled decaBDE in male rats. One group of rats was dosed orally by gavage with 2 µmol decaBDE/kg (1.9 mg/kg). Another group of rats was also dosed intravenously with unlabeled decaBDE at the same dose. At specific time intervals, blood samples from three rats per group were collected according to the following schedule: group 1 was sampled 1 hour and 24 hours after dosing, group 2 after 3 hours and 48 hours, group 3 after 6 hours and 72 hours, group 4 after 96 hours, group 5 after 120 hours, and group 6 after 144 hours. Plasma samples were extracted and decaBDE and its metabolites were quantified. Results from animals orally dosed with unlabeled decaBDE indicated the mean C_{max} in plasma reached 264 pmol/mL (253 ng/g) at 6 hours after dosing and AUC was 12 nmol × hour/mL (11.5 µg × hour/mL). The i.v. data indicated that the clearance was 0.60 mg/minute-kg, and the apparent volume of distribution at steady state was 1.4 L/kg.

Half-lives were determined from the slope of the total concentration-time curve by linear regression. The disposition of decaBDE in male rats after oral dosing was described by a two-compartment model with $t_{1/2\beta}$ and $t_{1/2\gamma}$ values of 6.9 hours and 51 hours, respectively. The results from the i.v. study were consistent with a three-compartment model with half-lives ($t_{1/2\alpha}$, $t_{1/2\beta}$, and $t_{1/2\gamma}$) of 1.6 hours, 12 hours, and 58 hours, respectively (Sandholm et al., 2003). This value is lower than the 15-day half-life for humans estimated by Thuresson et al. (2006).

Huwe and Smith (2007) modeled the distribution and elimination half-lives of parent BDE-209 and several debrominated nonhydroxylated metabolites based on assays of plasma, excreta, liver, and carcass over a 21-day period that followed 21 days of dosing with 0.36 µg/day. The BDE-209 data showed the best fit to a biphasic depletion pattern. The distribution half-lives were 1.0 and 1.2 days for liver and plasma, respectively. The elimination half-lives for the same tissues were 20.2 and 75.9 days, respectively. A plasma first order half-life for octaBDE-207 (7.9 days) was twice that of BDE-209 (3.9 days). In the liver, the first order half-life estimates for congeners 208, 191, and 197 (~6 days) were greater than those for 206 and 207 (~1 day). First-order estimates for 207 and 196 were intermediate (4–5 days). These values reflect the behavior of the parent BDE-209 and the debrominated, nonhydroxylated metabolites after absorption, distribution, and excretion but do not reflect the distribution and elimination of the hydroxylated metabolites.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Limited information is available on the absorption, distribution, metabolism, and excretion of decaBDE 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 (PBPK) models is not possible at this time.

4. HAZARD IDENTIFICATION

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

Studies of decaBDE levels in occupationally exposed groups or studies of decaBDE in human biological media did not include surveillance of health endpoints.

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

Inhalation toxicological studies of decaBDE in experimental animals are not available.

4.2.1. Short-term and Subchronic Studies

The short-term and subchronic studies are summarized in Table 4-1.

4.2.1.1. *Studies in Rats*

Zhou et al. (2001) conducted a study in weanling rats to investigate the mechanism(s) by which decaBDE interferes with thyroid hormone homeostasis. In this study, Long-Evans female rats (eight animals/dose group) were orally administered decaBDE (>98% purity) in corn oil at doses of 0, 0.3, 1, 3, 10, 30, 60, or 100 mg/kg-day for 4 consecutive days. Body weights were recorded and dosing volumes adjusted daily. Animals were sacrificed 1 day after the last dose. Serum total thyroxine (T_4) and triiodothyronine (T_3), serum thyroid stimulating hormone (TSH), and hepatic enzyme activities (EROD, a marker for CYP-1A1; PROD, a marker for CYP-2B1; and T_4-uridine diphosphate glucuronyl transferase [T_4-UDPGT]) were measured (see section 3.3 for data on EROD and PROD). Short-term treatment with decaBDE did not cause any visible signs of toxicity or any effects on body-weight gain or liver-to-body-weight ratios at any dose level. DecaBDE (up to 100 mg/kg-day) had no effect on serum T_4, T_3, or TSH concentration or on hepatic UDPGT activity. Based on these observations, the highest dose of 100 mg/kg-day is identified as the no-observed-adverse-effect level (NOAEL).

Carlson (1980) conducted a study to evaluate the induction of xenobiotic metabolism in rats, following short-term administration of decaBDE. Groups of four male Sprague-Dawley rats were dosed orally with 0.1 mmol/kg-day (96 mg/kg-day) of decaBDE in corn oil for 14 days. The decaBDE was synthesized by the complete bromination of diphenyl ether and was reported to be of "high purity." These animals were probably dosed every other day since the protocol stated that livers were collected from animals sacrificed 24 hours after the last (seventh) dose.

Table 4-1. Oral short-term and subchronic toxicity studies for decaBDE in laboratory animals

Study	Species, sex, and sample size	Route, dose, and duration	Observed effects	NOAEL	LOAEL[a]	Comments
Zhou et al. (2001)	Rat, Long-Evans, female weanling rats, 8/dose group	Gavage (>98% purity); 0, 0.3, 1, 3, 10, 30, 60, or 100 mg/kg-day; 4 days	No observed dose-related effects on body weight or liver weight or changes in T_3 or T_4 levels	100 mg/kg-day	Not identified	
NTP (1986)	Rat, F344/N, male and female, 5/sex/dose group	Diet (99% purity); 0, 472, 928, 1,846, 4,569, or 9,326 mg/kg-day in males; 0, 538, 1,061, 2,137, 5,323, or 10,853 mg/kg-day in females; 14 days	None	9,326 mg/kg-day in males; 10,853 mg/kg-day in females	Not identified	Survival, final mean body weights not adversely affected. Clinical signs or gross pathological effects not noted.
NTP (1986)	Rat, F344/N, male and female, 10/sex/dose group	Diet (97–99% purity); 0, 191, 372, 781, 1,536, or 3,066 mg/kg-day in males; 0, 238, 504, 967, 1,955, or 3,944 mg/kg-day in females; 13 weeks	None	3,066 mg/kg-day in males; 3,994 mg/kg-day in females	Not identified	Survival, final mean body weights not adversely affected. Clinical signs or gross pathological effects not noted.
NTP (1986)	Mouse, B6C3F1, male and female, 5/sex/dose group	Diet (99% purity); 0, 1,027, 2,143, 4,246, 10,536, or 20,994 mg/kg-day in males; 0, 1,146, 2,286, 4,627, 11,348, or 23,077 mg/kg-day in females; 14 days	None	20,994 mg/kg-day in males; 23,077 mg/kg-day in females	Not identified	Survival, final mean body weights not adversely affected.
NTP (1986)	Mouse, B6C3F1, male and female, 10/sex/dose group	Diet (97–99% purity); 0, 666, 1,355, 2,659, 5,278, or 10,233 mg/kg-day in males; 0, 702, 1,437, 2,899, 5,687, or 11,566 mg/kg-day in females; 13 weeks	None	10,233 mg/kg-day in males; 11,566 mg/kg-day in females	Not identified	Survival, final mean body weights not adversely affected. Clinical signs or gross pathological examination did not show any effect.

[a]LOAEL = lowest-observed-adverse-effect level.

Detoxification of O-ethyl O-p-nitrophenyl phenylphosphonothioate, methylation of p-nitroanisole, levels of NADPH cytochrome c reductase and cytochrome P-450 (CYP-450), and activities of UDPGT and benzo[a]pyrene hydroxylase were determined in hepatic cytosol or microsomes. Serum sorbitol dehydrogenase measurements were also performed on blood samples collected from the tail vein. No significant changes were observed in these enzyme activities. DecaBDE significantly increased the liver-to-body-weight ratio, indicating liver enlargement. Sorbitol dehydrogenase activity in the serum was not altered by decaBDE, suggesting that liver necrosis had not occurred, although other common serum markers for liver damage were not measured. Because of limitations in the study design, these results are not adequate for use in the health assessment of decaBDE.

NTP (1986) conducted a 14-day study in rats exposed to decaBDE. F344/N rats (five animals/sex/dose) were fed diets containing 0, 5,000, 10,000, 20,000, 50,000, or 100,000 ppm decaBDE (99% purity). Based on the reported body weight information NTP (1986) and U.S. EPA (1988) default food intake values for F344 male (0.018 kg food/day) and female (0.014 kg food/day) rats, the corresponding estimated average daily doses were 0, 472, 928, 1,846, 4,569, or 9,326 mg/kg-day in male rats and 0, 538, 1,061, 2,137, 5,323, or 10,853 mg/kg-day in female rats. Animals were observed daily and were weighed on days 1, 7, and 14. At the end of the exposure period, animals were necropsied and several organs and tissues were examined histologically. No mortality was observed in the rats during the course of the study. Exposure to decaBDE did not cause any clinical signs of toxicity or adversely affect the final mean body weights. Gross pathological effects were not noted in any animal at any dose level. The results of this study indicated a NOAEL of 9,326 mg/kg-day in male rats and 10,853 mg/kg-day in female rats.

The subchronic effects of decaBDE (97–99% purity) on rats were also investigated in a 13-week study (NTP, 1986). Groups of F344/N rats (10/sex/dose) were administered decaBDE in the diet at concentrations of 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm for 13 weeks. Based on body-weight information in the NTP (1986) report and U.S. EPA (1988) default food intake values for F344 male (0.018 kg food/day) and female (0.014 kg food/day) rats, the corresponding estimated average daily doses were 0, 191, 372, 781, 1,536, or 3,066 mg/kg-day in male rats and 0, 238, 504, 967, 1,955, or 3,944 mg/kg-day in female rats. Animals were observed twice daily and body weight, feed consumption, clinical signs, and behavior were recorded once a week. A necropsy was performed on all animals, including those killed in extremis, with the exception of those excessively autolyzed or cannibalized. Histologic examination was performed on major organs and tissues from control and high-dose groups. No mortality was observed in rats fed decaBDE, and no clinical signs of toxicity were noted. Compound-related changes in body weight and feed consumption were not observed, and no

gross or macroscopic pathological effects were noted in any animal examined. The results indicate a NOAEL of 3,066 mg/kg-day in male rats and 3,944 mg/kg-day in female rats.

4.2.1.2. *Studies in Mice*

A 14-day study (NTP, 1986) was also conducted in mice. B6C3F1 mice (five animals/sex/dose) were fed diets containing 0, 5,000, 10,000, 20,000, 50,000, or 100,000 ppm decaBDE (99% purity). Based on the reported body weight information (NTP, 1986) and U.S. EPA (1988) default food intake values for B6C3F1 male (0.0057 kg food/day) and female (0.0048 kg food/day) mice, the estimated average daily doses were 0, 1,027, 2,143, 4,246, 10,536, or 20,994 mg/kg-day in male mice and 0, 1,146, 2,286, 4,627, 11,348, or 23,077 mg/kg-day in female mice. Animals were observed daily and were weighed on days 1, 7, and 14. Necropsy was performed at the end of the exposure period, and several organs and tissues were examined histologically. Exposure to decaBDE up to 20,994 mg/kg-day in males and 23,077 mg/kg-day in females showed no effects on survival or body weight, and there were no clinical signs of toxicity. No compound-related gross pathological effects were noted in any animal in any group. The results of this study indicate a NOAEL of 20,994 mg/kg-day in male mice and 23,077 mg/kg-day in female mice.

B6C3F1 mice (10 animals/sex/dose) were fed diets containing 0, 3,100, 6,300, 12,500, 25,000, or 50,000 ppm decaBDE (97–99% purity) for 13 weeks (NTP, 1986). Based on the mouse body weight information reported (NTP, 1986) and U.S. EPA (1988) default food intake values for B6C3F1 male (0.0057 kg food/day) and female (0.0048 kg food/day) mice, the corresponding estimated average daily doses were 0, 666, 1,355, 2,659, 5,278, or 10,233 mg/kg-day in males and 0, 702, 1,437, 2,899, 5,687, or 11,566 mg/kg-day in females. Animals were observed twice daily and body weights, feed consumption, clinical signs, and behavior were monitored once a week. Necropsy was performed on all animals, including those killed in extremis, with the exception of those excessively autolyzed or cannibalized. Histologic examination was performed on the organs and tissues from control and high-dose groups. Only one male and one female mouse fed 12,500 ppm died in the course of the study. There were no clinical signs of toxicity, and no compound-related effects on body weight and feed consumption were observed. No gross or macroscopic pathological effects were noted in any animal at any dose. The results of this study indicated a NOAEL of 10,233 mg/kg-day in males and 11,566 mg/kg-day in females.

4.2.2. Chronic Studies and Cancer Bioassays

NTP (1986) investigated the relationship between ingestion of decaBDE in rats and mice and tumor development. These chronic oral studies are summarized in Table 4-2. The decaBDE used in both rats and mice was 94–97% pure, with no detectable brominated dioxins or furans.

The major impurities in the decaBDE test material were isolated and identified as two unspecified congeners of nonaBDE.

4.2.2.1. *Study in Rats*

Groups of 7- to 8-week-old male and female F344/N rats (50/sex/dose) were exposed to decaBDE (94–97% purity) in the diet at concentrations of 0, 25,000, or 50,000 ppm for 103 weeks (NTP, 1986). Average daily doses of decaBDE as reported in the study were 0, 1,120, or 2,240 mg/kg-day for male rats and 0, 1,200, or 2,550 mg/kg-day for female rats. The animals were observed twice daily and clinical signs were recorded once per week. Animals were weighed once a week for the first 12 weeks, once per month thereafter until week 100 or 101, then every 2 weeks. Mean body weights were calculated for each group. In addition, feed consumption, morbidity, and mortality were monitored throughout the study period. Animals found moribund and animals that survived to the end of the study period were sacrificed. Complete necropsy was performed on all animals, including those found dead during the course of the study, unless they were excessively autolyzed or cannibalized. Gross and microscopic examinations were performed on major organs or tissues.

No clinical signs of toxicity were observed in the treated rats. There were no significant differences in the mean body weight and feed consumption between treated and control animals. Survival of low-dose male rats was significantly lower than that of the controls after week 102, but the decrease was not considered to be compound related because the reduction in survival occurred late in the study and there was a lack of a dose effect.

At necropsy, several nonneoplastic changes were observed. In the liver, an increase in the incidence of thrombosis was observed in high-dose male rats (1/50, 0/50, 9/49), with no such increase noted in low-dose males or in any female at any dose level. A dose-dependent, but not statistically significant, increase in the incidence of degeneration of the liver was also observed in treated male rats at incidence rates of 13/50, 19/50, and 22/49 in the control, low-dose, and high-dose groups, respectively. No liver degeneration was observed in female rats. In the spleen, an increased incidence of fibrosis was seen in males at low dose (8/50) and high dose (13/49) compared with 5/49 in controls, indicating a dose-dependent increase that was statistically significant only in the high-dose group. Hematopoiesis was observed at an increased incidence in the spleen of female rats (control 12/49, low dose 24/48, high dose 17/50), but the increase was not dose dependent or statistically significant at any dose level. No such increases were observed in males. In the mandibular lymph node, lymphoid hyperplasia increased in males in a dose-dependent manner (4/50, 6/50, and 13/49 in the control, low dose, and high dose, respectively), but the incidence reached statistical significance only at the high dose.

Table 4-2. Oral chronic toxicity studies for decaBDE in laboratory animals

Study	Species, sex, and sample size	Route, dose, and duration	Observed effects	NOAEL	LOAEL[a]	Comments
NTP (1986)	Rat, F344/N, male and female, 50/sex/dose group	Diet (94–97% purity); 0, 1,120, or 2,240 mg/kg-day for males; 0, 1,200, or 2,550 mg/kg-day for females; 2 years	Increased incidences of thrombosis (1/50, 0/50, 9/49) and degeneration (13/50, 19/50, 22/49) in the liver of high-dose male rats; dose-dependent increase in splenic fibrosis in males with statistical significance at high dose; dose-dependent increase in lymphoid hyperplasia of the mandibular lymph nodes in males with statistical significance at high dose Dose-related increase in liver neoplastic nodules statistically significant in low- and high-dose males and in high-dose females	1,120 mg/kg-day in males 2,550 mg/kg-day in females	2,240 mg/kg-day in males Not identified	Males, but not females, exhibited effects.
NTP (1986)	Mouse, B6C3F1, male and female, 50/sex/dose group	Diet (94–97% purity); 0, 3,200, or 6,650 mg/kg-day for males; 0, 3,760, or 7,780 mg/kg-day for females; 2 years	Increased incidences of granulomas in the liver of treated males (with statistical significance only at low dose); centrilobular hypertrophy in males (with significance at low and high doses); dose-dependent increase in thyroid follicular cell hyperplasia (2/50, 10/50, 19/50) in males (significant at low and high doses); increase in the incidence of stomach ulcers in females at high dose Increased incidence of hepatocellular adenoma or carcinoma (combined) in low-dose males; marginally but not statistically significant increase in the thyroid gland follicular cell adenomas or carcinomas (combined) in males	Not available in males 3,760 mg/kg-day in females	3,200 mg/kg-day in males 7,780 mg/kg-day in females	

[a]LOAEL = lowest-observed adverse-effect level.

No incidence of lymphoid hyperplasia was reported in females at any dose level. An increased incidence of retinal degeneration was noted in low-dose female rats but not in high-dose females or males at any dose level. This lesion was attributed to greater exposure of the female rats to fluorescent light and was not considered to be treatment related. Acanthosis (increased thickness of the epithelial surface) of the forestomach was observed at increased incidence in treated males but not in females, but the incidence was not significant. Both male and female rats demonstrated a dose-dependent decrease in the incidence of C-cell hyperplasia in the thyroid gland (12/50, 6/49, and 2/49 in males and 14/50, 7/49, and 2/50 in females at control, low-dose, and high-dose groups, respectively). Decreases in the C-cell hyperplasia would not be considered biologically adverse since other thyroid effects were not observed in these rats. No other significant pathological changes were observed.

Histopathologic examination revealed a significant increase in the incidence of neoplasia in the rat, and several organs or tissues were adversely affected. There was a dose-dependent increase in the incidence of neoplastic nodules in the livers of both male and female rats. The incidence of these lesions was 1/50, 7/50, and 15/49 in males and 1/50, 3/49, and 9/50 in females in control, low-dose, and high-dose groups, respectively. The incidence reached statistical significance at both treatment doses in males and at the high dose in females.

At the time the NTP (1986) study was conducted, the term neoplastic nodule was used to describe abnormal cellular masses in the livers of rats characterized by loss or distortion of normal cellular architecture (Maronpot et al., 1986). Some of those nodules would now be described as benign hepatocellular adenomas in rats (Wolf and Mann, 2005). However, there is no complete equivalency between the neoplastic nodule of the past and the hepatocellular adenoma term of today. Some of the neoplastic nodules from the NTP (1986) study might now be classified as foci of cellular alteration or hyperplasia rather than adenomas (Maronpot et al., 1986). Adenomas and foci of cellular alteration are considered to be preneoplastic lesions, whereas hyperplastic lesions represent secondary nonneoplastic changes (Maronpot et al., 1986). The assumption that the hepatic neoplastic nodules from the NTP (1986) bioassay are equivalent to hepatic adenomas under the current NTP lexicon is a conservative interpretation of the data.

A slight increase in mononuclear cell leukemia in treated rats was observed with incidences in males of 30/50, 33/50, and 35/50 at control, low, and high doses, respectively, and in females of 14/50, 21/50, and 18/50 at control, low, and high doses, respectively. However, the increase was not considered to be biologically significant because of the marginal and insignificant increase in treated rats of both sexes and the high incidence in control rats. The incidence of hepatocellular carcinomas was low in male and female rats and was apparently not compound related. Other tumors observed included splenic sarcomas, pancreatic acinar cell adenomas, carcinomas in Zymbal's gland, and osteosarcomas, but these were considered to be of

no significance because of high historical incidence, lack of dose-response trend in the tumor incidence, or lack of statistically significant responses.

Based on the results from this 2-year study in rats (NTP, 1986), exposure to decaBDE in the diet did not cause compound-related effects on survival or any significant effects on body weight or food consumption. However, treatment resulted in several nonneoplastic changes at the high dose, including thrombosis and degeneration of the liver in males, fibrosis in the spleen in males, and lymphoid hyperplasia in the mandibular lymph node in males. Dose-dependent increases in the incidence of neoplastic nodules in liver of males and females provide some evidence of carcinogenicity of decaBDE in rats. Based on these results and on increased incidence of thrombosis and degeneration in the liver, splenic fibrosis, and lymphoid hyperplasia of the mandibular lymph nodes, a NOAEL for systemic toxicity was 1,120 mg/kg-day and the lowest-observed-adverse-effect level (LOAEL) was 2,240 mg/kg-day for males. Female rats appeared to be less sensitive to the systemic toxicity of decaBDE at the doses used in this study. Based on this finding, the NOAEL for systemic toxicity in females was 2,550 mg/kg-day, with no LOAEL established. The dose-related increase in nonmalignant liver tumors (neoplastic nodules) in both males and females provides some evidence of carcinogenicity of decaBDE in rats.

4.2.2.2. *Study in Mice*

Groups of 9-week-old male and female B6C3F1 mice (50/sex/dose) were administered decaBDE (94–97% purity) in the diet at doses of 0, 25,000, or 50,000 ppm for 103 weeks (NTP, 1986). Average daily doses of decaBDE as reported in the study were 0, 3,200, or 6,650 mg/kg-day in male mice and 0, 3,760, or 7,780 mg/kg-day in female mice. The animals were observed twice daily, and clinical signs were recorded once per week. Animals were weighed once a week for the first 12 weeks and once per month thereafter. In addition, feed consumption, morbidity, and mortality were monitored throughout the study period. All survivors were sacrificed during weeks 112–113. Complete necropsy was performed on all animals, including those found dead during the course of the study, unless they were excessively autolyzed or cannibalized. Gross and microscopic examinations were performed on major organs or tissues.

There were no significant differences in survival among any groups of mice of either sex at terminal sacrifice. However, loss of control male mice (presumably due to fighting) was significant during the first 15 months of the study. There were no compound-related clinical signs of toxicity or changes in body weight or food consumption.

At necropsy, several nonneoplastic changes were observed in tissues of treated mice. In the liver, the incidence of granulomas in control, low-, and high-dose mice was 8/50, 22/50, and 12/50 in males and 23/50, 27/50, and 24/50 in females, respectively. Although increases were observed in the treated animals of both sexes compared with controls, no consistent dose

response for this effect was evident, and the effect was not associated with a statistically significant positive trend. Thus, the toxicological significance of this effect is unclear. Significant increases in the incidence of centrilobular hypertrophy were also observed in the liver of male mice at high and low doses (0/50, 34/50, and 32/50 in the control, low dose, and high dose, respectively). Although the increases were comparable in the low- and high-dose males and were not observed in female mice at any dose level, the high incidence in the treated males indicates that the effect was treatment related. In the thyroid gland, a dose-dependent and statistically significant increase (at both dose levels) in the incidence of follicular cell hyperplasia was observed in males (2/50, 10/50, and 19/50, respectively, in the control, low-dose, and high-dose groups). In the females, the incidence was increased in the low- (9/50) and high- (7/49) dose groups compared to 4/50 in the control group, but the increase was not dose dependent or statistically significant at any dose level. Only high- but not low-dose female mice exhibited a significant increase in the incidence of stomach ulcers (1/50, 1/50, and 8/50 in the control, low-dose, and high-dose females, respectively). In males, the incidence in treated mice was comparable with that in control mice: 5/49, 3/50, and 5/50 in the control, low-dose, and high-dose males, respectively.

Histopathological examination revealed a significantly increased incidence of neoplasia in mice, with the liver appearing to be the main target organ for decaBDE. In the liver, increases (although not statistically significant) in the incidence of hepatocellular adenoma were observed at similar rates in low- and high-dose males (4/50, 12/50, and 12/50 in control, low-dose, and high-dose groups, respectively). Other changes observed in male mice included an increased incidence of hepatocellular carcinoma (5/50, 14/50, and 8/50 in the control, low-dose, and high-dose groups, respectively) that was not statistically significant at either dose. The combined incidence of hepatocellular adenomas or carcinomas in male mice, after correction for survival, significantly increased at low dose but not at high dose; the responses amounted to 8/50, 22/50, and 18/50 in control, low-dose, and high-dose groups, respectively. As indicated in the study report, these findings may have been influenced by the large number of early deaths in control male mice compared with treated mice, thereby decreasing the significance of the hepatocellular adenomas or carcinomas seen in the treated male mice. Consequently, the apparent statistically significant increase in the hepatocellular adenomas and carcinomas (combined) at the high dose (18/50 versus 8/50) based on regular rate comparison test (i.e., Fisher's exact test) was no longer significant when the life table test or incidental tumor test was used to control for intercurrent mortality. The trend tests from the latter two tests also failed to show statistical significance in the combined hepatocellular adenomas or carcinomas in male rats. The incidence of hepatocellular adenomas or carcinomas (combined) in female mice was not statistically significant (8/50, 13/50, and 13/50 in control, low-, and high-dose mice, respectively). In the thyroid gland, follicular cell adenomas or carcinomas (combined) were slightly, but not

significantly, increased in treated mice of both sexes over the corresponding control mice; the incidence was 0/50, 4/50, and 3/50 in males and 1/50, 3/50, and 3/50 in females in the control, low-, and high-dose groups, respectively.

These results from the NTP (1986) chronic carcinogenicity study in mice show that decaBDE treatment caused liver granulomas, liver hypertrophy, and thyroid gland follicular cell hyperplasia in males only and stomach ulcers only in females. Males, but not females, had a statistically significant increase in the incidence of hepatocellular adenomas and carcinomas at the low dose but not at the high dose. Based on these results and on increased incidence of liver granulomas and centrilobular hypertrophy and statistically significant and dose-dependent increases in the thyroid gland follicular cell hyperplasia in male mice administered 3,200 mg/kg-day or higher, no NOAEL for systemic effects for males was established. Female mice were less sensitive to the systemic toxicity of decaBDE at the doses used in this study; however, the IRIS assessment authors identified a NOAEL for a portal-of-entry effect for females of 3,760 mg/kg-day and a LOAEL of 7,780 mg/kg-day based on an increase in the incidence of stomach ulcers.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Reproductive/developmental toxicity studies are not available in humans by the oral or inhalation routes of exposure. No reproductive/developmental animal toxicity studies were identified by the inhalation route of exposure. Oral reproductive and developmental studies in experimental animals are summarized in Table 4-3.

4.3.1. Studies in Rats

Hardy et al. (2002) conducted a developmental toxicity study in rats by using a composite of three lots of a commercial flame retardant (97.34% decaBDE and 2.66% nonaBDE and octaBDE) produced by three manufacturers. In this study, Sprague-Dawley rats (25 mated females per dose group) were administered decaBDE in corn oil by gavage at doses of 0, 100, 300, or 1,000 mg/kg-day during gestation days 0 through 19. Dams were observed daily for morbidity, mortality, and signs of injury. Maternal body weight, body-weight gain, and food consumption were monitored. Dams were sacrificed on day 20 of gestation, and liver weights, gravid uterine weights, and the number of corpora lutea, implants, fetuses, and resorptions were recorded. The placenta and fetuses were examined for gross abnormalities, and histologic examinations were performed.

All dams survived decaBDE treatment until scheduled sacrifice. There were no adverse treatment-related effects observed in maternal clinical findings, body weight, or body-weight gain. Although a slight but statistically significant increase in food consumption was observed at 1,000 mg/kg-day at time intervals up to day 12 of gestation, the authors did not consider this indicative of an adverse effect of treatment.

Table 4-3. Oral developmental and reproductive studies for decaBDE in laboratory animals

Study	Species, sex, and sample size	Route, dose, and duration	Observed effects	NOAEL	LOAEL	Comments
Hardy et al. (2002)	Rat, Sprague-Dawley, pregnant female, 25/dose group	Gavage (97.34% purity); 0, 100, 300, or 1,000 mg/kg-day; gestation days 0–19	No statistically significant maternal or developmental treatment-related effects	1,000 mg/kg-day	Not identified	
Viberg et al. (2007)	Rat, Sprague-Dawley, male, 20 rats from 3–5 litters/treatment group	Single dose gavage (>98% purity in 20% fat emulsion); 0, 6.7, or 20.1 mg/kg on PND 3	Significant dose-related disruption in habituation (changes in locomotion, rearing, and total activity) at 2 months in rats, following exposure to 6.7 and 20.1 mg/kg on PND 3	Not identified	6.7 mg/kg	Single dose experiment
Viberg et al. (2003a)	Mouse, NMRI, male, 10 mice from 3–5 litters/ treatment group	Single oral gavage (>99% purity in 20% fat emulsion); 0, 2.22, or 20.1 mg/kg (PNDs 3 and 19); 0, 1.34, 13.4, or 20.1 mg/kg (PND 10)	Significant dose-related disruption in habituation (changes in locomotion, rearing, and total activity) at 2, 4, and 6 months in mice, following exposure to 20.1 mg/kg on PND 3	2.22 mg/kg	20.1 mg/kg	Single dose experiment
Tseng et al. (2006)	Mouse, CD-1, male, 50/dose group	Oral gavage (98% purity in corn oil); 0, 10, 100, 500, or 1,500 mg/kg-day; PNDs 21–70	Reduced amplitude of sperm lateral head displacement, reduced sperm mitochondrial membrane potential, increased sperm H_2O_2 generation	100 mg/kg-day	500 mg/kg-day	
Rice et al. (2007)	Mouse, C57BL6/J, 3 males and 3 females from 11–13 litters/treatment group	Micropipette administration (99.5% purity in 20% fat emulsion); 0, 6, or 20 mg/kg-day; PNDs 2–15	Decreased number of male and female pups performing the palpebral reflex on PND 14, increased struggling behavior on PND 20, effects on grip strength on PNDs 14 and 16, effects on locomotor activity on PND 70	Not identified	6 mg/kg-day	

No statistically significant differences were observed in maternal absolute or relative liver weights between treatment and control groups. At necropsy, gross examination of the dams revealed no adverse effect of treatment with decaBDE. Number of dams with viable fetuses, mean number of corpora lutea, number of implantation sites, percent preimplantation loss per dam, number of viable fetuses, and gravid uterine weights were not adversely affected by decaBDE treatment.

A statistically significant increase in the mean number of early resorptions per dam was observed in the 1,000 mg/kg-day group compared to controls. Based on the lack of a consistent dose response for this effect (the mean number of early resorptions per dam was 0.6, 0.6, 0.5, and 1.4 at 0, 100, 300, and 1,000 mg/kg-day, respectively), lack of a statistically significant positive trend associated with the effect, and the historically high incidence of this effect (0.5–1.4) for the laboratory, these effects are not considered to be of toxicological significance. Examination of the results indicated a marginal increase in the postimplantation loss/dam of 7 and 9% at 300 and 1,000 mg/kg-day, respectively, compared with 4% in controls and at 100 mg/kg-day. However, this effect was not associated with a statistically significant positive trend. A slight, but statistically not significant, decrease in the percentage of viable fetuses per implant was seen
(96, 96, 93, and 91% in the control, 100, 300, and 1,000 mg/kg-day groups, respectively). Fetal body weights, crown-rump ratio, and fetal sex ratio were not different between treatment and control groups. No adverse decaBDE treatment-related effects were identified during fetal external, skeletal, or visceral examinations. DecaBDE treatment, therefore, did not produce any evidence of maternal or developmental toxicity up to the highest dose tested of 1,000 mg/kg-day. The NOAEL for maternal and developmental toxicity in this study was 1,000 mg/kg-day, the highest dose tested.

BDE-209 (>98% purity) in a fat emulsion was administered by gavage to 3-day-old Sprague-Dawley rats at 0, 6.7, or 20.1 mg/kg (Viberg et al., 2007). A total of 20 rats were picked from three to five different litters in each treatment group. Motor activity was measured for a 60-minute period, divided into three 20-minute periods, in rats at the age of 2 months. Motor activity tests measured locomotion (horizontal movement), rearing (vertical movement), and total activity (all types of vibration within the test cage [i.e., those caused by rat movements, shaking/tremors, and grooming]). There were no clinical signs of toxicity in the BDE-209-treated rats at any given time during the experimental period, nor was there any significant difference in body-weight gain or adult weight between controls and rats treated with BDE-209. In control rats, there was a distinct decrease in locomotion, rearing, and total activity at 2 months of age, indicating habituation in response to the diminishing novelty of the test chamber over the 60-minute test period. Habituation is defined as the ability of the animals to adapt to a new environment and is characterized as initial investigation and exploration of their surroundings

followed by gradual acclimatization and acceptance of the new area. Two-month-old rats exposed to 20.1 mg/kg BDE-209 on PND 3 displayed significantly less activity for all three behavioral variables during the first 20-minute test period compared with controls, while during the third 20-minute period (40–60 minutes) they were significantly more active than the control animals. Rats receiving the low dose of BDE-209 (6.7 mg/kg) showed significantly increased locomotion activity during the second 20-minute period, significantly decreased rearing activity during the first and second 20-minute periods, and significantly higher total activity during the first and second 20-minute periods compared with the control rats. The LOAEL in this study was 6.7 mg/kg for significant changes in spontaneous motor behavior (locomotion, rearing, and total activity) in 2-month-old rats given BDE-209 on PND 3.

Immediately after the spontaneous behavior tests, nicotine-induced behavior was studied to determine whether changes in spontaneous behavior in adult rats neonatally exposed to BDE-209 included effects on development of the cholinergic system and thereby altered the response in the adult animal to the cholinergic agent nicotine (Viberg et al., 2007). The rats were given a single subcutaneous injection of 80 µg nicotine base/kg, a dose known to cause an increase in activity in experimental animals, and were immediately tested again for nicotine-induced motor behavior with regard to locomotion, rearing, and total activity during another 60-minute period divided into three 20-minute periods.

Pair-wise testing between the nicotine-injected and saline-injected rats showed, as expected, a significant increase in response to nicotine in the neonatally vehicle-treated rats during the first 20-minute period (60–80 minutes) for all three variables (locomotion, rearing, and total activity). In contrast, the nicotine-injected rats exposed to 20.1 mg/kg of BDE-209 on PND 3 showed significantly decreased activity for all three tests (locomotion, rearing, and total activity) during the first 20-minute period (60–80 minutes) compared with the rats neonatally exposed to the high BDE-209 dose and injected with saline. The authors concluded that neonatal exposure to BDE-209 on PND 3 affects adult spontaneous behavior and also affects the cholinergic system, seen as changes in the adult rats' response to the cholinergic agent nicotine.

4.3.2. Studies in Mice

Viberg et al. (2003a) assessed the potential of decaBDE to affect the developing central nervous system. The neurotoxic effects of decaBDE on spontaneous motor behavior of NMRI male mice were investigated in adult animals exposed to a single oral dose as neonates. Uptake of radiolabel by the brain of the neonatal mice orally administered ^{14}C-labeled BDE-209 on PND 3, 10, or 19 (i.e., at different stages of neonatal mouse brain development) was also measured to determine if there were age-related differences in tissue toxicokinetics that might correlate with the neurodevelopmental effects evaluated.

In this behavioral study, 3-day-old and 19-day-old male mice were given a single dose of 0, 2.22, or 20.1 mg/kg body weight decaBDE (purity estimated to be >99%) in a 20% (weight/weight) emulsion vehicle of egg lecithin-peanut oil and water. Ten-day-old mice received 0, 1.34, 13.4, or 20.1 mg/kg. The spontaneous behavior test (measuring locomotion, rearing, and total activity) was conducted in 10 mice randomly selected from the litters in each treatment group at 2, 4, and 6 months of age. There were three to five litters (four to seven males per litter) in each of the dose groups. The behavior variables were measured for a 60-minute period divided into three consecutive 20-minute periods. In order to study time-dependent changes in habituation, an habituation ratio was calculated by dividing the motor behavior measures from the 40- to 60-minute observation period by those from the 0- to 20-minute period and multiplying by 100 for each of the three different variables: locomotion, rearing, and total activity. The habituation ratios from 2-, 4-, and 6-month-old mice within each treatment group were then compared. An increase in habituation ratio indicates decreased capability to habituate to a novel environment. Data for the three spontaneous behavior variables and habituation ratio are only available in graphic form and could not be used for quantitative assessment.[1]

Treatment with decaBDE caused no clinical signs of toxicity at any time during the experimental period. Body weight and body-weight gain were not significantly different between decaBDE- and vehicle-treated mice in the three different age groups. Control mice treated on PND 3, 10, or 19 exhibited normal habituation profiles. Pair-wise testing between adult mice exposed to 20.1 mg/kg on PND 3 and control groups indicated significant changes in all three spontaneous behavior variables at 2, 4, and 6 months of age. For the first 20 minutes, mice receiving 20.1 mg/kg displayed significantly less activity for locomotion, rearing, and total activity compared with controls. During the third 20-minute period, exposure of mice to 20.1 mg/kg on PND 3 caused significantly more activity for locomotion, rearing, and total activity than the controls at 2, 4, and 6 months. The only effect noted in mice exposed to 2.22 mg/kg was a significant decrease in total activity in the first 20-minute test period compared with the controls at 2 months of age. However, total activity returned to control level during the third 20-minute period. The lower dose of 2.22 mg/kg did not elicit any significant differences in these three variables compared with controls at 4 months of age. Lower activity was observed at 2.22 mg/kg during the first 20-minute period for the rearing variable at 6 months of age compared with controls, again returning to control level during the third 20-minute period. Mice exposed neonatally up to 20.1 mg on either PND 10 or 19 did not show any significant differences in any of the variables after 2, 4, or 6 months compared with controls. The authors

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

indicated that the absence of effects on spontaneous activity in mice treated on PNDs 10 and 19 suggests that there is a critical window for the induction of the observed behavioral disturbances.

The habituation ratio calculated from the three spontaneous motor behavior variables (locomotion, rearing, and total activity) significantly increased after 2, 4, and 6 months of age in mice exposed on PND 3 to 20.1 mg/kg. Mice exposed on PND 3 to 2.22 mg/kg did not show a significant decrease in habituation capability with age. The decrease observed in the habituation capability in the adult mice exposed neonatally to the high dose indicated that the neurotoxic effect of neonatal decaBDE exposure was persistent and also worsened with age. The NOAEL in this study was 2.22 mg BDE-209/kg, and the LOAEL was 20.1 mg/kg for significant changes in spontaneous motor behavior and decreased habituation capability for locomotion, rearing, and total activity, worsening with increasing age.

Neonatal exposure to ^{14}C-decaBDE (specific activity 17.5 mCi/mmol) in mice on PND 3, 10, or 19 revealed that the labeled substance was absorbed and distributed to the brain, heart, and liver. However, the amounts in the liver 24 hours after dosing on PNDs 3, 10, and 19 were 12.6, 9.4, and 5.8%, respectively. The lower amount of BDE-209 measured as the dosing day occurred later suggests that gastrointestinal absorption may have decreased as the gastrointestinal tract matured over the first 19 days after birth. Only a small fraction of the dose reached the brain or heart 24 hours after dosing, especially with dosing on day 19 (see Viberg et al. [2003a] in section 3.2.2). However, results indicated that the amount of radioactivity increased significantly in the brain over a 7-day period in animals exposed on PNDs 3 and 10 but not in those exposed on PND 19. The radioactivity in the liver and heart was the same or decreased during the 7-day period following oral administration on PNDs 3, 10, and 19. The observed increase in radioactivity in the brain over the 7-day period suggests slow transport of decaBDE and/or its metabolites to the brain, which may account for the fact that administration on day 3 caused a more significant impact on habituation than administration on PND 10 or 19. The radiolabel data showed that the amount of decaBDE in the brain 24 hours after dosing on PNDs 3, 10, and 19 was 0.5, 0.4, and 0.06%, respectively. The radioactivity was significantly greater 7 days after BDE-209 administration on PNDs 3 and 10 compared with 24 hours after administration. The amount of radioactivity measured in the brain 7 days after dosing on PNDs 3 and 10 (PNDs 10 and 17) was 0.7 and 1.1%, respectively. There was no change in the amount of radioactivity measured in the brain of animals 7 days after dosing on PND 19.

Rice et al. (2007) examined the effects of BDE-209 (99.5% purity) on developmental milestones, sensorimotor behaviors, serum T_4 levels, and locomotor activity in male and female C57BL6/J mice administered doses of 0, 6, or 20 mg/kg-day. The BDE-209, in a 20% egg lecithin/peanut oil/water emulsion, was administered to the pups from PNDs 2–15 by using a micropipette to avoid gavaging neonatal animals. There were no delays in postnatal developmental milestones (pinna detachment, incisor eruption, eye opening, vaginal opening, or

testes descent) from BDE-209 treatment in male or female mice. There were no effects of exposure on anogenital distance or crown-rump length.

The authors developed a special functional observational battery (FOB), suitable for neonatal mice, to examine a series of home-cage, reflexive, and sensorimotor behaviors, measured on PND 14, 16, 18, or 20. Three of the FOB endpoints were affected by BDE-209 exposure: palpebral reflex, forelimb grip, and struggling behavior during handling. On PND 14, significantly fewer male and female pups in the 6 and 20 mg/kg-day groups performed the palpebral reflex compared with controls. On PNDs 14 and 16, fewer male pups in the 20 mg/kg-day group performed an effective forelimb grip compared with same-sex controls. Male and female pups in the 6 mg/kg-day group struggled significantly more on PND 20, while they were being handled for the FOB, compared with controls and the high-dose group.

Rice et al. (2007) also examined the locomotor activity of male and female mice in a novel environment over a 2-hour period on PND 70 and at approximately 1 year of age (one male and one female from each of 9–13 litters were evaluated). Locomotor activity declined over the course of the 2-hour assessment in PND 70 males and in females in the control and treated groups. However, the rate of decline was significantly different ($p = 0.04$) in male mice exposed to 6 and 20 mg/kg-day compared with control animals, an effect that was most pronounced in the high-dose males during the first 1.5 hour of the 2-hour activity session. There was a non-dose-related decline in the locomotor activity in the female mice during the 2-hour activity session. Unlike the males, they became hypoactive compared with the controls. There was no effect of BDE-209 treatment on the locomotor activity of the 1-year old male and female mice.

The effect of BDE-209 treatment on serum T_4 in PND 21 offspring was also investigated in this study. A dose-related reduction in serum T_4 in comparison with controls occurred in males but not in females.

The LOAEL in this study was 6 mg/kg-day, the lowest dose tested, for decrease in the percent of male and female pups performing the palpebral reflex on PND 14, for increased struggling behavior of male mice on PND 20, for decreased T_4 levels in male mice, and for effects on locomotor activity of male mice on PND 70.

Tseng et al. (2006) studied the effects of BDE-209 on mouse sperm function, DNA content, and histopathology of the testes. CD-1 male mice (50/dose group) were fed decaBDE (98% purity) by gavage in corn oil at 0, 10, 100, 500, or 1,500 mg/kg-day from PNDs 21–70. Body weight, body-weight gain, and absolute and relative weights of the testis, epididymis, cauda epididymis, and seminal vesicle of treated animals were not significantly different from controls at sacrifice on PND 71. Morphology of the testicular tissues appeared normal in all treated groups compared with controls. DNA content in testis cells was unaffected by treatment with BDE-209. No significant differences in sperm motility (expressed as the ratio between the

number of motile sperm and total number of sperm), sperm count, or morphology were seen among the groups exposed to BDE-209 and the controls. Sperm motion velocity parameters were measured, including curvilinear velocity, straight-line velocity, and amplitude of the lateral head displacement (ALH). The only effect seen on sperm motion velocity was a significant decrease in ALH at 500 and 1,500 mg/kg-day. Significant increase in the generation of hydrogen peroxide in the sperm of sexually mature mice occurred at 500 and 1,500 mg/kg-day. The mitochondrial membrane potential (MMP) of sperm cells, a predictor of sperm fertility potential, was assessed using a lipophilic cationic compound, JC-1, which possesses the ability to differentially label mitochondria with high and low membrane potential (Gravance et al., 2001). Mice exposed to 500 and 1,500 mg/kg-day were found to have a significant decrease in high MMP sperm. In addition, the MMP was negatively and significantly associated with the generation of sperm hydrogen peroxide. The NOAEL in this study was 100 mg/kg-day and the LOAEL 500 mg/kg-day for decrease in ALH and MMP and increased generation of hydrogen peroxide in the sperm of adult mice.

4.4. OTHER ENDPOINT-SPECIFIC STUDIES

4.4.1. Receptor Site Interactions

Some studies of halogenated aromatic compounds show that they 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 polychlorinated biphenyls (PCBs) suggest that PBDEs might activate the aryl hydrocarbon receptor (AhR) and the estrogen receptor (ER). Based on the data from the well-studied PCBs and dioxins, the activation of these receptors is associated with immunosupression, reproductive effects, and carcinogenesis (Klaassen, 1996; Bock, 1994), all endpoints of interest for PBDEs. Studies of receptor interactions of BDE-209 with the AhR, ER, pregnane X receptor (PXR), and steroid X receptor (SXR) are summarized in the sections that follow. As a group, these studies indicate that the ability of BDE-209 to activate signal transduction responses via the receptors studied is minimal or negligible.

4.4.1.1. *Aryl Hydrocarbon Receptor Studies*

Although in vivo studies investigating mechanisms of liver toxicity in animals have not been identified, several in vitro systems have evaluated the ability of decaBDE to interact with the AhR by using liver tissues or cell lines. Brown et al. (2004) investigated the potential of decaBDE to bind to and activate the AhR (using a gel retardation assay) and the ability of decaBDE to induce the expression of dioxin-responsive genes (using the reporter gene based

Chemical-Activated Luciferase eXpression [CALUX] assay). The decaBDE used in this study was 85.5% purity, and the identities of the other components present in the preparation were not reported. Incubation of decaBDE with hepatic cytosol prepared from guinea pigs and subsequent incubation with [^{32}P]-labeled dioxin-responsive element (DRE)-containing oligonucleotide, followed by resolution of the ligand:AhR:[^{32}P]DRE complex by gel retardation analysis, indicated that decaBDE (20 µM) stimulated AhR-DNA binding but to a much lower level (26%) than 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). DecaBDE demonstrated some activity in the CALUX assay. Based on the results from gel retardation and reporter gene assays, decaBDE may induce AhR-dependent DNA binding and gene expression but with very low potency if the activity observed is solely attributable to decaBDE. However, the low but measurable activity may have been caused by contaminants since the test material was impure.

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

Villeneuve et al. (2002) evaluated the dioxin-like potency of decaBDE (99% purity) at environmentally relevant concentrations (up to 500 ng/mL) by using in vitro luciferase assays with H4IIE-luc recombinant rat hepatoma cells. DecaBDE did not induce AhR-dependent gene expression. The authors calculated that most PBDEs, including decaBDE, were at least 10,000 times less potent than TCDD for inducing AhR-mediated responses in vitro.

The affinity of decaBDE for rat hepatic AhR was evaluated through competitive binding assays (Chen et al., 2001). Results indicated that decaBDE has no AhR-binding activity. The ability of decaBDE to induce hepatic CYP-450 enzyme activity by means of EROD assays in human, rat, chick, and rainbow trout cells was also determined in this study. Incubation of decaBDE (of unspecified purity) with cultures of primary rat hepatocytes, primary chick embryo hepatocytes, two human cell lines (hepatoma HepG2 and intestinal Caco-2), and one rat cell line (hepatoma H4IIE) showed that decaBDE did not induce EROD activity in any cell line tested. Moreover, decaBDE was almost completely inactive in a gel retardation assay, suggesting that decaBDE did not activate the AhR.

Pullen et al. (2003) investigated the effect of decaBDE on CYP-450 activity in mouse hepatocytes. Incubation of decaBDE (of unspecified purity) with hepatocytes isolated from C57BL/6 mice for 24 hours did not induce CYP-1A1 (measured as EROD activity), indicating that decaBDE does not act as a specific ligand for the AhR and, thus, exerts no effect via the

AhR pathway as do many of the polycyclic aromatic hydrocarbons. Together, these studies suggest that decaBDE has very limited potential to activate the AhR signal transduction pathway, which is an important step in the mode of action through which many persistent aromatic hydrocarbons impact cell regulation and cell maintenance (Klaassen, 1996).

4.4.1.2. *Estrogen Receptor Studies*

ER-mediated (estrogenic) effects of decaBDE at environmentally relevant concentrations were investigated by Villeneuve et al. (2002) in an in vitro luciferase assay system. Incubation of decaBDE (99% purity) at concentrations up to 500 ng/mL with MVLN recombinant human breast carcinoma cells for 72 hours showed that decaBDE did not exhibit estrogenic effects. Most PBDEs, including decaBDE, were at least 50,000 times less potent than 17β-estradiol (E2) for inducing ER-mediated gene expression. This study also examined the ability of decaBDE to displace E2 or testosterone from serum proteins in a competitive hormone displacement assay. Incubation of decaBDE at concentrations up to 2.5 µg/mL in the presence of ^{3}H-estradiol or ^{3}H-testosterone and hormone-stripped carp serum for 15 hours indicated that decaBDE did not cause any significant displacement of these hormones in vitro. These results suggest that decaBDE and other PBDEs are not likely to elicit E2-mediated gene expression at the exposure levels currently observed in fish and wildlife.

4.4.1.3. *Other Receptors*

Pacyniak et al. (2007) conducted a study of the PXR and its human counterpart SXR by using HepG2 cells transvested with the appropriate complementary DNA (cDNA), the receptor response elements, and a luciferase reporter vector. The cultured cells were exposed to 0, 0.1, 1, 10, or 100 µM concentrations of BDE-209. With the PXR there was a very slight dose-related increase in relative luciferase activity that achieved significance for only the highest concentration. With the SXR, there was no impact of luciferase for any concentration other than the 100 mM preparation. Accordingly, BDE-209 appears to be a weak inducer for PXR and SXR. The authors also compared the response in PXR knock-out mice (10–12 weeks old) with the wild-type mice and found that CYP-3A11 was induced to a greater extent in the wild-type animals, indicating that BDE-209 seems to be a ligand for the PXR. However, even in the knock-out mice, some CYP-3A11 protein was identified, suggesting that the PXR was not the only receptor affected by BDE-209. The authors suggested that the persisting CYP-3A11 protein could be related to activation of the constitutive androstane receptor (CAR).

4.4.2. Immunological Studies

No in vivo studies that specifically evaluated the immunosuppressive effects of decaBDE have been identified. However, in chronic dietary studies, decaBDE has been found to induce

histological changes in lymphoid organs, including the spleen (fibrosis) and mandibular lymph nodes (hyperplasia) in male rats at a dose of 2,240 mg/kg-day (NTP, 1986). Although these histopathological changes may play an important role in flagging decaBDE for immunotoxicity, they are at the high end of the spectrum of doses evaluated, and the lack of studies to investigate the functional effects on immune cells from exposure to decaBDE precludes complete assessment of its immunotoxic potential.

Only one study investigated the immunotoxic effects of decaBDE on intact lymphocytes. Pullen et al. (2003) isolated splenocytes from C57BL/6 mice and incubated the cells in culture with decaBDE (purity not specified). DecaBDE was not cytotoxic to these cells at 3 µmol/L. Treatment of cells for 48 hours with 3 µmol/L decaBDE did not cause attenuation of interleukin-2-receptor α chain (CD25) expression (evaluated by immunohistochemistry and confocal microscopy or laser scanning cytometry). The lack of attenuation of CD25 expression suggests that decaBDE is not likely to alter a surface marker (which is essential for lymphocyte proliferation during immune response) and may not affect the immune system in an immunosuppressive manner. Pullen et al. (2003) also evaluated the potential of decaBDE to alter cytokine production. Results showed that decaBDE did not have any effect on the production of the cytokines IFN-γ, IL-2, IL-6, and IL-10, indicating that decaBDE does not interfere with the cells' ability to synthesize protein.

4.4.3. Genotoxicity

In vitro studies were conducted to evaluate the mutagenic potential of decaBDE (NTP, 1986). DecaBDE was tested in *Salmonella typhimurium* TA98, TA100, TA1535, or TA1537 strains up to 10,000 µg/plate in the presence or absence of exogenous metabolic system (S9 fractions) prepared from the livers of Aroclor 1254-induced male Sprague-Dawley rats or male Syrian hamsters. Results indicated that decaBDE was not mutagenic in the salmonella assay systems.

The mutagenicity of decaBDE at doses of up to 10 µg/mL was evaluated in L5178Y/TK$^{+/-}$ mouse lymphoma cell assay system in the absence or presence of S9 prepared from the livers of Aroclor 1254-induced male F344/N rats. DecaBDE did not induce mutagenic potential in this assay system. DecaBDE at doses up to 500 µg/mL did not induce sister-chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in the absence or presence of S9 prepared from the livers of Aroclor 1254-induced male Sprague-Dawley rats (NTP, 1986).

Results from these studies provide evidence that parent decaBDE in the presence or absence of an exogenous liver metabolic system does not react directly or indirectly with DNA to cause gene mutations, DNA damage, or chromosomal effects.

4.5. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.5.1. Oral

Several studies have been conducted to evaluate the effects of decaBDE and to elucidate its mode of action. Long-term dietary studies conducted by NTP (1986) have identified nonneoplastic liver and thyroid histopathology, such as liver thrombosis and degeneration in male rats, and liver granulomas, centrilobular hypertrophy, and thyroid follicular cell hyperplasia in male mice.

Neurobehavioral developmental effects on habituation were seen in adult male rats (Viberg et al., 2007) and adult male mice (Viberg et al., 2003a) given a single dose of BDE-209 during the postnatal period. Male and female mice pups given BDE-209 during the neonatal period showed early effects on palpebral reflex, forelimb grip, and struggling behavior. Locomotor activity was also affected in young adult male mice (Rice et al., 2007).

Behavioral disturbances observed in the mature rats and mice exposed to decaBDE as neonates (Rice et al., 2007; Viberg et al., 2007, 2003a) raise concern about possible developmental neurotoxicity in children. In the study by Viberg et al. (2003a), neonatal male mice were given a single dose of decaBDE (up to 20.1 mg/kg) in a fat emulsion on PNDs 3, 10, or 19 and spontaneous motor behavior tests (measuring locomotion, rearing, and total activity) were conducted in the mice at 2, 4, or 6 months of age. In addition, an habituation ratio was also determined for each behavior variable at the tested ages. Exposure of 3-day-old mice to 20.1 mg/kg decaBDE resulted in significant dose-related changes in all three spontaneous behavior variables at 2, 4, and 6 months of age. However, adult mice exposed neonatally up to 20.1 mg on either PND 10 or 19 did not show any significant differences in any of the variables after 2, 4, or 6 months compared with controls. In the study in rats (Viberg et al., 2007), significant dose-related disruption in habituation (changes in locomotion, rearing, and total activity) was observed in 2-month-old rats, following exposure to BDE-209 at 6.7 and 20.1 mg/kg on PND 3.

Rice et al. (2007) used a similar approach in evaluating locomotor activity in groups of 9–13 male and female C57BL6/J mice exposed to 6 or 20 mg/kg-day BDE-209 from PNDs 2–15 and observed after placement in a novel environment on PND 70 during a 2-hour period. The exposed males showed greater activity than the controls throughout the observation period. Early in the period, the exposed male mice were hyperactive compared with controls; the difference between the exposed animals and controls declined with time but locomotor activity remained greater than that for the controls at the end of the 2-hour observation period. In the case of the females, the activity of all groups was similar for the first 45 minutes of exposure but the females became less active than the controls thereafter. The linear slope for the decline in activity among the exposed males was significantly different from that of controls; the 20 mg/kg group was affected to a greater extent than the 6 mg/kg group. The females on PND 70 and both

sexes when examined at 1 year after birth did not display similar dose-related differences in locomotor activity.

Rice et al. (2007) also examined a number of developmental and behavioral parameters during the dosing period and for five days after dosing ceased. BDE-209 exposure did not impact the developmental endpoints evaluated (pinnae detachment, incisor eruption, eye opening, vaginal opening, testes descent). The behavioral parameters studied were selected as measures of home-cage, motor, and sensorimotor behaviors. There was no apparent impact on most measures of behavior; however, palpebral reflex, forelimb grip, and struggling during handling appeared to be impacted by BDE-209 exposure. There was a significantly poorer performance of the 20 mg/kg-day group in the palpebral reflex test on PND 14 and in the grip test (males only) on PND 16. On PND 20, the struggling behavior of the group exposed to 6 mg/kg was significantly increased compared with the controls and 20 mg/kg-day group.

The most striking dose-related trend in the Rice et al. (2007) study was the locomotive activity profile of males at PND 70. The direction of this trend differed from that observed by Viberg et al. (2007, 2003a). In the Rice et al. (2007) study, activity in the exposed and control mice decreased across the observation period, while in the Viberg et al. (2007, 2003a) studies activity increased across the observation period for the exposed animals compared with the controls. However, the exposure and testing conditions varied between the two studies and, thus, the two sets of results are not directly comparable.

4.5.2. Inhalation

No data are available on the toxicity of decaBDE by the inhalation route of exposure.

4.5.3. Mode-of-Action Information

Impaired development of the cholinergic system during the postnatal “brain growth spurt” period has been offered as a plausible hypothesis for the observed neurodevelopmental impact of the PBDEs on adult responses to a novel environment (Viberg et al., 2007, 2003b). The authors described a critical window of vulnerability in development of the cholinergic system during postnatal development that in mice occurs in the first few weeks after birth with a peak at PND 10 (Viberg et al., 2003b). They hypothesize that any agent that caused a deficit in cholinergic receptors during this period could cause a hypoactive response to cholinergic stimulants, including exposure to a novel environment in adulthood.

Viberg et al. (2007) tested this hypothesis in Sprague-Dawley rats. Two-month-old rats that had been exposed to vehicle, 6.7 mg/kg, or 20 mg/kg BDE-209 on PND 3 were given a subcutaneous injection of nicotine or saline solution. The vehicle control animals and animals in the 6.7 mg/kg dose group receiving the nicotine demonstrated the typical hyperactive response of mature rats when compared with the saline-injected rats during the 60–80 minute observation

period. Those given the 20.1 mg/kg dose were markedly hypoactive compared with the saline controls. These observations provide some support for the hypothesis that impaired prenatal development of the cholinergic system may play a role in the habituation deficits observed in adult rats and mice that were prenatally exposed to decaBDE during the critical window for system development.

Other studies by the Eriksson/Viberg research team using PBDE compounds with four, five, and six bromines have shown effects on locomotion and habituation that are similar to those observed for the deca compound. However, in the study of BDE-99, when the timing of exposure was varied, the window of greatest vulnerability occurred on day 10 rather than on days 3 or 19 (Eriksson et al. 2002). Thus, the results for BDE-99 are different from those for decaBDE reported in Viberg et al. (2003a). However, the data from the radiolabel component of the study provide a rationale for the difference in the apparent window of vulnerability. Two contributing factors are observed. First, absorption from the gastrointestinal tract decreases as the neonatal mice age during their first few weeks of life. Thus, less of the decaBDE reaches the liver for distribution on days 10 and 19 than on day 3. Secondly, distribution to the brain is slow. The amount of radiolabel in the brain is higher (0.7%) when measured 7 days after dosing on PND 3 than when measured 24 hours after dosing (0.5%). The radioactivity measured 7 days after dosing was also higher than when measured 24 hours after dosing on PND 10 (0.4% at 24 hours; 1.1% at 7 days). Less than 0.1% of the label reaches the brain when the compound is administered on day 19. While evidence exists that demonstrates BDE-209, as well as other PBDEs, interact at the neurological level, data are inadequate to determine the mode of action for BDE-209.

DecaBDE caused thyroid gland follicular cell hyperplasia and thyroid tumors in male mice, effects that are indicative of thyroid toxicity (NTP, 1986). Based on these effects, decaBDE may share the general property of organohalogen compounds in which in vivo exposure in rodents results in reduction of serum total and free thyroid hormone (T_4) levels (Legler and Brouwer, 2003). Thyroid hormone disruption has been hypothesized to arise from at least two different mechanisms, one of which is the induction of liver enzymes of different enzyme families, including CYP-1A1 and CYP-2B (McDonald, 2002). Induction of liver UDPGTs may increase the rate of T_4 conjugation and excretion, which is believed to be a mechanism for TCDD-induced thyroid effects. An increase in the metabolism of T_4 will result in enhanced excretion and thus a drop in its circulating levels. The other mechanism involves alteration of thyroid homeostasis through hormone mimicry. Potential thyroid toxicants may be metabolized by liver enzymes to metabolites that compete with the thyroid transport protein, transthyretin, a key protein involved in the transport of T_4 through the blood and into the developing tissues in rodents.

Zhou et al. (2001) evaluated the interference of decaBDE with thyroid hormone homeostasis in weanling rats exposed to decaBDE (>98% purity) up to 100 mg/kg-day for 4 consecutive days. Results indicated that decaBDE did not affect hepatic enzyme activities or serum T_4, T_3, TSH, and hepatic UDPGT activity at doses up to 100 mg/kg-day. Rice et al. (2007) also evaluated T_4 levels in the blood of C57BL6/J mice (1 male, 1 female/litter) on PND 21 following exposure to 0.6 or 20 mg/kg-day BDE during PNDs 2–15. In this instance, there was a dose-related reduction in the T_4 levels for males but not females. Despite the possibility of thyroid hormone involvement in the neurodevelopmental impact of BDE-209 on the habituation response in male mice exposed to a single dose on PND 3, there are no mode-of-action data that link thyroid hormones to the neurobehavioral observations reported by Viberg et al. (2003a).

In the chronic dietary studies conducted by NTP (1986), decaBDE has been found to induce histologic changes in lymphoid organs, including the spleen (fibrosis) and mandibular lymph nodes (hyperplasia), in rats. These histopathologic changes could flag decaBDE as a potential immunotoxicant. No in vivo functional immunotoxicity studies were identified. Results from an in vitro study investigating the immunotoxic effects of decaBDE indicated that decaBDE did not show any attenuation of CD25 expression or changes in cytokine production (Pullen et al., 2003). As such, decaBDE is unlikely to affect the immune system in an immunosuppressive manner.

Several in vivo and in vitro studies have evaluated decaBDE for its potential to activate the AhR signal transduction pathway and to induce gene expression, resulting in increased phase I and phase II metabolism. Taken together, the in vivo and in vitro studies suggest that decaBDE has very low or no potential to bind the AhR. The inability of decaBDE to elicit CYP-450 induction or AhR binding and gene expression, indicating lack of potential to activate the AhR signal transduction pathway, suggests that decaBDE may cause liver toxicity through a different mechanism and that the observed liver effects are not an adaptive effect related to enzyme induction. Results from the Morck et al. (2003) study indicated high concentration of tissue-bound radioactivity in the liver, and the radioactivity was assumed to be bound to macromolecules. The authors asserted that binding occurred via reactive metabolites. Formation of such metabolites may be responsible for the liver toxicity observed on exposure to decaBDE. However, the enzymes responsible for decaBDE metabolism and reactive intermediates have not been identified. The existing data show tissue binding and are therefore not adequate to elucidate a potential role in the mode of action.

A number of studies have also indicated that brominated flame retardants may interfere with estrogen pathways by binding to and activating ERs (Legler and Brouwer, 2003). No in vivo studies were identified that evaluated the specific estrogenic effects of decaBDE. Furthermore, specific effects attributable to estrogenic dysregulation were not identified in the

available animal studies, although some developmental toxicity endpoints were observed. Mechanistic studies do not support effects of decaBDE on ER pathways.

4.6. EVALUATION OF CARCINOGENICITY

4.6.1. Summary of Overall Weight of Evidence

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the descriptor "suggestive evidence of carcinogenic potential" is appropriate for decaBDE. This descriptor of the database is appropriate when the weight of evidence is suggestive of carcinogenicity and a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion. This descriptor covers a spectrum of evidence associated with varying levels of concern for carcinogenicity, ranging from a positive cancer result in the only study on an agent to a single positive cancer result in an extensive database that includes negative studies in other species.

The weight of evidence of human carcinogenicity of decaBDE is based on (1) no studies of cancer in humans exposed to decaBDE; (2) a statistically significant increase in incidence of neoplastic nodules in the liver of low- and high-dose male rats and high-dose female rats; (3) a significantly increased incidence of hepatocellular adenoma or carcinoma (combined) in male mice at the low dose and marginally increased incidence at high dose; (4) a nonsignificantly increased incidence of hepatocellular adenoma or carcinoma (combined) in female mice; (5) a slightly greater (but statistically not significant) incidence of thyroid gland adenomas or carcinomas (combined) in dosed male and female mice; (6) a significantly increased incidence in male mice, at both doses, of follicular cell hyperplasia, considered by many as a precursor to thyroid tumors; and (7) an apparent absence of genotoxic potential.

Based on the results of the chronic and genotoxicity studies, NTP (1986) concluded that there was "some evidence of carcinogenicity" for male and female rats as shown by increased incidences of neoplastic nodules of the liver in low-dose males and high-dose groups of each sex. There was "equivocal evidence of carcinogenicity" for male mice as shown by increased incidences of hepatocellular adenomas or carcinomas (combined) in only the low-dose group and slight increase of thyroid gland follicular cell adenomas or carcinomas (combined) in both dosed groups. There was "no evidence of carcinogenicity" for female mice. Several nonneoplastic lesions were observed at increased incidences, the most notable being thyroid gland follicular cell hyperplasia in male mice. The weight of experimental evidence is on the strong end of the spectrum for the descriptor "suggestive evidence of carcinogenic potential," since there is suggestive evidence that decaBDE is carcinogenic for more than one species, sex, and site. Based on the weight of evidence, a dose-response assessment of the carcinogenicity of decaBDE is deemed appropriate

4.6.2. Synthesis of Human, Animal, and Other Supporting Evidence

No information is available on the carcinogenicity of decaBDE in humans. Chronic dietary studies of decaBDE have been conducted in rats and mice (NTP, 1986), and carcinogenicity data are presented in Tables 4-4 and 4-5. DecaBDE caused substantial dose-related increases in neoplastic nodules in rats with statistical significance at the high dose in both sexes (Table 4-4). There were no statistically significant changes in hepatocellular carcinoma in either sex. Although there was a positive trend in mononuclear cell leukemia in male rats, the increase was marginal and not considered to be biologically significant due to the unusually high incidence in controls.

In mice, insignificant increases in hepatocellular adenoma occurred in males at low (3,200 mg/kg-day) and high (6,650 mg/kg-day) doses compared with controls. The incidence of hepatocellular carcinoma was not significantly increased in male mice at either dose. In these male mice, however, significantly increased hepatocellular adenomas or carcinomas (combined) were observed in the low-dose group but only marginally increased in the high-dose group. An increased incidence of hepatocellular adenoma and carcinoma was also observed in the female mice; however, these changes were not statistically significant in either treated dose group. There was also a slight (but not statistically significant) increase in the incidence of thyroid tumors in mice of both sexes, but this was not observed in rats. The biological relevance of this finding in mice is supported by the accompanying increased incidence of follicular cell hyperplasia in male mice; the increase in hyperplasia in females was not statistically significant.

The increased incidence of neoplastic nodules of the liver observed in male and female rats constitutes some evidence of carcinogenicity in treated rats, and this observation is supported by the significantly increased incidence of hepatocellular adenoma and carcinoma (combined) in low-dose male mice and nonsignificantly increased incidence in female mice. Although the study results from each individual species/sex combination were judged to be either “some” or “equivocal” evidence of carcinogenicity, a common target organ (liver) in two species and both sexes indicates a potential for decaBDE to cause cancer in treated animals. In addition to the liver cancer, slight increases in thyroid follicular cell tumors in mice were accompanied by hyperplasia. Based on the increasing agreement that hyperplasia is a stage of the thyroid follicular cell carcinogenic process (NAS, 2000; Hard, 1998; U.S. EPA, 1998b; Hill et al., 1989), decaBDE would be considered as eliciting a carcinogenic effect in a second target organ (i.e., the thyroid) in mice.

4.6.3. Mode-of-Action Information

Genotoxicity studies have found that decaBDE does not appear to be mutagenic since it did not induce gene mutations in *S. typhimurium* strains or in mouse L5178Y lymphoma cells. Also, decaBDE did not induce sister-chromatid exchanges or chromosomal aberrations in

Chinese hamster ovary cells in the presence or absence of metabolic activation systems (NTP, 1986).

There are no other studies of the carcinogenic mode of action of decaBDE. The mode of action is unknown.

Table 4-4. Incidence of liver neoplastic nodules and carcinomas in rats fed decaBDE for 2 years

		Males			Females		
Dose groups	**ppm**	**0**	**25,000**	**50,000**	**0**	**25,000**	**50,000**
	mg/kg-day	**0**	**1,120**	**2,240**	**0**	**1,200**	**2,550**
Number examined		**50**	**50**	**50**	**50**	**50**	**50**
Liver neoplastic nodules							
Overall rates		1/50 (2%)	7/50 (14%)[a]	15/49 (31%)[a]	1/50 (2%)	3/49 (6%)	9/50 (18%)[a]
Adjusted rates		2.9%	27.1%	52.7%	2.5%	9.1%	24.4%
Terminal rates		1/35 (3%)	6/24 (25%)	13/26 (50%)	1/40 (3%)	3/33 (9%)	7/34 (21%)
Week of first observation		104	89	87	104	104	87
Historical incidence		3.5%			2.6%		
Hepatocellular carcinoma							
Overall rates		1/50 (2%)	1/50 (2%)	1/49 (2%)	0/50 (0%)	2/49 (4.1%)	0/50 (0%)
Liver neoplastic nodule or hepatocellular carcinoma							
Overall rates		2/50 (4%)	8/50 (16%)[a]	15/49 (31%)[a]	1/50 (2%)	5/49 (10%)	9/50 (18%)[a]
Adjusted rates		5.2%	31.1%	52.7%	2.5%	15.2%	24.4%
Terminal rates		1/35 (3%)	7/24 (29%)	13/26 (50%)	1/40 (3%)	5/33 (15%)	7/34 (21%)
Week of first observation		97	89	87	104	104	87

[a]Statistically significantly different from controls ($p < 0.05$), based on life table tests and incidental tumor tests.

Source: NTP (1986).

Table 4-5. Incidence of nonneoplastic or neoplastic lesions in the liver and thyroid gland of mice fed decaBDE for 2 years

		Males			Females		
Dose groups	**ppm**	**0**	**25,000**	**50,000**	**0**	**25,000**	**50,000**
	mg/kg-day	**0**	**3,200**	**6,650**	**0**	**3,760**	**7,780**
Number examined		**50**	**50**	**50**	**50**	**50**	**50**
Hepatocellular adenoma							
Overall rates		4/50 (8%)	12/50 (24%)	12/50 (24%)	5/50 (10%)	10/50 (20%)	7/50 (14%)
Adjusted rates		19.0%	46.2%	39.0%	16.8%	31.2%	21.9%
Terminal rates		3/19 (16%)	11/25 (44%)	7/24 (29%)	4/27 (15%)	9/31 (29%)	7/32 (22%)
Week of first observation		81	100	60	83	102	103
Hepatocellular carcinoma							
Overall rates		5/50 (10%)	14/50 (28%)	8/50 (16%)	3/50 (6%)	4/50 (8%)	7/50 (14%)
Adjusted rates		20.7%	42.9%	26.8%	10.7%	12.1%	20.8%
Terminal rates		1/19 (5%)	8/25 (32%)	4/24 (17%)	2/27 (7%)	3/31 (10%)	6/32 (19%)
Week of first observation		81	72	76	101	93	96
Hepatocellular adenoma or carcinoma							
Overall rates		8/50 (16%)	22/50 (44%)[a]	18/50 (36%)	8/50 (16%)	13/50 (26%)	13/50 (26%)
Adjusted rates		33.9%	67.7%	56.5%	26.7%	39.1%	39.1%
Terminal rates		4/19 (21%)	15/25 (60%)	11/24 (46%)	6/27 (22%)	11/31 (35%)	12/32 (38%)
Week of first observation		81	72	60	83	93	96
Thyroid gland							
Hyperplasia, follicular cell		2/50 (4%)	10/50 (20%)[a]	19/50 (38%)[a]	4/50 (8%)	9/50 (18%)	7/49 (14%)
Follicular cell adenoma							
Overall rates		0/50 (0%)	3/50 (6%)	3/50 (6%)	1/50 (2%)	3/50 (6%)	2/49 (4%)
Adjusted rates		0%	10.8%	12.5%	2.9%	7.8%	6.3%
Terminal rates		0/19 (0%)	2/25 (8%)	3/24 (13%)	0/27 (0%)	1/31 (3%)	2/32 (6%)
Week of first observation			90	103	95	80	103
Follicular cell adenoma or carcinoma (combined)							
Overall rates		0/50	4/50 (8%)	3/50 (6%)	1/50 (2%)	3/50 (6%)	3/49 (6%)
Adjusted rates		0%	14.7%	12.5%	2.9%	7.8%	9.4%
Terminal rates		0	3/25 (12%)	3/24 (13%)	0/27 (0%)	1/31 (3%)	3/32 (9%)
Week of first observation			90	103	95	80	103

[a]Statistically significantly different from controls ($p < 0.05$), based on life table tests and incidental tumor tests.

Source: NTP (1986).

4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.7.1. Possible Childhood Susceptibility

As discussed in section 4.3.1, a gavage study (Hardy et al., 2002) in which rats were exposed in the diet to decaBDE (97.34% purity) during gestation days 0–19 reported no effects on number of dams with viable fetuses, mean number of corpora lutea, number of implantation sites, percent preimplantation loss per dam, number of viable fetuses, gravid uterine weights, fetal body weights, crown-rump ratio, and fetal sex ratio. No effects were reported during external, skeletal, or visceral examination of rat fetuses. The NOAEL in this study was 1,000 mg/kg-day, the highest dose tested. In a study in male mice (Tseng et al., 2006) exposed to BDE-209 from PNDs 21–70 (see section 4.3.2), effects were seen on sperm function, DNA content, and histopathology of the testes at 500 mg/kg-day but not at 100 mg/kg-day.

In a study in rats (Viberg et al., 2007), exposure of the animals to the lowest dose tested of 6.7 mg/kg-day BDE-209 on PND 3 resulted in significant changes in spontaneous motor behavior (locomotion, rearing, and total activity) in 2-month-old rats. In the study of Rice et al. (2007), effects on the palpebral reflex, struggling behavior and locomotor activity were seen in mice given the lowest dose of 6 mg/kg-day BDE-209 on PNDs 2–15.

Neurobehavioral alterations were observed in mature mice when male mice were exposed to 20.1 mg/kg decaBDE as neonates on PND 3 but not on PNDs 10 and 19 (Viberg et al., 2003a). A significant increase in the amount of radioactivity in the brain on day 7 after exposure was noted when the neonates were exposed to ^{14}C-decaBDE on PNDs 3 and 10 but not on PND 19 (Viberg et al., 2003a). The increase in the radioactivity in the brain, coupled with the behavioral disturbances on exposure to decaBDE on PND 3, suggests that differences may exist in the absorption and distribution of decaBDE among the 3-, 10-, and 19-day-old neonates. In addition, differences exist in the timing of the brain growth spurt in rodents (first few weeks after birth) and humans (starts in the sixth month of gestation and continues through the first 2 years of life after birth). The significance of this difference with regard to the potential for effects on children from exposure to BDE-209 is not clear. Moreover, whether other targets of decaBDE (thyroid and liver) are more sensitive in children is unknown since the enzymes responsible for decaBDE metabolism have not been identified and the mechanisms for age-dependent kinetic differences are not completely clear. Although no studies were identified that investigated placental transfer, decaBDE has been found in human breast milk, suggesting that newborns and infants have a potential source of exposure via this route.

4.7.2. Possible Gender Differences

All absorption studies following oral administration of decaBDE were conducted in male rats, precluding a comparison of kinetics in males versus females. There were no clear gender-dependent differences in decaBDE toxicity in short-term and subchronic studies conducted in

both sexes of rats and mice (NTP, 1986). However, long-term exposure studies to decaBDE indicated that male rats may be more sensitive than females. Male rats exhibited nonneoplastic lesions, including liver thrombosis and degeneration, splenic fibrosis, and lymphoid hyperplasia, and male mice showed increased incidences of liver granulomas, hepatocellular hypertrophy, and thyroid follicular cell hyperplasia, while female rats and mice appeared to be refractory to these systemic effects of decaBDE at the tested doses (NTP, 1986). One exception was that female mice had a higher incidence of stomach ulcers than their male counterparts. Although a significantly increased incidence of liver neoplastic nodules was observed in both sexes of rats (NTP, 1986), hepatocellular carcinomas or adenomas were not significantly increased in male mice only. No gender-specific effects on reproductive organs were identified.

The study of Rice et al. (2007) investigated several endpoints in male and female mice. In PND 21 offspring of mice treated with decaBDE on PNDs 2–15, a dose-related reduction in serum T_4 was observed in males but not females. Development of forelimb grip strength was delayed at PND 16 in exposed males in the high-dose group but not in females. Struggling behavior was more apparent in males than in females, and effects on locomotor activity were observed on PND 70 in male mice but not in female mice.

Overall, it appears that males may be more susceptible to some effects of decaBDE compared to females, although this is not true for all endpoints. Underlying mechanisms for the apparent differences in susceptibility have not been identified.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

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

Epidemiological studies or case reports are not available for decaBDE.

Available animal studies of repeated oral exposure to decaBDE include a 4-day study in rats (Zhou et al., 2001), 14-day studies in rats and mice (NTP, 1986), 13-week studies in rats and mice (NTP, 1986), and 2-year studies in rats and mice (NTP, 1986). In addition, decaBDE was also tested for its toxicity in reproductive and developmental studies (Rice et al., 2007; Viberg et al., 2007, 2003a; Tseng et al., 2006; Hardy et al., 2002). The results from these studies are summarized in Tables 4-1, 4-2, and 4-3. Among the available studies, several potential principal studies for deriving an RfD were identified: the 2-year chronic studies in rats and mice (NTP, 1986) and neurodevelopmental studies in rats and mice (Rice et al., 2007; Viberg et al., 2007, 2003a).

NTP (1986) reported a study of the chronic oral toxicity and carcinogenicity of decaBDE in rats. In the liver, an increase in the incidence of thrombosis was observed in high-dose male rats (2,240 mg/kg-day). A dose-dependent but statistically not significant increase in degeneration of the liver was also observed in treated male rats at incidences of 13/50, 19/50, and 22/49 in the control, low-dose, and high-dose groups, respectively. In the spleen, increased incidence of fibrosis was seen in males at the low dose (8/50) and high dose (13/49) compared to 5/49 in controls; the effect was statistically significant only in the high-dose group. In the mandibular lymph node, lymphoid hyperplasia increased in males in a dose-dependent manner, but the incidence reached statistical significance only at the high dose. Based on these results, the NOAEL for systemic toxicity was 1,120 mg/kg-day, and the LOAEL was 2,240 mg/kg-day in male rats based on increased incidence of thrombosis and degeneration in the liver, splenic fibrosis, and lymphoid hyperplasia of the mandibular lymph nodes. Female rats appeared to be refractory to the systemic toxicity of decaBDE at the doses used in this study. Therefore, the NOAEL for systemic toxicity in females was 2,550 mg/kg-day, with no LOAEL established.

The observed systemic toxicity of decaBDE in the 2-year study in rats is supported by observed liver effects at higher doses in a 2-year study in mice (NTP, 1986). Significant increases in the incidence of centrilobular hypertrophy were also observed in the liver of male mice at low and high doses (34/50, and 32/50, respectively) in comparison to controls (0/50). In the thyroid gland, a dose-dependent and statistically significant increase (at both dose levels) in the incidence of follicular cell hyperplasia was observed in male mice (control, 2/50; low dose, 10/50; high dose, 19/50). In the females, this incidence increased in the low- and high-dose groups compared with the control group, but the increase was not dose dependent or statistically

significant at any dose level. High-dose female mice exhibited a significant increase in the incidence of stomach ulcers. Based on these results, no NOAEL for males was established. The LOAEL, based on increased incidence of centrilobular hypertrophy and a statistically significant and dose-dependent increase in thyroid gland follicular cell hyperplasia in male mice, was 3,200 mg/kg-day or greater. Similar to the study in rats (NTP, 1986), female mice appeared to be refractory to the systemic toxicity of decaBDE at the doses used in this study. The study established a NOAEL of 3,760 mg/kg-day for portal-of-entry effects for females and a LOAEL of 7,780 mg/kg-day based on a significant increase in the incidence of stomach ulcers.

Viberg et al. (2003a) reported functional neurobehavioral effects of single-dose exposures to decaBDE. The neurotoxic effects of decaBDE on spontaneous motor behavior were investigated in adult NMRI male mice exposed to a single oral dose of decaBDE as neonates on PND 3, 10, or 19 (i.e., at different stages of neonatal mouse brain development). Pair-wise testing between adult mice exposed on PND 3 and control groups indicated significant dose-related changes in the habituation ratio calculated from three behavior variables (locomotion, rearing, and total activity) in mice exposed to 20.1 mg/kg and evaluated at 2, 4, and 6 months of age; no statistically significant effect was seen at 2.22 mg/kg. Adult mice exposed neonatally up to 20.1 mg/kg on either PND 10 or 19 did not show any significant differences in any of the variables after 2, 4, or 6 months compared with controls. The authors indicated that the absence of changes in spontaneous activity in mice treated on PNDs 10 and 19 appears to suggest that there is a critical window for the induction of behavioral disturbances. The decrease observed in the habituation capability in the adult mice exposed neonatally to the high dose indicated that the neurotoxic effect of neonatal decaBDE exposure was persistent and also worsened with age. Based on these observations, the NOAEL in this study was 2.22 mg/kg and the LOAEL was 20.1 mg/kg. In the study in rats (Viberg et al., 2007), significant dose-related disruption in habituation (changes in locomotion, rearing, and total activity) was observed in 2-month-old rats, following exposure to BDE-209 at 6.7 and 20.1 mg/kg on PND 3. A NOAEL was not identified in this study.

Rice et al. (2007) examined the effects of BDE-209 (99.5% purity) on developmental milestones, sensorimotor behaviors, serum T_4 levels and locomotor activity in male and female C57BL6/J mice administered doses of 0, 6, or 20 mg/kg-day from PNDs 2–15. An FOB suitable for neonatal mice was developed to examine a series of homecage, reflexive, and sensorimotor behaviors, measured on PND 14, 16, 18, or 20. Three of the FOB endpoints were affected by BDE-209 exposure: palpebral reflex, forelimb grip, and struggling behavior during handling. On PND 14, significantly fewer male and female pups in the 6 and 20 mg/kg-day groups performed the palpebral reflex compared with controls. On PNDs 14 and 16, fewer male pups in the 20 mg/kg-day group performed an effective forelimb grip compared with same-sex controls. Male and female pups in the 6 mg/kg-day group struggled significantly more on PND 20, while

they were being handled for the FOB, compared with controls and the high-dose group. Locomotor activity in PND 70 males and females in the control and treated groups declined over the course of the 2-hour assessment. However, the rate of decline was significantly different ($p = 0.04$) in male mice exposed to 6 and 20 mg/kg-day compared with control animals. The effect was most pronounced in the high-dose males during the first 1.5 hours of the 2-hour activity session. A dose-related reduction in serum T_4 in comparison with controls occurred in PND 21 males but not in females. The LOAEL in this study was 6 mg/kg-day, the lowest dose tested, for decrease in the percent of male and female pups performing the palpebral reflex on PND 14, for increased struggling behavior of male mice on PND 20, for decreased T_4 levels in male mice, and for effects on locomotor activity of male mice on PND 70.

Viberg et al. (2007) administered BDE-209 by gavage to 3-day-old Sprague-Dawley rats at 0, 6.7, or 20.1 mg/kg. A total of 20 rats were picked from three to five different litters in each treatment group. Motor activity, measured as locomotion (horizontal movement), rearing (vertical movement), and total activity (all types of vibration within the test cage [i.e., those caused by rat movements, shaking/tremors, and grooming]) was evaluated for a 60-minute period, divided into three 20-minute periods, in rats at the age of 2 months. In control rats, there was a distinct decrease in locomotion, rearing, and total activity, indicating habituation in response to the diminishing novelty of the test chamber over the 60-minute test period, at 2 months of age. Two-month-old rats exposed to 20.1 mg/kg BDE-209 on PND 3 displayed significantly less activity for all three behavioral variables during the first 20-minute test period compared with controls, while during the third 20-minute period (40–60 minutes) they were significantly more active than the control animals. Rats receiving the low dose of BDE-209 (6.7 mg/kg) showed significantly increased locomotion activity during the second 20-minute period, significantly decreased rearing activity during the first and second 20-minute periods, and significantly higher total activity during the first and second 20-minute periods compared with the control rats. The LOAEL in this study was 6.7 mg/kg for significant changes in spontaneous motor behavior (locomotion, rearing, and total activity) in 2-month-old rats given BDE-209 on PND 3.

Of the three neurobehavioral studies, only Viberg et al. (2003a) identified a NOAEL. The NOAEL identified in Viberg et al. (2003a) was lower than the LOAELs identified in the neurobehavioral studies conducted by Viberg et al. (2007) and Rice et al. (2007). The Viberg et al. (2003a) study was therefore selected as the principal study for deriving the RfD. However, several considerations were weighed in making this decision. Concerns regarding the study design raise potential issues about full reliance on this study. The dosing regimen did not include gestation and lactation exposure (U.S. EPA, 1998a); only single doses were given. The study was conducted in male mice only. The protocol was unique and did not conform to health effects test guidelines for neurotoxicity screening battery or developmental neurotoxicity studies

(U.S. EPA, 1998a, c). While the study design appears to identify a developmental window of susceptibility, it is not adequate to determine the effect of longer dosing. However, the fact that the effects were observed after a single dose is a situation that increases concern regarding the impact of BDE-209 on neurological development. 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 were used for the behavioral testing (10 mice were randomly selected from three to five different litters in each treatment group). Increasing the number of samples from each litter may bias the analyses towards false positives. The observed neurobehavioral effects may be attributable to differences in pups born to a single dam rather than related to treatment. Another concern regarding the study design was the limited number of neurobehavioral parameters that were assessed; the authors measured only indices related to motor activity (locomotion, rearing, and total activity). The absence of a full FOB that evaluates neurological and behavioral signs limits the ability to correlate the reported effects with other FOB parameters. Data from the FOB utilized in the Rice et al. (2007) study, also in mice, mitigate some concern related to its absence in the Viberg et al. (2003a) study.

As indicated in the *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), it is assumed that an agent that produces detectable adverse neurotoxic effects in experimental animal studies will pose a potential hazard to humans. For BDE-209, in the absence of human evidence, data from experimental animal studies are used as the basis for the RfD.

While study design limitations cloud the utility of this study, several additional considerations support the use of these data. Acute exposure to a highly lipophilic and long half-life chemical, such as BDE-209, will result in exposure that lasts much longer than just acutely. In addition, there are a wide variety of brain structures that have very limited critical windows during development. These short critical windows translate to susceptible periods of exposure that can be very short.

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-, penta-, and hexaBDEs (Kuriyama et al., 2005; Viberg et al., 2005, 2004a, b, 2003b, 2002; Ankarberg, 2003; Branchi et al., 2002; Eriksson et al., 2002, 2001). In addition, the study of Rice et al. (2007) strengthens the evidence that links the behavioral effects seen in mice to neonatal BDE-209 exposure. Therefore, the observed neurobehavioral effects in the Viberg et al. (2003a) study in mice and the Viberg et al. (2007) study in rats are biologically plausible, and exposure to BDE-209 may pose a potential hazard to humans (U.S. EPA, 1998a).

Viberg et al. (2003b) have suggested that the animals are vulnerable only during the postnatal brain growth spurt that occurs in the first few weeks after birth in mice. In the case of

decaBDE, the identification of PND 3 as the most vulnerable window for exposure can be explained by the toxicokinetic data showing apparent absorption decreases during the first few weeks of life and slow distribution to the brain when the dosing occurs on PND 3 or 10. Although some radiolabel reaches the brain in the 24 hours after dosing, the amount increases over the 7-day period after dosing. After dosing on PND 19, less than 0.1% of the dose was found in the brain initially and after 7 days. These observations would explain the absence of effects in animals exposed later in the postnatal period as adults.

Taken together, these considerations support the use of the Viberg et al. (2003a) study as a potential critical study for deriving the RfD for BDE-209.

5.1.2. Methods of Analysis

The published information presented by Viberg et al. (2007, 2003a) could not be used for benchmark dose (BMD) modeling; mean values for motor activity (locomotion, rearing, and total activity), habituation capability for 2-, 4-, and 6-month-old mice or rats, and standard deviations are only displayed graphically, and such values cannot be read with any accuracy from the graphs. Therefore, the NOAEL of 2.22 mg/kg is used as a point of departure for estimating the oral RfD for BDE-209.

DecaBDE has very low water solubility and a relatively high K_{ow}; therefore, preferential distribution to adipose tissue might be expected. This property raises concerns for accumulation of decaBDE in the body as a result of repeated exposure. However, limited data on the tissue distribution of decaBDE in experimental animals indicate that it is not readily distributed to adipose tissue (Morck et al., 2003). The limited deposition in the adipose tissue suggests that chronic accumulation may not be significant for BDE-209. The optimal way to address this issue would be to use a PBPK model to estimate the steady-state body burden in the lipid compartment after repeated exposure and examine the dose-response relationship based on the internal dose at the target tissues. No PBPK models of decaBDE were identified for humans or animals, and data on internal doses from existing toxicity studies are not adequate to support dose-response analysis. In the absence of these data, a dose-response analysis based on body burden was not included in this assessment.

5.1.2.1. *Benchmark Dose Modeling*

Although the NOAEL from the Viberg et al. (2003a) study was selected as the critical endpoint for the RfD derivation, the dose-response data from the NTP (1986) study were modeled to determine the lower bound BMD (BMDL) for systemic toxicity. Details of the BMD modeling results are presented in Appendix B and summarized in this section. The following data sets from the NTP (1986) 2-year rat and mouse studies were selected for BMD modeling: thrombosis in the liver, liver degeneration, fibrosis in the spleen, and lymphoid hyperplasia in

male rats; centrilobular hypertrophy in the livers; and follicular cell hyperplasia in the thyroid of male mice.

The BMD modeling was conducted using EPA's BMD software (BMDS) version 1.3.2. The dose levels showing a change compared with control values exhibited responses at levels near 10%; therefore, all of the BMD analyses were conducted with the benchmark response (BMR) set to a 10% extra risk (U.S. EPA, 2000b). For each data set, all the dichotomous models, including gamma, logistic, log-logistic, multistage, probit, log-probit, quantal-linear, quantal-quadratic, and Weibull, available in the BMDS were used. BMD model fit was evaluated based on the goodness-of-fit *p* value (indicating global model fit), Akaike Information Criterion (AIC) (indicating model fit when controlling for number of model parameters), as well as local chi-square residual (indicating the model fit at the data point close to the preset BMR). Based on the model output, the models providing best model fit to the data were selected to estimate BMD_{10} and $BMDL_{10}$. The BMD analyses results are summarized in Table 5-1.

Based on the comparison of BMD modeling results for all the potential critical effects observed in chronic rat and mouse studies, the lowest $BMDL_{10}$ is 406 mg/kg-day for liver degeneration effect in male rats. This value is much higher than the NOAEL of 2.22 mg/kg for the neurobehavioral changes observed in Viberg et al. (2003a). Because the graphic habituation data from the Viberg et al. (2003a) publication could not be used for BMD modeling, the NOAEL of 2.22 mg/kg is used as a point of departure for calculating the oral RfD.

Table 5-1. BMDs for potential critical effect from chronic rat and mouse studies

Endpoint	Reference	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Thrombosis in the liver of male rats	NTP (1986)	1,120	2,240	2,125	1,738
Liver degeneration in male rats	NTP (1986)	2,240	N/A[a]	765	406
Splenic fibrosis in male rats	NTP (1986)	1,120	2,240	1,446	1,001
Lymphoid hyperplasia in male rats	NTP (1986)	1,120	2,240	1,538	1,165
Centrilobular hypertrophy in the liver of male mice	NTP (1986)	N/A	3,200	No model fit	–
Follicular cell hyperplasia in the thyroid of male mice	NTP (1986)	N/A	3,200	1,670	1,190

[a]N/A = not available or cannot be estimated.

Source: NTP (1986).

5.1.3. RfD Derivation

Based on the neurobehavioral effects observed in the Viberg et al. (2003a) study, the NOAEL of 2.22 mg/kg is used as the point of departure for calculating the RfD. The following uncertainty factors (UFs) (U.S. EPA, 2002) are applied to the NOAEL of 2.22 mg/kg: 10 for

extrapolating animal data to humans (UF_A interspecies variability), 10 for susceptible human subpopulation (UF_H interhuman variability), and 3 for extrapolating from the single-dose to a lifetime exposure (UF_S). The total composite UF = 10 × 10 × 3 = 300.

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

A default intraspecies UF_H of 10 was applied to account for variations in susceptibility within the human population (intrahuman variability). This factor accounts for the segment of the human population that may be more sensitive than the general population to exposure to BDE-209. A default value is warranted because insufficient information is currently available to assess human-to-human variability in BDE-209 toxicokinetics or toxicodynamics.

A threefold UF_S was used to adjust for exposure duration. For BDE-209, the principal study identified endpoints that, for the most part, reflect specific aspects of developmental physiology. The hypothesized window of susceptibility, proposed by the study authors, is based on the observation that the developmental neurotoxic effects observed following exposure to BDE-209 on PND 3 will not occur once the toxicokinetics of intestinal uptake and excretion have matured and the animal brain is developmentally less active (outside the window of susceptibility). The Eriksson/Viberg group has suggested that the period of maximum vulnerability for the developing cholinergic system that coincides with the most pronounced neurodevelopmental effects from BDE-99 exposure is from PNDs 10–14. However, for BDE-209 the developmental neurotoxic effects were noted following exposure on PND 3 rather than on PNDs 10 or 19. It is likely that two factors contributed to this difference in the timing for the peak period of vulnerability. First, absorption from the gastrointestinal tract decreases as the neonatal mice age during their first few weeks of life; thus, less of the BDE-209 reaches the liver for distribution on PNDs 10 and 19 than on PND 3. Secondly, distribution to the brain is slow. The amount of radiolabel in the brain is 1.4- and 2.8-fold greater 7 days after dosing compared with 24 hours after dosing on PNDs 3 and 10. Even less reaches the brain when the BDE-209 is administered on day 19. The UF_S was viewed as a dosing duration adjustment rather than simply a comparison of the effects of a subchronic to a chronic exposure. A threefold UF_S was applied because the critical study dosed the animals only once within the hypothesized critical window, not because the chronic exposures would have exacerbated the impact on habituation.

A UF_L for LOAEL-to-NOAEL extrapolation was not applied because a NOAEL was used as the point of departure.

A UF_D to account for deficiencies in the available decaBDE database was not necessary. Available animal studies on repeated oral exposure to decaBDE include 14-day studies in rats and mice (NTP, 1986), 13-week studies in rats and mice (NTP, 1986), and 2-year studies in rats and mice (NTP, 1986). A developmental and behavioral toxicity study in male and female mice was available (Rice et al., 2007), as well as a behavioral study in male rats (Viberg et al., 2007). A 7-week study (Tseng et al., 2006) of sperm functions in mice was also available. In addition, a standard developmental toxicity study in rats (Hardy et al., 2002) was identified for decaBDE. No multigeneration reproductive toxicity study or other study of reproductive function is available for pure decaBDE. This array of studies results in potential uncertainty regarding the reproductive toxicity of decaBDE. However, this potential uncertainty is adequately accounted for based on the following considerations. First, none of the well-conducted longer-term dosing studies identified effects on male or female reproductive organs. Second, no developmental or reproductive effects were observed at doses up to 1,000 mg/kg-day in rats (Hardy et al., 2002) and 100 mg/kg-day in mice (Tseng et al., 2006). The absence of effects in the available longer-term and developmental studies indicates that at least some aspects of reproductive organ toxicity or function are not affected at doses much higher than those that resulted in the neurological effects in neonates (Viberg et al., 2003a), although effects on mating and fertility are not evaluated in these studies.

The other potential uncertainty in the database factor is immunotoxicity from exposure to decaBDE. The potential immunotoxicity was indicated by the observations of significant increases in spleen fibrosis and lymphoid hyperplasia in male rats treated with a high dose of decaBDE (NTP, 1986). However, no such changes occurred in female rats or in either sex of treated mice (NTP, 1986). In addition, an in vitro immunotoxicity study (Pullen et al., 2003) with mouse splenocytes suggested that decaBDE is not likely to affect the immune system in an immunosuppressive manner nor the production of the cytokines by these cells. Moreover, the proposed point of departure is based on the developmental neurobehavioral changes and immunotoxicity is not likely to occur at the current point of departure of 2.22 mg/kg, which is 500-fold lower than the NOAEL for histopathologic changes in rat spleen and lymphoid in the NTP (1986) study.

Based on the choice of the critical effect and UF selections, the oral RfD for decaBDE is calculated as follows:

$$\begin{aligned} RfD &= NOAEL \div UF \\ &= 2.22 \text{ mg/kg} \div 300 \\ &= 0.007 \text{ mg/kg-day or } 7 \text{ µg/kg-day} \end{aligned}$$

5.1.4. Previous RfD Assessment

An IRIS health assessment of decaBDE is available (U.S. EPA, 1989). The RfD is based on the study of Kociba et al. (1975) in which male and female Sprague-Dawley rats (25/sex/dose group) were treated with a commercial decaBDE (77.4% decabromodiphenyl oxide, 21.8% nonaBDE, and 0.8% octaBDE) at doses of 0, 0.01, 0.1, or 1.0 mg/kg-day for 2 years. Parameters examined were hematology, clinical chemistry, food consumption, organ weight, body weight, and incidence of histopathologic lesions. No significant differences among treatment and control groups were found. The NOAEL in this study was 1.0 mg/kg-day, the highest dose tested. A UF of 100 was applied to the NOAEL for both the expected intra- and interspecies variability to the toxicity of this chemical in lieu of specific data, resulting in a RfD of 0.01 mg/kg-day or 10 µg/kg-day. Confidence in the RfD was considered low (The low doses and number of animals used in this study have been questioned as to their adequacy to determine the carcinogenic potential of this commercial decaBDE [NTP, 1986]). The previous RfD was not based on the NTP (1986) study because the final NTP report was not available at the time.

The National Research Council of the National Academies of Science (NAS, 2000) derived a RfD for decaBDE based on the NTP (1986) study. The LOAEL for liver thrombosis and degeneration in male rats was 2240 mg/kg-day and the NOAEL 1120 mg/kg-day. The following UFs were applied: 10 for extrapolation from animals to humans, 10 for intraspecies variation, and 3 for database deficiency. Applying this total UF of 300 to the NOAEL of 1120 mg/kg-day gives a RfD of 4.0 mg/kg-day. Confidence in this provisional RfD was considered medium to low.

The Agency for Toxic Substances and Disease Registry (ATSDR, 2004) derived an intermediate oral minimal risk level (MRL) of 10 mg/kg-day based on the Hardy et al. (2002) study. No effects on any maternal or fetal endpoints were observed in any dose group. The NOAEL in this study was the highest dose tested of 1000 mg/kg-day. Applying a total UF of 100 (10 for extrapolation from animals to humans and 10 for human variability), an intermediate oral MRL of 10 mg/kg-day was derived.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

No data are available for deriving a RfC for decaBDE.

5.3. CANCER ASSESSMENT

5.3.1. Choice of Study/Data—with Rationale and Justification

As discussed in section 4.6, the weight of evidence of human carcinogenicity of decaBDE is based on (1) no studies of cancer in humans exposed to decaBDE; (2) a significantly increased incidence of neoplastic nodules in the liver of low- and high-dose male rats and high-dose female rats; (3) a significantly increased incidence of hepatocellular adenoma or carcinoma

(combined) in male mice at low dose and marginally increased incidence at high dose; (4) a nonsignificantly increased incidence of hepatocellular adenoma or carcinoma (combined) in female mice; (5) a slight (but statistically not significant) increase in incidence of thyroid gland adenomas or carcinomas (combined) in both male and female mice; (6) a significantly increased incidence in male mice of follicular cell hyperplasia, considered by many as a precursor to thyroid tumors; and (7) an apparent absence of genotoxic potential. All of the data supporting carcinogenicity were obtained from chronic studies in rats and mice (NTP, 1986).

As discussed in section 4.6, the weight of evidence suggests that decaBDE shows "suggestive evidence of carcinogenic potential." Support for tumorigenic effects in the liver of varying strengths is found in both sexes of rats and mice. In the NTP (1986) study, decaBDE caused a dose-dependent increased incidence of liver neoplastic nodules in rats with statistical significance at the high dose in both sexes (Table 4-4), indicating some evidence of carcinogenicity in treated rats. However, there were no significant changes in hepatocellular carcinoma in either sex. In mice, nonsignificant increases in hepatocellular adenoma or carcinoma occurred in male mice at low (3,200 mg/kg-day) and high (6,650 mg/kg-day) doses compared with controls. However, combined incidence of hepatocellular adenoma or carcinoma increased significantly in the low-dose group but not in the high-dose group. Increased incidence of hepatocellular adenoma and carcinoma was also observed in the female mice; however, these changes were not statistically significant. Similarly, although the increases in thyroid tumors were not statistically significant in either sex of mice, they were supported by increases in a precursor to thyroid tumors, follicular cell hyperplasia.

Therefore, the NTP (1986) chronic studies in mice and rats were considered the basis for the quantitative cancer assessment. Liver and thyroid tumors were considered as possible bases for the quantitation. To select the most sensitive cancer endpoint as the point of departure to derive a cancer risk value, benchmark modeling was conducted for each tumor endpoint or related precursor (i.e., thyroid hyperplasia). The BMD analysis and corresponding results are summarized in the following sections.

5.3.2. Dose Adjustments

For the chronic rat study (NTP, 1986), the original oral doses were reported as 0, 1,120, and 2,240 mg/kg-day for males and 0, 1,200, and 2,550 mg/kg-day for females. Since there are not enough toxicokinetic data for conducting a quantitative interspecies extrapolation, a default dose conversion was used. Based on EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), corresponding human equivalent doses (HEDs) were calculated by scaling animal daily applied doses experienced for a lifetime in proportion to body weight raised to the 0.75 power.

A body weight $(bw)^{3/4}$ scaling factor was used to convert the points of departure in the rat study to HEDs, in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). This procedure presumes that equal doses in these units (i.e., in $mg/kg^{3/4}$-day), when administered daily over a lifetime, will result in equal lifetime risks of the critical effect across mammalian species (U.S. EPA, 1992). The HED may be calculated as follows (U.S. EPA, 2005a, 1992):

$$\text{HED (mg/kg-day)} = \text{dose in animals (mg/kg-day)} \times (bw_a/bw_h)^{0.25}$$

where:

HED = human equivalent dose

dose = average daily dose in animal study

bw_a = animal body weight (kg)

bw_h = reference human body weight (70 kg)

Based on reported weekly body weight data for each dose group for male rats, average lifetime body weights were calculated as 383.9, 385.2, and 380.7 g for the control, low-, and high-dose groups, respectively. Using the calculated average lifetime rat body weight and a default human body weight of 70 kg, the corresponding HEDs were calculated as 0, 305.1, and 608.3 mg/kg-day, respectively. Similarly, for female rats, average lifetime body weights were calculated as 247.9, 246.2, and 242.1 g for the control, low-, and high-dose groups, respectively. Based on the calculated body weight and default human body weight of 70 kg, corresponding HEDs were calculated as 0, 292.2, and 618.4 mg/kg-day, respectively.

For the chronic mouse study (NTP, 1986), the only dose-response data on carcinogenicity available were obtained from male mice; therefore, the dose conversion was conducted for male mice only. The original oral doses for male mice were reported as 0, 3,200, and 6,650 mg/kg-day. Since there are not enough toxicokinetic data for conducting a quantitative interspecies extrapolation, a default dose conversion was used. Based on reported weekly body weight data for each dose group, average lifetime body weights were calculated as 37.3, 37.4, and 37.0 g for control, low-, and high-dose groups, respectively. Based on these data on average lifetime mouse body weight and a default human body weight of 70 kg, corresponding HEDs were calculated by scaling animal daily applied doses experienced for a lifetime in proportion to body weight raised to the 0.75 power. The estimated HEDs are 0, 486.5, and 1,008.6 mg/kg-day, respectively.

5.3.3. Extrapolation Method(s)

U.S. EPA BMDS (version 1.40b) was used in the cancer endpoint BMD analysis. Following the guidelines for evaluation of data on tumorigenic responses, the cancer model

(a multistage model with a calculation function for slope factor) was used to calculate effective doses (EDs) for a tumor response within the range of observation and the lower confidence estimate on that dose. The lower confidence limit of the estimated dose for a BMR similar to the response observed in the low-dose group is used as a point of departure to estimate an oral slope factor (U.S. EPA, 2005a, b).

All potential tumor endpoints with statistically significant responses in the rat and mouse chronic studies (NTP, 1986) were modeled in order to identify the most sensitive point of departure. Animals dying prior to week 53, before the first appearance of tumors, were censored from the group totals to adjust the incidence rates for early mortality. To adjust for differential mortality among treated male rats, censored data on neoplastic nodules or the combined neoplastic nodules and carcinomas in rats of both sexes were also modeled. The data on combined hepatic carcinomas and adenomas in male mice were modeled despite the fact that they did not show a good dose-response relationship because they were significantly increased in comparison with controls. A detailed description of the BMD modeling results is presented in Appendix C. The estimated cancer slope factors based on estimated EDs and their 95% lower confidence limits using the multistage model for each cancer endpoint are summarized in Table 5-2.

Table 5-2. Cancer slope factors derived from BMDs for neoplastic effect from chronic rat and mouse studies using the multistage model

Endpoint	Species	Cancer slope factor $(mg/kg\text{-}day)^{-1}$
Neoplastic nodules in the liver	Male rat	0.0007
Neoplastic nodules or carcinomas (combined) in the liver	Male rat	0.0007
Neoplastic nodules in the liver	Female rat	0.0004
Neoplastic nodules or carcinomas (combined) in the liver	Female rat	0.0005
Follicular cell hyperplasia in the thyroid	Male mouse	0.0005
Adenomas or carcinomas (combined) in the liver	Male mouse	0.0005

Source: NTP (1986).

Neoplastic nodules and carcinomas were also modeled together because the two lesions could have a similar mode of action. Based on the estimated ED for each cancer endpoint, the most sensitive response is neoplastic nodule or carcinoma (combined) in the liver of male rats. The ED_{12} from the lower end of the range of observation is 263 mg/kg-day, and the corresponding 95% lower confidence limit for the effective dose (LED_{12}) is 178 mg/kg-day. For neoplastic nodules or carcinomas in the liver of female rats, both censored and uncensored data resulted in higher LEDs than for this effect in male rats; therefore, the LED in females was not used as the point of departure. Similarly, the estimated LED for the follicular cell hyperplasia in the thyroid of male mice is also higher than that of neoplastic nodules or carcinomas (combined)

in the male rats. Thus, the lowest LED, 178 mg/kg-day for neoplastic nodules or carcinomas (combined) in the male rats, is selected as the point of departure for deriving an oral cancer slope factor. However, as stated earlier (section 4.2.2.1), the term neoplastic nodule is no longer used for rat liver tumors, and it is not possible to know if all lesions originally categorized as neoplastic nodules were preneoplastic under current guidelines. Some may have been nonneoplastic hyperplasia. Accordingly, there is some uncertainty in the calculated slope factor that should be considered when it is applied in quantitative risk assessment.

5.3.4. Oral Slope Factor

Although no human studies were available, two chronic rodent studies provide suggestive evidence of decaBDE-induced carcinogenicity. The data from these studies are adequate to support a quantitative cancer dose-response assessment. Even though the available evidence is suggestive of human carcinogenic potential, there is very limited information exploring the mode of action for any of the tumors reported in the animal chronic studies. While decaBDE was not mutagenic or genotoxic in various in vitro studies, there are no data to support alternative mode-of-action hypotheses. In the absence of such data, a mode of action for BDE-209 could not be determined; therefore, extrapolation from the point of departure to lower doses is conducted by using a linear approach.

Based on a comparison of estimated EDs for all the cancer endpoints observed in rat and mouse chronic studies (NTP, 1986), the neoplastic nodules or carcinomas (combined) in the liver of treated male rats are the most sensitive endpoint. Therefore, the LED_{12} of 178 mg/kg-day estimated for this endpoint is used as a point of departure for calculating the cancer slope factor.

For linear extrapolation, a straight line is drawn from the point of departure expressed as an HED to the origin to give a probability of extra risk. The slope of the line expresses extra risk per dose unit. For linear extrapolation, the slope of the line is $0.12/LED_{12}$. The central estimate, ED_{12}, of exposure at 12% extra risk is $0.12/ED_{12}$. The slope of the linear extrapolation from the central estimate ED_{12} is 5×10^{-4}, which is derived using 0.12/(263 mg/kg-day). For neoplastic nodules or carcinomas, the resulting oral cancer slope factor is 7×10^{-4} per mg/kg-day, which is derived using 0.12/(178 mg/kg-day). Since some of the neoplastic nodules might not progress to carcinomas, this oral risk estimate errs on the conservative side. Based on this slope factor, the dose associated with an excess cancer risk (risk-specific dose [RSD]) value is approximately as follows: RSD at 10^{-6} is 1 µg/kg-day; RSD at 10^{-5} is 10 µg/kg-day; and RSD at 10^{-4} is 100 µg/kg-day.

Doses for excess cancer risks of approximately 5×10^{-6} or lower would be protective of neurodevelopmental effects since the RfD established on the basis of these effects is 7 µg/kg-day (see section 5.1.3). As explained above, there is some uncertainty in this slope factor because

some of the neoplastic nodules might have been nonneoplastic hyperplasia rather than preneoplastic foci of cellular alteration or hepatic adenomas. The combination of the hyperplastic nodules and carcinomas for the dose-response analysis leads to a conservative but protective slope factor.

5.3.5. Previous Cancer Assessment

The carcinogenicity of decaBDE was evaluated in IRIS (U.S. EPA, 1989). DecaBDE was classified in Group C, "possible human carcinogen," according to EPA cancer guidelines (U.S. EPA, 1986b). The basis of this classification was lack of human carcinogenicity data and limited evidence of carcinogenicity in animals, namely, significantly increased incidences of neoplastic liver nodules in male and female rats and increased incidences of hepatocellular adenomas or carcinomas (combined) in male mice (NTP, 1986). A quantitative estimate of carcinogenic risk from oral exposure was not derived in the IRIS assessment because the final NTP (1986) report was not available at the time.

On the basis of the carcinogenicity studies in F344/N rats and B6C3F1 mice, NTP (1986) concluded that there was "some evidence of carcinogenicity" for male and female rats as shown by increased incidences of neoplastic nodules of the liver in low-dose males and high-dose groups of both males and females. There was "equivocal evidence of carcinogenicity" for male mice as shown by increased incidences of hepatocellular adenomas or carcinomas (combined) in the low-dose group and of thyroid gland follicular cell adenomas or carcinomas (combined) in both dosed groups. There was "no evidence of carcinogenicity" for female mice receiving low or high dose of decaBDE in the diet. Several nonneoplastic lesions were observed at increased incidences, the most notable being thyroid gland follicular cell hyperplasia in male mice.

The potential carcinogenicity of decaBDE was evaluated by NAS (2000) on the basis of the NTP (1986) study. Based on lack of human data and limited evidence of carcinogenicity in animals (i.e., significant increased incidences of neoplastic liver nodules in male and female rats and increased incidences of hepatocellular adenomas or carcinomas in male mice), NAS (2000) concluded that decaBDE is a possible carcinogen in rats but has not concluded that it is a carcinogen in humans. Based on the incidence of hepatic neoplastic nodules in male rats (NTP, 1986), a cancer slope factor of 9×10^{-4} per mg/kg-day was derived, applying the multistage model to censored data to adjust for differential mortality among treated rats.

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

6.1. HUMAN HAZARD POTENTIAL

Studies of toxicokinetics of decaBDE reveal that the chemical can be absorbed by the oral route to a limited extent, does not accumulate in tissues, and undergoes clearance, largely as a result of metabolism in the liver and excretion in the bile.

Short-term and subchronic studies demonstrated low toxicity from oral exposure to decaBDE with NOAELs of 3,000 mg/kg-day or higher. NTP (1986) conducted a chronic toxicity and carcinogenicity dietary study in F344 rats. DecaBDE caused an increase in the incidence of thrombosis in the liver in high-dose male rats (2,240 mg/kg-day). A dose-dependent, but insignificant, increase in the incidence of degeneration of the liver was also observed in treated male rats. In the spleen, a dose-dependent increase (statistically significant in the high-dose group) in the incidence of fibrosis was observed in males. In the mandibular lymph node, lymphoid hyperplasia increased in males in a dose-dependent manner, but the incidence reached statistical significance only at the high dose. Histopathology examination also revealed a dose-dependent increase in the incidence of neoplastic nodules in the liver in both male and female rats. Female rats appeared to be refractory to the systemic toxicity of decaBDE at the doses used in this study.

The observed toxicity of decaBDE in the 2-year study in rats is further supported by the 2-year mouse study conducted by NTP (1986). Significant increases in the incidence of centrilobular hypertrophy were observed in the liver of treated male mice. In the thyroid gland, a dose-dependent and statistically significant increase (at all dose levels) in the incidence of follicular cell hyperplasia was observed in male mice. In the females, the incidence increased in the low- and high-dose groups compared with the control group, but the increase was not statistically significant at any dose level. Female mice in the high-dose group exhibited a significant increase in the incidence of stomach ulcers. In addition, there were significant increases in the combined incidence of hepatocellular adenomas or carcinomas at both low and high doses in male mice. In the thyroid gland, follicular cell adenomas or carcinomas (combined) were slightly, but not significantly, increased in treated mice of both sexes. Similar to female rats, female mice appeared to be refractory to the systemic toxicity of decaBDE.

DecaBDE also has been shown to induce behavioral changes in several studies in mice and rats (Viberg et al., 2007, 2003a; Rice et al., 2007). In the principal study selected, Viberg et al. (2003a) investigated the neurotoxic effects of decaBDE on spontaneous motor behavior of adult NMRI male mice when these animals were exposed to a single oral dose as neonates on PND 3, 10, or 19 (i.e., at different stages of neonatal mouse brain development). Pair-wise

testing between adult mice exposed on PND 3 and control groups indicated significant dose-related changes in all three spontaneous behavior variables at 2, 4, and 6 months of age. Adult mice exposed neonatally up to 20.1 mg on either PND 10 or 19 did not show any significant differences in any of the variables. These data suggested that there was a critical window for the induction of behavioral disturbances, and the neurotoxic effect of neonatal decaBDE exposure was persistent and also worsened with age in male mice.

The appropriate hazard descriptor for decaBDE is "suggestive evidence of carcinogenic potential" (U.S. EPA, 2005a, b). The weight-of-evidence of human carcinogenicity of decaBDE is based on (1) no studies of cancer in humans exposed to decaBDE; (2) a statistically significant increase in incidence of neoplastic nodules and a slight increase in incidence of carcinomas (not statistically significant) in the liver of low- and high-dose male rats and high-dose female rats; (3) a significantly increased incidence of hepatocellular adenoma or carcinoma (combined) in male mice at the low dose and marginally increased incidence at the high dose; (4) a nonsignificantly increased incidence of hepatocellular adenoma or carcinoma (combined) in female mice; (5) a slightly greater (but statistically not significant) incidence of thyroid gland adenomas or carcinomas (combined) in dosed male and female mice; (6) a significantly increased incidence in male mice, at both doses, of follicular cell hyperplasia, considered by many as a precursor to thyroid tumors; and (7) an apparent absence of genotoxic potential.

The weight of experimental evidence is on the strong end of the spectrum for the descriptor "suggestive evidence of carcinogenic potential," since there is suggestive evidence that decaBDE is carcinogenic for more than one species, sex, and site. Based on the weight of evidence, a dose-response assessment of the carcinogenicity of decaBDE is deemed appropriate.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

BMD modeling was conducted for all the potential critical effects observed in the rat and mouse chronic studies, including thrombosis in the liver, liver degeneration, fibrosis in the spleen, and lymphoid hyperplasia in male rats, as well as centrilobular hypertrophy in the livers and follicular cell hyperplasia in the thyroid of male mice. The data on neurobehavioral effects in the Viberg et al. (2003a) study could not be modeled because the data were presented graphically rather than numerically and thus were not amenable to BMD analysis. The lowest $BMDL_{10}$ in rodent chronic studies is 383 mg/kg-day for liver degeneration effect in male rats. This value is much higher than the NOAEL level of 2.22 mg/kg for the neurobehavioral changes observed in mice (Viberg et al., 2003a). Therefore, the NOAEL of 2.22 mg/kg is used as a point of departure for calculating the oral RfD. A composite UF of 300 was applied to this effect level to account for the interspecies uncertainty (10-fold), intraspecies variation (10-fold), and

extrapolation of a single dose to a lifetime exposure (3-fold), yielding an RfD of 0.007 mg/kg-day or 7 µg/kg-day.

The available database included acute, short-term, subchronic, and chronic studies in two species. Also, there are developmental and reproductive studies (Tseng et al., 2006; Hardy et al., 2002) in addition to the critical neurobehavioral study. Confidence in the principal study (Viberg et al., 2003a) is low because the study used a single dose during a period of neurodevelopmental vulnerability. In addition, only limited tests on motor activity were conducted in only male mice in the critical study by Viberg et al. (2003a). This study is strengthened by the results of Rice et al. (2007), where behavioral effects were identified in neonatally exposed PND 70 mice following a repeated postnatal dose protocol, and also by the results of the Viberg et al. (2007) study in rats. The Viberg et al. (2003a) study provides information on the most sensitive endpoint; therefore, using the NOAEL from this study as a point of departure to calculate the RfD is expected to provide adequate protection for humans.

The overall confidence in the RfD assessment of BDE-209 is low.

6.2.2. Cancer/Oral

Although no human studies were available, two chronic rodent studies (NTP, 1986) provide suggestive evidence of decaBDE-induced carcinogenicity. The data from these studies are adequate to support a quantitative cancer dose-response assessment. There is very limited information exploring the mode of action for any of the tumors reported in the animal chronic studies. DecaBDE was not mutagenic or genotoxic in several in vitro studies, and there are no data to support a nongenotoxic mode-of-action hypothesis. In the absence of such data, extrapolation from the point of departure to lower doses is most appropriate using a linear approach.

Based on a comparison of estimated EDs for all the cancer endpoints observed in rat and mouse chronic studies (NTP, 1986), the neoplastic nodule or carcinoma (combined) in the treated rats is the most sensitive endpoint. Therefore, the LED_{12} of 178 mg/kg-day estimated for this endpoint is used as a point of departure for calculating the cancer slope factor. For neoplastic nodules or carcinomas (combined), the resulting oral cancer slope factor is 7×10^{-4} per mg/kg-day. This slope factor assumes that all neoplastic nodules were preneoplastic cellular changes with the potential to become malignant. Based on this slope factor, the doses associated with excess cancer risks of 10^{-4}, 10^{-5}, and 10^{-6} are approximately 100, 10, and 1 µg/kg-day, respectively. There is some uncertainty in this estimate, given the fact that the tissues identified as neoplastic nodules may have included some lesions now categorized as nonneoplastic hyperplasia (Maronpot et al., 1986).

7. REFERENCES

ACC (American Chemistry Council). (2002) Voluntary Children's Chemical Evaluation Program (VCCEP). Data summary. Decabromodiphenyl ether (decabromodiphenyl oxide, DBDPO). Prepared by the American Chemistry Council's Brominated Flame Retardant Industry Panel, Arlington, VA; December 17. Available online at http://regulations.gov; Document ID EPA-HQ-OPPT-2004-0085-0032 in docket "Certain polybrominated diphenylethers; proposed significant new use rule."

Ankarberg, E. (2003) Neurotoxic effects of nicotine during neonatal brain development. Comprehensive summaries of Uppsala Dissertations from the Faculty of Science and Technology 907. Acta Universitatis Upsaliensis, Uppsala, Sweden.

ATSDR (Agency for Toxic Substances and Disease Registry). (2004) Toxicological profile for polybrominated biphenyls and polybrominated diphenyl ethers. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. Available online at http://www.atsdr.cdc.gov/toxpro2.html.

Bock, KW. (1994) Arylhydrocarbon of dioxin receptor: biologic and toxic responses. Rev Physiol Biochem Pharmacol 125:1–42.

Bradman A; Fenster, L; Sjodin, A; et al. (2007) Polybrominated diphenyl ether levels in the blood of pregnant women living in an agriculture community in California. Environ Health Perspect 115(1):71–74.

Branchi, I; Alleva, E; Costa, LG. (2002) Effects of perinatal exposure to a polybrominated diphenyl ether (PBDE 99) on mouse neurobehavioural development. Neurotoxicol 23(3):375–384.

Brown, DJ; Overmeire, IV; Goeyens, L; et al. (2004) Analysis of Ah receptor pathway activation by brominated flame retardants. Chemosphere 55:1509–1518.

Carlson, GP. (1980) Induction of xenobiotic metabolism in rats by short-term administration of brominated diphenyl ethers. Toxicol Lett 5:19–25.

Cetin, B; Odabasi, M. (2005) Measurement of Henry's law constants of seven polybrominated diphenyl ether (PBDE) congeners as a function of temperature. Atmos Environ 39:5273–5280.

Chen, G; Konstantinov, AD; Chittim, BG; et al. (2001) Synthesis of polybrominated diphenyl ethers and their capacity to induce CYP-1A by the Ah receptor mediated pathway. Environ Sci Technol 35:3749–3756.

Darnerud, PO; Eriksen, GS; Johannesson, T; et al. (2001) Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. Environ Health Perspect 109:49–68.

ECB (European Chemicals Bureau). (2003) Bis(pentabromophenyl) ether. Summary risk assessment report. Luxembourg, Belgium: Office for Official Publications of the European Communities. Available online at http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/SUMMARY/decasum013.pdf.

el Dareer, SM; Kalin, JR; Tillery, KF; et al. (1987) Disposition of decabromodiphenyl ether in rats dosed intravenously or by feeding. J Toxicol Environ Health 22(4):405–415.

Eriksson, P; Jakobsson, E; Fredriksson, A. (2001) Brominated flame retardants: a novel class of developmental neurotoxicants in our environment? Environ Health Perspect 109(9):903–908.

Eriksson, P; Viberg, H; Jakobsson, E; et al. (2002) A brominated flame retardant, 2,2',4,4',5-pentabromodiphenyl ether: uptake, retention, and induction of neurobehavioural alterations in mice during a critical phase of neonatal brain development. Toxicol Sci 67(1):98–103.

Fischer, D; Hooper, K; Athanasiadou, M; et al. (2006) Children show highest levels of polybrominated diphenyl ethers in a California family of four: a case study. Environ Health Perspect 114(10):1581–1584.

Gravance, CG; Garner, DL; Miller, MG; et al. (2001) Fluorescent probes and flow cytometry to assess rat sperm integrity and mitochondrial function. Reprod Toxicol 15:5–10.

Hakk, H; Larsen, G; Bergman, A; et al. (2002) Binding of brominated diphenyl ethers to male rat carrier proteins. Xenobiotica 32(12):1079–1091.

Hard, GC. (1998) Recent developments in the investigation of thyroid regulation and thyroid carcinogenesis. Environ Health Perspect 106(8):427–436.

Hardy, ML. (2002) A comparison of the properties of the major commercial PBDPO/PBDE product to those of major PBB and PCB products. Chemosphere 46:717–728.

Hardy, ML; Schroeder, R; Biesemeier, J; et al. (2002) Prenatal oral (gavage) developmental toxicity study of decabromodiphenyl oxide in rats. Int J Toxicol 21(2):83–91.

Hill, RN; Erdreich, LS; Paynter, OE; et al. (1989) Thyroid follicular cell carcinogenesis. Fund Appl Toxicol 12:629–697.

Hughes, MF; Edwards, BC; Mitchell, CT; et al. (2001) In vitro dermal absorption of flame retardant chemicals. Food Chem Toxicol 39(12):1263–1270.

Huwe, JK; Smith, DJ. (2007) Accumulation, whole-body depletion, and debromination of decabromodiphenyl ether in male Sprague-Dawley rats following dietary exposure. Environ Sci Technol 41:2371–2377.

Jakobsson, K; Thuresson, K; Rylander, L; et al. (2002) Exposure to polybrominated diphenyl ethers and tetrabromobisphenol A among computer technicians. Chemosphere 46:709–716.

Klaassen, CD; ed. (1996) Casarett and Doull's toxicology: the basic science of poisons. 5th edition. New York, NY: McGraw-Hill; pp. 47–48.

Kociba, RJ; Frauson, LO; Humiston, CG; et al. (1975) Results of a two-year dietary feeding study with decabromodiphenyl oxide (DBDPO) in rats. J Combust Toxicol 2:267–285. (Cited in Darnerud et al., 2001).

Kodavanti, RS; Ward, TR; Ludewig, G; et al. (2005) Polybrominated diphenyl ether (PBDE) effects in rat neuronal cultures: ^{14}C-PBDE accumulation, biological effects, and structure-activity relationships. Toxicol Sci 88(1):181–192.

Kuriyama, SN; Talsness, CE; Grote, K; et al. (2005) Developmental exposure to low dose PBDE 99: effects on male fertility and neurobehavior in rat offspring. Environ Health Perspect 113:149–154.

Legler, J; Brouwer, A. (2003) Are brominated flame retardants endocrine disruptors? Environ Int 29:879–885.

Maronpot, RR; Montgomery, CA; Boorman, GA; et al. (1986) National Toxicology Program nomenclature for hepatoproliferative lesions of rats. Toxicol Pathol 14(2):263–273.

McDonald, TA. (2002) A perspective on the potential health risks of PBDEs. Chemosphere 46:745–755.

Morck, A; Hakk, H; Orn, U; et al. (2003) Decabromodiphenyl ether in the rat: absorption, distribution, metabolism, and excretion. Drug Metab Disp 31:900–907.

NAS (National Academy of Sciences). (2000) Toxicological risks of selected flame-retardant chemicals. National Research Council, Commission on Life Sciences, Board on Environmental Studies and Technology, Committee on Toxicology, Subcommittee on Flame-Retardant Chemicals. Washington, DC: National Academy Press.

NRC (National Research Council). (1983) Risk assessment in the federal government: managing the process. Washington, DC: National Academy Press.

NTP (National Toxicology Program). (1986) Toxicology and carcinogenesis studies of decabromodiphenyl oxide (CAS No. 1163-19-5) in F344/N rats and B6C3F1 mice (feed studies). Public Health Service, U.S. Department of Health and Human Services; NTP TR 309. Available from the National Institute of Environmental Health Sciences, Research Triangle Park, NC, and online at http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr309.pdf.

Pacyniak, EK; Cheng, X; Cunningham, MK; et al. (2007) The flame retardants, polybrominated diphenyl ethers, are pregnane X receptor activators. Toxicol Sci 97(1):94–102.

Peters, AK; van Londen, K; Bergman, A; et al. (2004) Effects of polybrominated diphenyl ethers on basal and TCDD-induced ethoxyresorufin activity and cytochrome P450-1A1 expression in MCF-7, HepG2, and H4 IIE cells. Toxicol Sci 82:488–496.

Pullen, S; Boecker, R; Tieg, G. (2003) The flame retardants tetrabromobisphenol A and tetrabromobisphenol A-bisallylether suppress the induction of interleukin-2 receptor α chain (CD25) in murine splenocytes. Toxicology 184:11–22.

Rice, DC; Reeve, EA; Herlihy, A; et al. (2007) Developmental delays and locomotor activity in the C57BL6/J mouse following neonatal exposure to the fully-brominated PBDE, decabromodiphenyl ether. Neurotoxicol Teratol 29:511–520.

Sandholm, A; Emanuelsson, B-M; Klasson-Wehler, E. (2003) Bioavailability and half-life of decabromodiphenyl ether (BDE-209) in rat. Xenobiotica 33(22):1149–1158.

Schecter, A; Pavuk, M; Papke, O; et al. (2003) Polybrominated diphenyl ethers (PBDEs) in U.S. mothers' milk. Environ Health Perspect 111(14):1723–1729.

She, J; Holden, A; Sharp, M; et al. (2007) Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in breast milk from the Pacific Northwest. Chemosphere 67:S307–S317.

Sjodin, A; Hagmar, L; Klasson-Wehler, E; et al. (1999) Flame retardant exposure: polybrominated diphenyl ethers in blood from Swedish workers. Environ Health Perspect 107(8):643–648.

Sjodin, A; Patterson, DG, Jr; Bergman, A. (2001a) Brominated flame retardants in serum from U.S. blood donors. Environ Sci Technol 35(19):3830–3833.

Sjodin, A; Carlsson, H; Thuresson, K; et al. (2001b) Flame retardants in indoor air at an electronics recycling plant and at other work environments. Environ Sci Technol 35:448–454.

Sjodin, A; Patterson, DG, Jr; Bergman, A. (2003) A review on human exposure to brominated flame retardants—particularly polybrominated diphenyl ethers. Environ Internat 29:829–839.

Staskal, DF; Hakk, H; Bauer, D; et al. (2006) Toxicokinetics of polybrominated diphenyl ether congeners 47, 99, 100, and 153 in mice. Toxicol Sci 94(1):28–37.

Thuresson, K; Bergman, A; Jakobsson, K (2005) Occupational exposure to commercial decabromodiphenyl ether in workers manufacturing or handling flame-retarded rubber. Environ Sci Technol 39:1980–1986.

Thuresson, K; Hoglund, P; Hagmar, L; et al. (2006) Apparent half-lives of hepta- to decabrominated diphenyl ethers in human serum as determined in occupationally exposed workers. Environ Health Perspect 114(2):176–181.

Tseng, LH; Lee, CW; Pan, MH; et al. (2006) Postnatal exposure of the male mouse to 2,2',3,3',4,4',5,5',6,6'-decabrominated diphenyl ether: decreased epididymal sperm functions without alterations in DNA content and histology in testis. Toxicology 224:33–43.

U.S. EPA (Environmental Protection Agency). (1986a) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006–34012. Available online at http://www.epa.gov/iris/backgr d.htm.

U.S. EPA (Environmental Protection Agency). (1986b) Guidelines for carcinogen risk assessment. Federal Register 51(185):33992–34003. Available online at http://www.epa.gov/cancerguidelines.

U.S. EPA (Environmental Protection Agency). (1986c) Proliferative hepatocellular lesions of the rat: review and future use in risk assessment. Risk Assessment Forum, Washington, DC; EPA/625/3-86/011. Available from the National Technical Information Service, Springfield, VA, PB87-178711, and online at http://nepis.epa.gov/EPA/html/Pubs/pubtitleORD.htm.

U.S. EPA (Environmental Protection Agency). (1988) Recommendations for and documentation of biological values for use in risk assessment. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH; EPA/600/6-87/008. Available from the National Technical Information Service, Springfield, VA, PB88-179874/AS, and online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855.

U.S. EPA (Environmental Protection Agency). (1989) Decabromodiphenyl ether (CASRN 1163-19-5). Integrated Risk Information System (IRIS). National Center for Environmental Assessment, Washington, DC. Available online at http://www.epa.gov/iris.

U.S. EPA (Environmental Protection Agency). (1991) Guidelines for developmental toxicity risk assessment. Federal Register 56:63798–63826. Available online at http://www.epa.gov/ncea/raf/rafguid.htm.

U.S. EPA (Environmental Protection Agency). (1992) Draft report: a cross-species scaling factor for carcinogen risk assessment based on equivalence of mg/kg3/4/day. Federal Register 57(109):24152–24173.

U.S. EPA. (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity studies. Federal Register 59(206):53799. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Office of Research and Development, Washington, DC; EPA/600/8-90/066F. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA (Environmental Protection Agency). (1995) Use of the benchmark dose approach in health risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/R-94/007. Available from the National Technical Information Service, Springfield, VA, PB95-213765, and online at http://cfpub.epa.gov/ncea/raf/raf_pubtitles.cfm?detype=document&excCol=archive.

U.S. EPA (Environmental Protection Agency). (1996) Guidelines for reproductive toxicity risk assessment. Federal Register 61:56274–56322. Available online at http://www.epa.gov/ncea/raf/rafguid.htm.

U.S. EPA (Environmental Protection Agency). (1998a) Guidelines for neurotoxicity risk assessment. Federal Register 63:26926–26954. Available online at http://www.epa.gov/ncea/raf/rafguid.htm.

U.S. EPA (Environmental Protection Agency). (1998b) Assessment of thyroid follicular cell tumors. Risk Assessment Forum, Washington, DC; EPA/630/R-97/002. Available from the National Technical Information Service, Springfield, VA, PB98-133119, and online at http://nepis.epa.gov/EPA/html/Pubs/pubtitleORD.htm.

U.S. EPA (Environmental Protection Agency). (1998c) Health effects test guidelines: neurotoxicity screening battery. Office of Prevention, Pesticides and Toxic Substances, Washington, DC; OPPTS 870.6200; EPA 712-C-98-238. Available online at http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-6200.pdf.

U.S. EPA (Environmental Protection Agency). (2000a) Science policy council handbook: risk characterization. Office of Science Policy, Office of Research and Development, Washington, DC. EPA/100-B-00-002. Available online at http://www.epa.gov/OSA/spc/pdfs/prhandbk.pdf.

U.S. EPA (Environmental Protection Agency). (2000b) Benchmark dose technical guidance document [external review draft]. Risk Assessment Forum, Washington, DC; EPA/630/R-00/001. Available online at

http://cfpub.epa.gov/ncea/cfm/
nceapublication.cfm?ActType=PublicationTopics&detype=DOCUMENT&subject=BENCHMARK+DOSE&subjtype=TITLE&excCol=Archive.

U.S. EPA. (2000c) Supplementary guidance for conducting for health risk assessment of chemical mixtures. Risk Assessment Forum, Washington, DC; EPA/630/R-00/002. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA (Environmental Protection Agency). (2002) A review of the reference dose and reference concentration processess. Risk Assessment Forum, Washington, DC; EPA/630/P-02/002F. Available online at http://cfpub.epa.gov/ncea/raf/raf_pubtitles.cfm?detype=document&excCol=archive.

U.S. EPA (Environmental Protection Agency). (2004) Decabromodiphenyl ether. Substance Registry System. U.S. Environmental Protection Agency, Washington, DC. Available online at http://www.epa.gov/srs.

U.S. EPA (Environmental Protection Agency). (2005a) Guidelines for carcinogen risk assessment. Federal Register 70:17765–18717. Available online at http://www.epa.gov/cancerguidelines.

U.S. EPA (Environmental Protection Agency). (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, Washington, DC; EPA/630/R-03/003F. Available online at http://www.epa.gov/cancerguidelines.

U.S. EPA (Environmental Protection Agency). (2006a) Science policy council handbook: peer review. 3rd edition. Office of Science Policy, Office of Research and Development, Washington, DC; EPA/100/B-06/002. Available online at http://www.epa.gov/OSA/spc/2peerrev.htm.

U.S. EPA (Environmental Protection Agency). (2006b) A framework for assessing health risk of environmental exposures to children. National Center for Environmental Assessment, Washington, DC, EPA/600/R-05/093F. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363.

Viberg, H; Fredriksson, A; Eriksson, P. (2002) Neonatal exposure to the brominated flame retardant 2,2',4,4',5-pentabromodiphenyl ether causes altered susceptibility in the cholinergic transmitter system in the adult mouse. Toxicol Sci 67(1):104–107.

Viberg, H; Fredriksson, A; Jakobsson, E; et al. (2003a) Neurobehavioral derangements in adult mice receiving decabromodiphenyl ether (PBDE 209) during a defined period of neonatal brain development. Toxicol Sci 76:112–120.

Viberg, H; Frederiksson, A; Eriksson, P. (2003b) Neonatal exposure to polybrominated diphenyl ether (PBDE 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. Toxicol Appl Pharmacol 192(2):95–106.

Viberg, H; Fredriksson, A; Jakobsson, E; et al. (2004a) Neonatal exposure to the brominated flame-retardant, 2,2',4,4',5-pentabromodiphenyl ether, decreases cholinergic nicotinic receptors in hippocampus and affects spontaneous behaviour in the adult mouse. Environ Toxicol Pharmacol 17:61–65.

Viberg, H; Fredriksson, A; Eriksson, P. (2004b) Investigations of strain and/or gender differences in developmental neurotoxic effects of polybrominated diphenyl ethers in mice. Toxicol Sci 81:344–353.

Viberg, H; Fredriksson, A; Eriksson, P. (2005) Deranged spontaneous behavior and decrease in cholinergic muscarinic receptors in hippocampus in the adult rat, after neonatal exposure to the brominated flame-retardant, 2,2',4,4',5-pentabromodiphenyl ether (PBDE 99). Environ Toxicol Pharmacol 20:283–288.

Viberg, H; Fredriksson, A; Eriksson, P. (2007) Changes in spontaneous behavior and altered response to nicotine in the adult rat, after neonatal exposure to the brominated flame retardant, decabrominated diphenyl ether (PBDE 209). Neurotoxicol 28:136–142.

Villeneuve, DL; Kannan, K; Priest, BT; et al. (2002) In vitro assessment of potential mechanism-specific effects of polybrominated diphenyl ethers. Environ Toxicol Chem 21(11):2431–2433.

Wolf, DC; Mann, PC. (2005) Confounders in interpreting pathology for safety and risk assessment. Toxicol Appl Pharmacol 202:302–308.

Zhou, T; Ross, DG; DeVito, MJ; et al. (2001) Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. Toxicol Sci 61:76–82.

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

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

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

EXTERNAL PEER REVIEWER COMMENTS

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

A. General Comments

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

Comment 1: Three reviewers stated that they were unaware of any other relevant studies that would contribute to the BDE-209 IRIS assessment. One reviewer identified potentially relevant additional literature:

Jones-Ortazo, HA; et al. (2005) Environ. Sci. Technol. 39:5121-5130
Wilford, BH; et al. (2005) Environ. Sci. Technol. 39:7027-7035
Schecter, A; et al. (2005) J. Toxicol. Environ. Health Part A 68:501-513
Hites, RA; et al. (2004) Environ. Sci. Technol. 38:4945-4949
Schecter, A; et al. (2006) Environ. Health Perspect. 114:1515-1520
Fischer, D; et al. (2006) Environ. Health Perspect. 114:1581-1584
Bradman, A; et al. (2007) Environ. Health Perspect. 115:71-74
Kodavanti, PRS; Derr-Yellin, EC. (2002) Toxicol. Sci. 68:451-457
Kodavanti, PRS; et al. (2005) Toxicol. Sci. 88:181-192
Reistad, T; Mariussen, E. (2005) Toxicol. Sci. 87:57-65
Reistad, T; et al. (2006) Arch. Toxicol. 80:785-796

Response: The Agency reviewed and evaluated the studies recommended by the reviewer and has included the relevant studies for BDE-209. Fischer et al. (2006), Bradman et al. (2007), and Kodavanti et al. (2005) were found to be relevant and were added to the document. The remaining studies suggested by the reviewer fell outside the scope of the IRIS assessment (i.e., exposure data, commercial mixtures). Additionally, a new literature search was conducted to ensure that recently published relevant studies are included in the IRIS assessment. Four studies were added to the "Toxicological Review" for BDE-209 as the result of the literature search (Huwe and Smith, 2007; Pacyniak et al., 2007; Rice et al., 2007; Thuresson et al., 2006). A description of these studies can be found in sections 3 and 4.

B. Oral Reference Dose (RfD) Values

Charge Question 2. *Have the rationale and justification for deriving RfDs on the basis of the neurobehavioral toxicity studies been transparently and objectively described in the draft*

toxicological reviews of BDE-47, -99, -153, and -209? Are there additional studies that should be considered for deriving the RfDs for any of the four PBDE congeners?

Comment 1: Three reviewers stated that the rationale for deriving the RfD based on the neurobehavioral toxicity studies was clearly and transparently described. Two reviewers stated that the neurobehavioral effects are the only toxic effects that have been observed consistently in PBDE rodent studies. One of these reviewers noted that, although the rat and mouse bioassays by NTP (1986) provide suitable data, the neurobehavioral studies appeared to provide the most appropriate dose-response data on which to base the health assessment. None of the reviewers suggested additional studies that should be considered for deriving the RfD.

Response: No response needed.

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

Comment 1: One reviewer supported the use of the Eriksson/Viberg neurobehavioral study as the basis for the derivation of the RfD, given the limited body of toxicological information available. Two reviewers noted that the studies are limited by the fact that they originated from the same laboratory. One reviewer was concerned that the experimental design of the principal study selected more than one pup per litter, ignoring the "litter" effect. Treating littermates as independent experimental units could confuse dose effects with litter effects. Another reviewer was concerned with the specificity of the neurobehavioral data for developmental neurotoxicity and suggested that independent confirmation of the endpoints is essential. One peer reviewer identified the use of a single sex (male mice) as a limitation of the critical study that had not been identified in the "Toxicological Review" discussion of study limitations. One of these reviewers stated that these limitations do not hinder the derivation of the RfD for BDE-209 but make the confidence low. Another reviewer noted that the neurobehavioral findings of this laboratory have been corroborated in a study examining BDE-99 (Kuriyama et al., 2005). None of the reviewers stated that the studies could not be used as the basis for the derivation of the RfD.

Response: The "Toxicological Review" contains a detailed summary of the concerns with the study design and methods utilized in the principal study (see section 5.1.1). A discussion of the use of only male mice in the study by Viberg et al. (2003a) has been added to the discussion in section 5.1.1 of the "Toxicological Review." Additionally, the neurobehavioral effects reported in Viberg et al. (2003a) are supported by a study in male and female mice that was published by

Rice et al. (2007) after the peer review, which used an expanded array of neurodevelopmental endpoints and dosing for 14 days. The neurodevelopmental findings from Viberg et al. (2003a) are also supported by an expanding body of literature for the BDE-47, -99 and -153 congeners (Viberg et al., 2007, 2005, 2004a, b, 2003b, 2002; Kuriyama et al., 2005; Eriksson et al., 2002, 2001; Branchi et al., 2002) that details changes in motor and cognitive activity in rodents following administration of single or repeated perinatal doses of PBDEs. Some of the concerns associated with the methodology of the Eriksson/Viberg neurobehavioral studies are alleviated by other studies (Rice et al., 2007; Kuriyama et al., 2005; Branchi et al., 2002) using more traditional methodologies that have generated toxic effects similar to those reported by Viberg et al. (2003a). Results reported by Viberg et al. (2003a) for BDE-209 are similar to results reported by Eriksson et al. (2001) for BDE-47, Viberg et al. (2004b) and Kuriyama et al. (2005) for BDE-99, and Viberg et al. (2003b) for BDE-153. Altogether, these studies support the findings of Viberg et al. (2003a) that exposure to BDE-209 in early developmental stages can result in lasting changes in the neurobehavioral activity of mice.

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

Comment 1: All four reviewers believed that the Viberg et al. (2003a) BDE-209 study was appropriate for determining the point of departure. One reviewer felt that these data were appropriate as long as the document emphasizes that the neurochemical data also show alterations in normal developmental patterns. Another reviewer noted that both Viberg et al. (2003a) and NTP (1986) provided data appropriate for determining a point of departure, with Viberg et al. (2003a) providing the lowest point of departure. None of the reviewers suggested an alternative study for determining the point of departure. Two reviewers explicitly stated that the strengths and weaknesses were identified and clearly presented.

Response: No response needed.

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

Comment 1: All four reviewers agreed with the selection of the neurobehavioral effects as critical effects and that these effects were appropriate for identifying a point of departure. One

of the reviewers felt that the neurochemical data also provided critical information and should be presented centrally rather than as supporting data. One reviewer stated that there was no correlation between PND of exposure and the concentration of the chemical in the brain. One reviewer added that decreased habituation might be as appropriate as or more appropriate than the habituation ratio as an indicator of toxicity, while another believed that the actual behavioral data, rather than the habituation ratio, should have been presented in the document. It was not clear for another reviewer why the actual data could not be recovered from the study authors to allow for dose-response modeling and BMD estimation, given that the studies were published fairly recently (2003). This reviewer recommended that the Agency attempt to recover the neurobehavioral toxicity data from the study authors.

Response: The evidence of neurochemical interactions and the potential relationship with the neurobehavioral effects are highlighted in the mode-of-action section of the document (section 4.5.3). The document presents the hypothesis proposed by the Eriksson/Viberg group in which the observed effects on locomotion and habituation are related to impaired development of the cholinergic system during the postnatal brain growth spurt; however, data are unavailable to determine the relevance of the neurochemical effects or to establish a mode of action. This conclusion has been added to section 4.5.3. In the case of BDE-209, the levels of radiolabel in the brain were higher following administration of equivalent doses on PND 3 or 10 compared to PND 19 (Viberg et al., 2003a). There are no measurements of levels of BDE-209 or the congeners in the brain or other tissues at the time of neurobehavioral testing at 2 or 4 months to show if any differences exist in the brain or other tissues at those time points.

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

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

Response: No response needed.

Comment 3: One reviewer noted that a BMDL would be better than a NOAEL for deriving an RfD.

Response: The Agency recognizes that a NOAEL can be limiting since it is highly dependent on the doses selected and the sample size and does not account for the dose-response curve. Alternatively, a BMDL is more independent of study design, takes into account the dose-response curve, and, therefore, is preferable to a NOAEL as a point of departure. However, the data in the principal study for BDE-209 only allows for the identification of a NOAEL. The reporting is insufficient to provide the necessary information to perform BMD modeling and the Agency's attempts to obtain the raw data were unsuccessful.

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

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

Comment 1: Two reviewers agreed that the document described the rationale and justification for each UF and another reviewer noted that the selection of the UFs was described in detail. The fourth reviewer felt that the document did not provide much explanation or justification for applying the default intraspecies UF_H for BDE-209.

Response: There is little information available on the effects of PBDEs in humans and in the absence of data there is no scientific rationale for moving away from the default value for the intraspecies UF_H. Additional explanation for the intraspecies UF_H was added to section 5.1.3.

Comment 2: One reviewer suggested decreasing the interspecies UF_A, considering the relatively specific and sensitive nature of the neurobehavioral and neurochemical measures compared with conventional endpoints. However, another reviewer felt that the 10-fold UF_A was justifiable based on the lack of data on the mode of action in animals and humans.

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

Comment 3: Two reviewers suggested lowering the intrahuman UF_H. One of these reviewers felt the relatively specific and sensitive nature of the neurobehavioral and neurochemical measures compared with conventional endpoints warranted a decrease in the UF_H. The other reviewer recommended decreasing the 10-fold UF_H to 3-fold based on the sensitivity of the test species population (neonates).

Response: The 10-fold UF_H for intraspecies uncertainty is retained based on the lack of information concerning the pharmacokinetics and mode of action of the BDE-209 in humans. In the absence of human data, the effects in potentially susceptible populations exposed to BDE-209 cannot be determined. Additional explanation for applying the default intraspecies UF_H was added to section 5.1.3.

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

Response: For BDE-209, the principal study identified endpoints that, for the most part, reflect specific aspects of developmental physiology. The hypothesized window of susceptibility, proposed by the study authors, is based on the observation that the developmental neurotoxic effects observed following exposure to BDE-209 on PND 3 will not occur once the toxicokinetics of intestinal uptake and excretion have matured and the animal brain is developmentally less active (outside the window of susceptibility). The Eriksson/Viberg group has suggested that the period of maximum vulnerability for the developing cholinergic system that coincides with the most pronounced neurodevelopmental effects from BDE-99 exposure is from PNDs 10–14. However, for BDE-209 the developmental neurotoxic effects were noted following exposure on PND 3 rather than on PNDs 10 or 19. It is likely that two factors

contributed to this difference in peak vulnerability. First, absorption from the gastrointestinal tract decreases as the neonatal mice age during their first few weeks of life, thus less of the BDE-209 reaches the liver for distribution on PNDs 10 and 19 than on PND 3. Secondly, distribution to the brain is slow. The amount of radiolabel in the brain is 1.4- and 2.8-fold greater 7 days after dosing compared with 24 hours after dosing on PNDs 3 and 10, respectively. Even less reaches the brain when the BDE-209 is administered on day 19. The UF_S was viewed as a dosing duration adjustment rather than simply a comparison of the effects of a subchronic to a chronic exposure. A threefold UF_S was applied because the critical study dosed the animals only once within the hypothesized critical window, not because the chronic exposures would have exacerbated the impact on habituation.

In response to the comment about possible effects as the result of a maternal pre-pregnancy body burden, the Agency notes that, although the principal study did not include prenatal exposure, the maternal uptake and retention of BDE-209 during the prenatal period would be lower than that of the pups during the postnatal period of vulnerability because of the differences in toxicokinetics for mature versus neonatal animals. Support for the UF_S is provided by the Hardy et al. (2002) study, where BDE-209 treatment did not produce any evidence of maternal or developmental toxicity up to the highest dose tested (1,000 mg/kg-day) despite exposure from gestation days 0 through 19.

Comment 5: Two reviewers recommended raising the onefold database UF_D to threefold. One of these reviewers based this decision on the use of a NOAEL rather than a BMDL as the point of departure. This reviewer felt that the absence of definitive data for dose-response modeling for the neurobehavioral effects reflects an inadequacy in the database, although if the endpoints could be modeled then a onefold UF_D is acceptable. The other reviewer suggested raising the UF_D to account for the lack of other developmental neurotoxicity studies.

Response: EPA's practice is to apply a database UF_D, generally ranging from 1–10, in the health assessment to account for the potential for deriving an underprotective RfD as a result of an incomplete characterization of a chemical's toxicity because of missing studies. In deciding to apply this factor to account for deficiencies in the available data set and in identifying its magnitude, EPA considers both the data lacking and the data available for particular organ systems as well as life stages. The database for BDE-209 contains subchronic and chronic toxicity studies as well as a traditional developmental study. Although the database lacks a two-generation reproductive study, the absence of effects in the available longer-term and developmental studies indicates that at least some aspects of reproductive organ toxicity or function are not affected at doses much higher than those that resulted in the neurological effects observed in neonates in the principal study. Therefore, the Agency retains the onefold database

UF_D. Calculating a BMDL rather than using a NOAEL for the point of departure would not impact the use of a database UF.

Comment 6: Two reviewers believed the database supports the determination of the RfD but stated that the overall confidence in the RfD assessment is low. Another reviewer believed the database is very poor and suggested that the RfDs be acknowledged as temporary while waiting for additional studies that increase confidence.

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

C. Body Burden Approach

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

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

Response: EPA examined the data on BDE-209 to determine if a body burden approach could be used for this congener during the development of the "Toxicological Review." It was determined that existing half-life, exposure, metabolite and mode-of-action data could not support a body burden calculation for this congener.

Charge Question 8. *Do you agree with the rationale described in the "Toxicological Review" of BDE-209 that the data on the window of susceptibility of the cholinergic receptors to BDE-209 tend to minimize body burden concerns?*

Comment 1: Three reviewers stated that the question was unclear. One reviewer accepted the concept as a basis for the experimental design, given the available information. A second reviewer stated that there was no direct evidence that BDE-209 directly affects cholinergic receptors and suggested that the mechanism of the interaction must be complex and indirect. A third reviewer stated that although there are no definitive data on mode of action, this hypothesis is plausible. This reviewer acknowledges that the data on the window of susceptibility of the

cholinergic receptors to BDE-209 are suggestive but believes there are too many other possibilities for mode of action for this rationale to minimize body burden concerns.

Response: Available mode-of-action data that describe the developmental neurotoxicity of BDE-209 are limited. The Eriksson/Viberg group, the principal study authors, have hypothesized that the observed effects on locomotion and habituation are related to impaired development of the cholinergic system during the postnatal brain growth spurt period based on studies of BDE-99 (Viberg et al., 2005, 2004a) and supported by studies with BDE-153 (Viberg et al., 2003b) and BDE-209 (Viberg et al, 2007, 2003a). They have further hypothesized that the sensitivity of the cholinergic system occurs in the vicinity of PND 10 and have tested this hypothesis by varying the time of dosing and observing differences in the habituation effect for BDE-99 and BDE-209 (Viberg et al., 2007; Eriksson et al., 2002). A difference in the impact of the time of dosing was observed between BDE-99 and BDE 209 and can be explained based on toxicokinetic factors (see section 4.5.3). The resulting deficit in cholinergic receptors persisted across the duration of testing and could cause an abnormal response to exposure to cholinergic stimulants in adulthood. The following statement has been added to the mode-of-action summary (section 4.5.3): "While evidence exists that demonstrates BDE-209, as well as other PBDEs, interacts at the neurological level, data are inadequate to determine the mode of action for BDE-209."

D. Carcinogenicity Assessment

Charge Question 9. *Is the weight of evidence for the carcinogenicity of BDE-209 in the draft "Toxicological Review" appropriately described? Are there additional studies that should be included?*

Comment 1: Three reviewers commented that the weight of evidence for carcinogenicity of BDE-209 was appropriately described in the "Toxicological Review." One reviewer stated that the carcinogenicity study was completely and methodically described. This reviewer noted that the carcinogenicity study is over 25 years old and utilized very high doses that led to some toxic effects and animal deaths. None of the reviewers were aware of additional studies that should be included in the cancer assessment for BDE-209.

Response: No response needed.

Charge Question 10. *Do the data support estimation of a cancer slope factor for BDE-209? If yes, is the rationale for the quantitative analysis objectively and transparently described,*

considering the uncertainty in the data and the suggestive nature of the weight of evidence? Have the rationale and justification for the use of the linear low-dose extrapolation been objectively and transparently presented?

Comment 1: One reviewer stated that the data appear to support estimation of a cancer slope factor, one reviewer stated that the data supports estimation, one reviewer did not specifically answer this question but, as mentioned below, did question the appropriateness of the study EPA used, and the fourth reviewer did not answer the question about the support for estimation of a cancer slope factor. One reviewer felt that the data supported the weight-of-evidence classification as "suggestive of the carcinogenic potential." One reviewer questioned the appropriateness of the NTP (1986) study for developing the cancer slope factor based on the utilization of very high doses and potentially limited absorption of BDE-209 and suggested that a new bioassay be conducted. This reviewer recognized the public comments on the carcinogenesis data, particularly the statements regarding the modest dose-response and the change, since the completion of the study, in criteria for classifying liver lesions in rats. This reviewer was less concerned with the scoring of neoplastic nodules as tumors because the nodules identified in chronic studies with exposure to a single compound are less likely to regress as compared with an initiation/promotion study. Regarding the change in classification criteria, another reviewer noted that the data supported the estimation of a cancer slope factor for BDE-209, with the assumption that the change in classification of the neoplastic nodules would not appreciably change the dose response. Two reviewers explicitly stated that the rationale for the quantitative analysis is transparently described. Two reviewers stated that the justification for the use of the linear low-dose extrapolation is objectively presented in the BDE-209 assessment. One reviewer noted that the document did not include a rationale or justification, other than a reference to EPA guideline documents (U.S. EPA, 2005a, b), for the use of linear low-dose extrapolation. None of the reviewers disagreed with the estimation of the cancer slope factor or the use of linear low-dose extrapolation.

Response: The Agency is aware of the deliberations concerning the significance of neoplastic nodules in interpreting cancer risk. In 1986, a significant change was made regarding the NTP nomenclature for hepatoproliferative lesions of rats: lesions previously combined under the diagnosis of "neoplastic nodules" were replaced with three terms: "hepatocellular hyperplasia," "foci of cellular alteration," and "hepatocellular adenoma." The term "hyperplasia" was reserved for proliferative lesions that were perceived to be secondary, nonneoplastic responses to degenerative changes in the liver. Foci of cellular alteration, hepatocellular adenoma, and hepatocellular carcinoma were considered to represent a spectrum of changes that are part of the carcinogenic process (Maronpot et al., 1986). It is recognized that not all neoplastic nodules

have the potential to progress to malignancy: some neoplastic nodules may regress following removal of a chemical stimulus, others may only be hyperplastic lesions, and still others may progress to hepatocellular carcinoma. Therefore, the exact contribution of neoplastic nodules to the overall incidence of carcinoma in the rat liver is not known with any certainty. However, an increase in neoplastic nodules in treated rats provides some indication that the liver may be at increased risk of cancer formation, and inclusion of the incidence of neoplastic nodules in the assessment of cancer risk is considered to be a conservative approach (U.S. EPA, 1986c). This change in nomenclature has been noted in the "Toxicological Review" in sections 4.2, 4.6.2, and 5.3.3 and in Table 4-2.

EPA acknowledges that toxicokinetic limitations on solubility and absorption may make external dose a poor indicator of systemic dose. However, there are no PBPK models for BDE-209 that might be used to estimate internal dose. Thus, the only parameter available for modeling dose-response is the external dose.

The justification for using the linear low-dose extrapolation for deriving a cancer slope factor for BDE-209 is based on the absence of data available to determine the mode of action for BDE-209. This rationale is provided in section 5.3.4.

Charge Question 11. *Are there alternative modeling approaches that should have been considered instead of or in addition to the linear low-dose extrapolation approach?*

Comment 1: None of the reviewers suggested that an alternative modeling approach should have been considered. One reviewer stated that all possible modeling approaches were explained and the model that best fit the experimental data was selected. Another reviewer noted that consideration of other modeling approaches is not necessary, considering the dose-response data are linear, the linear model adequately fits the data, and the data are inadequate to justify an alternative approach.

Response: No response needed.

Miscellaneous Comments

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

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

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

Response: The requested data were added to the descriptions of the pertinent studies (in section 3) when they were provided by the authors of the paper. Frequently, the position of the radiolabel was not specified. In a few cases the radiolabel was described as "uniform," suggesting that all carbons carried the radiolabel. If the authors of the paper used the term "uniform," it has been added to the discussion of the study. No change was made if the authors of the paper did not comment on the position of the radiolabel.

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

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

Comment 4: One reviewer suggested including proposed metabolic pathways to strengthen the fact that the lower brominated congeners could be obtained from the higher brominated ones.

Response: A diagram of a proposed metabolic pathway has been included as Figure 3-1 in section 3.3. The diagram is based on the metabolites that have been identified in several studies. The metabolic pathway is described as "proposed" and uncertainties are indicated. The

individual publications that provided data on metabolites are cited in the Figure 3-1. In cases where the data from one study are discussed in more than one section of the "Toxicological Review," a reference to the location of prior mention of the study is provided.

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

Response: Information is currently insufficient to identify the relative potency of the four congeners.

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

Response: An overview to the toxicokinetics section and summary paragraphs have been added to section 3.

Comment 7: One reviewer recommended improving the discussion of the possible debromination of BDE-209 to lower brominated congeners and questioned if environmental debromination should be mentioned.

Response: Discussion of the potential debromination of BDE-209 to lower-brominated congeners can be found in sections 3.2.2 and 3.3, based on evidence observed in several studies (Huwe and Smith, 2007; Morck et al., 2003; Sandholm et al., 2003; Zhou et al., 2001). Data for environmental debromination falls outside the scope of the IRIS health assessment.

Comment 8: One reviewer recommended presenting the receptor site interaction information in a summary table.
Response: Tables were added to the toxicological reviews for BDE-47, -99, and -153 because there were data for multiple congeners for the tetra, penta, and hexa congeners. In the case of BDE-209, there is only one PBDE congener and there was no need to have a table to summarize the data.

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

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

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

Response: The lipohilicity of the BDE-209 is acknowledged in the "Toxicological Review" as part of section 3 on toxicokinetics. It will be necessary to determine if absorption occurs via the chylomicrons along with the body lipids or via direct membrane transport before the full impact of the vehicle on absorption, distribution, metabolism, and elimination can be determined. The data are currently inadequate to determine the impact of the oily vehicle on the distribution and uptake of BDE-209.

PUBLIC COMMENTS

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

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

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

Comment 2: Three public commenters from one group questioned the selection of Viberg et al. (2003a) as the principal study for the derivation of the RfD, and another public commenter disagreed with the selection of Viberg et al. (2003a). Critiques of the study were submitted by the three public commenters questioning the methods utilized by the principal study authors. These public commenters were concerned that the purity of the radiolabeled BDE-209 in Viberg et al. (2003a) was not reported and suggested that observed effects may have been induced by impurities rather than the parent compound. Two of these public commenters felt that applying the intraspecies UF_H to account for sensitive populations when evidence for a sensitive subpopulation does not exist is overly conservative. One of the public commenters noted the use of inconsistent units of concentration within the document.

Response: The Agency has included a detailed summary of the concerns with the experimental design and methods utilized in the principal study (see section 5.1.1). These issues were raised during the external peer review of the BDE-209 IRIS assessment. The peer reviewers acknowledged the limitations and concerns with the study; however, all of the peer reviewers agreed that this study was appropriate for derivation of the RfD for BDE-209 and that its limitations were transparently discussed in the "Toxicological Review." Additionally, the neurobehavioral effects reported in Viberg et al. (2003a) are supported by other data for BDE-209 (Rice et al., 2007) and by an expanding body of literature for the PBDEs (Viberg et al., 2007, 2005, 2004a, b, 2003b, 2002; Kuriyama et al., 2005; Eriksson et al., 2002, 2001; Branchi et al., 2002) that details changes in motor and cognitive activity in rodents following administration of single or repeated perinatal doses of PBDEs. A summary of Rice et al. (2007) has been added to the BDE-209 "Toxicological Review" in section 4.3.2.

In response to the radiolabel concerns, the purities and positions of the radiolabels, the specific activities of the ^{14}C compounds, and the locations of radioactivity in the brain were added to the descriptions of the relevant studies (in section 3) when they were provided by the authors of the paper. Frequently, the position of the radiolabel was not specified and/or the radiolabel was inadequately described to allow for the detailed reporting. These data have been added to the document where available.

The 10-fold UF_H for intraspecies uncertainty is retained based on the lack of information concerning the pharmacokinetics and mode of action of the PBDEs in humans. In the absence of human data, the effects in potentially susceptible populations exposed to PBDEs cannot be determined. Additional explanation for applying the default intraspecies UF_H was added to section 5.1.3.

In response to the statement regarding the inconsistent reporting of units of concentration, the Agency notes that the doses and concentrations that are in the "Toxicological

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

Comment 3: One public commenter suggested a revision of the absorption, distribution, metabolism, and elimination sections (section 3) of the IRIS assessment for BDE-209. Additionally, another public group identified weaknesses in several studies within section 3.

Response: The toxicokinetics section (section 3) has been revised and updated to reflect recently published literature (Huwe and Smith et al., 2007; Pacyniak et al., 2007; Thuresson et al., 2006). An introduction to the toxicokinetics information has been added to section 3. The studies within this section have been reorganized to separate animal and human data in section 3.1.

Comment 4: One public commenter disagreed with the conclusion that the database for BDE-209 provides "suggestive evidence of carcinogenic potential." This commenter recommended adding a chronic study in rats administered BDE-209 (Kociba et al., 1975). Additionally, the public commenter noted the change in nomenclature for hepatoproliferative liver lesions in rats and the appropriateness of using the incidence of "neoplastic nodules" for quantitative cancer risk assessment.

Response: The external peer reviewers were asked to evaluate the weight of evidence for carcinogenic potential for BDE-209 and to determine if the database supported the estimation of a cancer slope factor. Three of the peer reviewers explicitly stated that the database was sufficient, and none of the reviewers disagreed with either the classification of "suggestive evidence of carcinogenic potential" or the quantitative cancer assessment for BDE-209. Kociba et al. (1975) administered a commercial grade BDE-209 that possesses a lower purity than the analytical grade utilized in the majority of the studies described in the IRIS health assessment. This study was not included for comparison since it falls outside the scope of the IRIS health assessment for the pure BDE-209 congener. Please see the response to the external peer review Comment 1, under Charge Question 10, regarding the change in criteria for the classification of hepatoproliferative liver lesions.

APPENDIX B: BENCHMARK DOSE ANALYSIS OF NONCANCER ENDPOINTS

The following data sets from the NTP (1986) 2-year rat and mouse studies were selected for BMD modeling: thrombosis in the liver, liver degeneration, fibrosis in the spleen, and lymphoid hyperplasia in male rats, as well as centrilobular hypertrophy in the liver and follicular cell hyperplasia in the thyroid of male mice.

BMD modeling was conducted by using EPA's BMDS, version 1.3.2. All the BMD analyses were conducted with the BMR set to a 10% response (U.S. EPA, 2000b). For each data set, all the available dichotomous models, including gamma, logistic, log-logistic, multistage, probit, log-probit, quantal-linear, quantal-quadratic, and Weibull, available in the BMDS were used. BMD model fit was evaluated based on the goodness-of-fit *p* value (indicating global model fit), local chi-square residual (indicating the model fit at the data point close to the preset BMR), as well as AIC (indicating model fit when controlling for number of model parameters). Based on the model output, the model(s) providing best model fit to the data were selected to estimate BMD_{10} and $BMDL_{10}$. In some cases, the BMDS gives exactly the same results for several different models. This occurs when model parameters are fixed at a boundary, yielding reduced models that are identical expressions for the probability of response. For example, when the power parameter in the Weibull or gamma model is fixed at 1 by BMDS or when the degree of the multistage model is set to 1 by the user, these models reduce to the quantal linear model. When this occurred, all the reduced models providing the same BMD results were considered as one model.

Liver Thrombosis in Male Rats

The data (1/50, 0/50, and 9/49 for control, low dose, and high dose, respectively) on thrombosis in the liver of male rats treated with decaBDE (NTP, 1986) were modeled with EPA BMDS. The reported oral doses were 0, 1,120, and 2,240 mg/kg-day, respectively. The data were modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table B-1.

Based on these results, the models that did not demonstrate significant lack of fit for the data were obtained from three models (shown in bold in the table): gamma, log-logistic, and Weibull. These models provided relatively high goodness-of-fit *p* values and low AIC values. Based on these considerations, an average value of the BMDs estimated from these three models is used. For thrombosis in the livers of male rats, the average BMD_{10} is 2,125 mg/kg-day and the average $BMDL_{10}$ is 1,738 mg/kg-day.

Table B-1. Summary of BMD modeling results for thrombosis in the liver of male rats

Model	Goodness-of-fit *p* value	AIC	BMD_{10}	$BMDL_{10}$
Gamma	0.31	61.9754	**2,047**	**1,717**
Log-logistic	0.32	61.9384	**2,161**	**1,741**
Logistic	0.04	65.0449	1,909	1,597
Multistage	0.07	66.1380	1,898	1,449.97
Log-probit	N/A[a]	63.9383	2,130	1,691.4
Probit	0.04	65.8039	1,869	1,513.96
Quantal-linear	0.02	69.4028	2,030	1,162.71
Quantal-quadratic	0.07	66.1380	1,898	1,449.97
Weibull	0.31	61.9384	**2,166**	**1,757**

[a]N/A = not applicable.

Source: NTP (1986).

Liver Degeneration in Male Rats

The data (13/50, 19/50, and 22/49 for control, low dose, and high dose, respectively) on degeneration in the liver of male rats treated with decaBDE (NTP, 1986) were modeled with EPA BMDS. The reported oral doses were 0, 1,120, and 2,240 mg/kg-day, respectively. The data were modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table B-2.

Table B-2. Summary of BMD modeling results for degeneration in the liver of male rats

Model	Goodness-of-fit *p* value	AIC	BMD_{10}	$BMDL_{10}$
Gamma	0.83	195.178	**779**	**422**
Log-logistic	0.89	195.148	**707**	**344**
Logistic	0.71	195.269	929	600
Multistage	0.83	195.178	**779**	**422**
Log-probit	0.51	195.555	1,161	718
Probit	0.72	195.259	914	584
Quantal-linear	0.83	195.178	**779**	**422**
Quantal-quadratic	0.42	195.779	1,363	962
Weibull	0.83	195.178	**779**	**422**

Source: NTP (1986).

Based on the goodness-of-fit *p* value, none of the models demonstrated lack of fit. The best model fit for the data was obtained from five models (shown in bold in the table): gamma, log-logistic, multistage, quantal-linear, and Weibull. These models provided high goodness-of-

fit *p* values and low AIC values. However, among the five models, gamma, multistage, quantal-linear, and Weibull were reduced to a single identical model. Based on these considerations, an average value of the BMDs estimated from the log-logistic model and the common reduced model are used. For degeneration in the livers of male rats, the average BMD_{10} is 765 mg/kg-day and the average $BMDL_{10}$ is 406 mg/kg-day.

Liver Degeneration in Male Rats (NTP, 1986)

```
10% BMR
 ======================================================================
              Log-Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20
$
              Input Data File: C:\BMDS\DATA\RATS.(d)
              Gnuplot Plotting File:  C:\BMDS\DATA\RATS.plt
                                                Thu Nov 25 12:11:49 2004
 ======================================================================
 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:

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

Dependent variable = resp1
Independent variable = Dose
Slope parameter is restricted as slope >= 1

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

User has chosen the log transformed model

Default Initial Parameter Values
background   =    0.26
intercept    =    -8.66331
slope        =    1

Asymptotic Correlation Matrix of Parameter Estimates

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

background  intercept

background        1     -0.69

intercept         -0.69 1
```

Parameter Estimates

Variable	**Estimate**	**Std. Err.**
background	0.262212	0.0603288
intercept	-8.75859	0.569357
slope	1	NA

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-95.5647			
Fitted model	-95.5739	0.0184518	1	0.8919
Reduced model	-97.5646	3.99968	2	0.1354

AIC: 195.148

Goodness of Fit

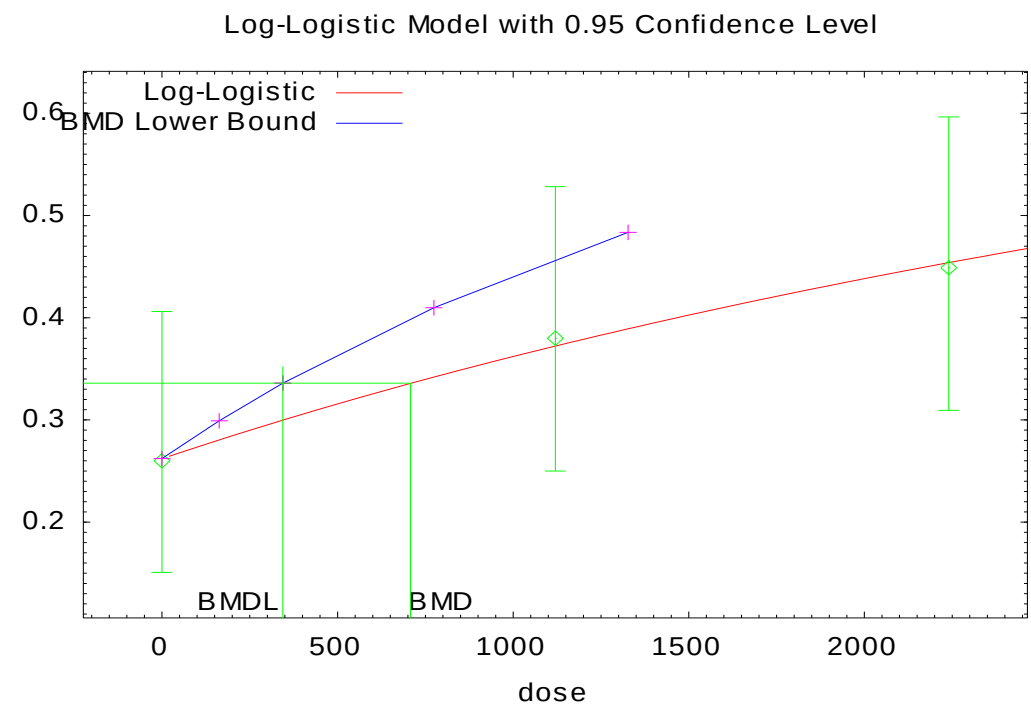
Dose	Est._Prob	Expected	Observed	Scaled Size	Residual
0.0000	0.2622	13.111	13	50	-0.03556
1120.0000	0.3726	18.630	19	50	0.1081
2240.0000	0.4543	22.259	22	49	-0.07432

Chi-square = 0.02 DF = 1 P-value = 0.8919

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95

BMD = 707.234
BMDL = 344.083



12:11 11/25 2004

Splenic Fibrosis in Male Rats

The data (5/49, 8/50, and 13/49 for control, low dose, and high dose, respectively) on fibrosis in the spleen of male rats treated with decaBDE (NTP, 1986) were modeled with EPA BMDS. The reported oral doses were 0, 1,120, and 2,240 mg/kg-day, respectively. The data were modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table B-3.

Table B-3. Summary of BMD modeling results for fibrosis in the spleen of male rats

Model	Goodness-of-fit *p* value	AIC	BMD_{10}	$BMDL_{10}$
Gamma	N/A[a]	139.172	1,464	700
Log-logistic	N/A	139.172	1,463	644
Logistic	0.92	137.183	**1,427**	**1,018**
Multistage	0.69	137.336	1,236	690
Log-probit	0.87	137.197	**1,519**	**1,017**
Probit	0.88	137.195	**1,393**	**968**
Quantal-linear	0.69	137.336	1,236	690
Quantal-quadratic	0.80	137.233	1,612	1,176
Weibull	N/A	139.172	1,470	700

[a]N/A = not applicable.

Source: NTP (1986).

Based on these results, the logistic, multistage, log-probit, probit, quantal-linear, and quantal-quadratic models did not show significant lack of fit for the data. The lowest AIC values were obtained with three models: logistic, log-probit, and probit (shown in bold in the table). Based on these considerations, an average value of the BMDs estimated from these three models is used. For fibrosis in the spleen of male rats, the average BMD_{10} is 1,446 mg/kg-day and the average $BMDL_{10}$ is 1,001 mg/kg-day.

Lymphoid Hyperplasia in Male Rats

The data (4/50, 6/50, and 13/49 for control, low dose, and high dose, respectively) on lymphoid hyperplasia in male rats treated with decaBDE (NTP, 1986) were modeled with EPA BMDS. The reported oral doses were 0, 1,120, and 2,240 mg/kg-day, respectively. The data were modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table B-4.

Based on these results, the models that adequately fit the data were the logistic, multistage, probit, quantal-linear, and quantal-quadratic. The quantal-quadratic model (shown in bold in the table) provided a relatively high goodness-of-fit *p* value and the lowest AIC value.

Based on these considerations, the BMD and BMDL estimates from this model are selected. For lymphoid hyperplasia of male rats, the BMD_{10} is 1,538 mg/kg-day and the $BMDL_{10}$ is 1,165 mg/kg-day.

Table B-4. Summary of BMD modeling results for lymphoid hyperplasia in male rats

Model	Goodness-of-fit *p* value	AIC	BMD_{10}	$BMDL_{10}$
Gamma	N/A[a]	127.266	1,601	765
Log-logistic	N/A	127.266	1,608	725
Logistic	0.62	125.512	1,404	1,063
Multistage	0.36	126.122	1,207	707
Log-probit	N/A	127.266	1,575	1,032
Probit	0.57	125.59	1,364	1,007
Quantal-linear	0.36	126.122	1,207	707
Quantal-quadratic	0.86	125.298	**1,538**	**1,165**
Weibull	N/A	127.266	1,620	765

[a]N/A = not applicable.

Source: NTP (1986).

Centrilobular Hypertrophy in the Livers of Male Mice

The data (0/50, 34/50, and 32/50 for control, low dose, and high dose, respectively) on centrilobular hypertrophy in the liver of male mice treated with decaBDE (NTP, 1986) were modeled with EPA BMDS. The reported oral doses were 0, 3,200, and 6,650 mg/kg-day, respectively. The data were modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table B-5.

Table B-5. Summary of BMD modeling results for centrilobular hypertrophy in the liver of male mice

Model	Goodness-of-fit *p* value	AIC	Local chi-square residual	BMD_{10}	$BMDL_{10}$
Gamma	0.005	140.455	2.48	479	389
Log-logistic	0.094	134.65	1.44	258	180
Logistic	0	166.133	3.87	1,286	1,046
Multistage	0.005	140.455	0.7	479	389
Log-probit	0.004	140.591	2.22	839	676
Probit	0	165.095	4.03	1,253	1,036
Quantal-linear	0.005	140.455	2.48	479	389
Quantal-quadratic	0	164.89	5.08	1,612	1,451
Weibull	0.005	140.455	2.48	479	389

Source: NTP (1986).

Based on the BMD modeling results from all the models used, none of the available dichotomous models in the BMDS provided satisfactory data fit because all the goodness-of-fit *p* values are less than 0.1. This unsatisfactory model fit is due to the nonmonotonic dose response for this particular endpoint. Therefore, BMD and BMDL estimated from these model fits cannot be used.

Follicular Cell Hyperplasia in the Thyroid of Male Mice

The data (2/50, 10/50, and 19/50 for control, low dose, and high dose, respectively) on follicular cell hyperplasia in the thyroid of male mice treated with decaBDE (NTP, 1986) were modeled with EPA BMDS. The reported oral doses were 0, 3,200, and 6,650 mg/kg-day, respectively. The data were modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table B-6.

Table B-6. Summary of BMD modeling results for follicular cell hyperplasia in the thyroid of male mice

Model	Goodness-of-fit *p* value	AIC	Local chi-square residual	BMD_{10}	$BMDL_{10}$
Gamma	N/A[a]	139.241	0	2,040	1,196
Log-logistic	N/A	139.241	<0.01	2,089	1,008
Logistic	0.34	138.147	0.70	2,989	2,430
Multistage	0.76	137.337	–0.09	**1,670**	**1,190**
Log-probit	0.66	137.436	0.34	2,561	1,977
Probit	0.44	137.846	0.59	2,792	2,265
Quantal-linear	0.76	137.337	–0.25	**1,670**	**1,190**
Quantal-quadratic	0.26	138.466	0.96	3,135	2,598
Weibull	N/A	139.241	0.00002	2,022	1,196

[a]N/A = not applicable.

Source: NTP (1986).

Based on these results, the logistic, multistage, log-probit, probit, quantal-linear, and quantal-quadratic models showed adequate fit of the data. The multistage and quantal-linear models (shown in bold in the table) provided adequate goodness-of-fit *p* values and the lowest AIC values. Since both models provided the same data fit, the BMDs estimated from these models are identical. For follicular cell hyperplasia in the thyroid of male mice, the BMD_{10} is 1,670 mg/kg-day and the $BMDL_{10}$ is 1,190 mg/kg-day.

APPENDIX C: BENCHMARK DOSE ANALYSIS OF CANCER ENDPOINTS

All the potential cancer endpoints in the rat and mouse chronic studies (NTP, 1986) expressed as responses from all the animals treated were modeled, and BMD modeling results are summarized below. All the cancer endpoints are modeled with the cancer model in the BMDS, version 1.40b.

Neoplastic Nodules in the Liver of Male Rats

In the rat chronic study (NTP, 1986), there was a dose-dependent increase in the incidences of neoplastic nodules in the livers of male rats. The incidences of the lesion were 1/50, 7/50, and 15/49 for control, low dose, and high dose, respectively. The original oral doses were 0, 1,120, and 2,240 mg/kg-day, and the corresponding HEDs are 0, 305.1, and 608.3 mg/kg-day, respectively. These incidence data on neoplastic nodules in male rats were modeled by using the multistage model as recommended for a tumorigenic endpoint, and the modeling results are summarized in Table C-1.

Table C-1. Summary of BMD modeling results for increase in neoplastic nodules in the liver of male rats

Model	Goodness-of-fit *p* value	AIC	$ED_{12.2}$	$LED_{12.2}$
Multistage	0.58	114.935	**250**	**174**

Source: NTP (1986).

The low-dose group resulted in an incidence of 7/50, which corresponds to 12.2% extra risk. Therefore, the cancer ED at an extra risk of 12.2% was estimated to be 250 mg/kg-day, and the corresponding LED is 174 mg/kg-day. The slope factor at the 12.2% extra risk is 0.0007 per mg/kg-day.

Neoplastic nodules in the liver of male rats (NTP, 1986)
BMR=12.2% extra risk

```
 ======================================================================
              Cancer Model. (Version: 1.2;  Date: 10/20/2005)
              Input Data File: C:\BMDS140B\UNSAVED1.(d)
              Gnuplot Plotting File:  C:\BMDS140B\UNSAVED1.plt
                                          Thu Dec 01 00:38:02 2005
 ======================================================================
 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = response
Independent variable = dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

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
Background  =     0.0063916
Beta(1)     =     0.000566746

Asymptotic Correlation Matrix of Parameter Estimates
            Background  Beta(1)
Background  1           -0.73
Beta(1)     -0.73       1

Parameter Estimates
                                          95.0% Wald Confidence Interval
 Variable        Estimate        Std. Err        Lower Conf.     Upper Conf.
                                                 Limit           Limit
 Background      0.0190343       0.127925        -0.231695       0.269764
 Beta(1)         0.000520805     0.000351251     -0.000167635    0.00120924


Analysis of Deviance Table
 Model            Log(likelihood)    # Param's   Deviance   Test      P-value
                                                            d.f.
 Full model       -55.3119           3
 Fitted model     -55.4674           2           0.311055   1         0.577
 Reduced model    -63.9318           1           17.2399    2
                                                                      0.0001805

AIC:         114.935
```

Goodness of Fit

Dose	Est._Prob	Expected	Scaled Observed	Size	Residual
0.0000	0.0190	0.933	1	49	0.070
305.1000	0.1632	8.158	7	50	-0.443
608.3000	0.2854	13.984	15	49	0.321

Chi^2 = 0.30 d.f. = 1 P-value = 0.5811

Benchmark Dose Computation

Specified effect = 0.122
Risk Type = Extra risk
Confidence level = 0.95

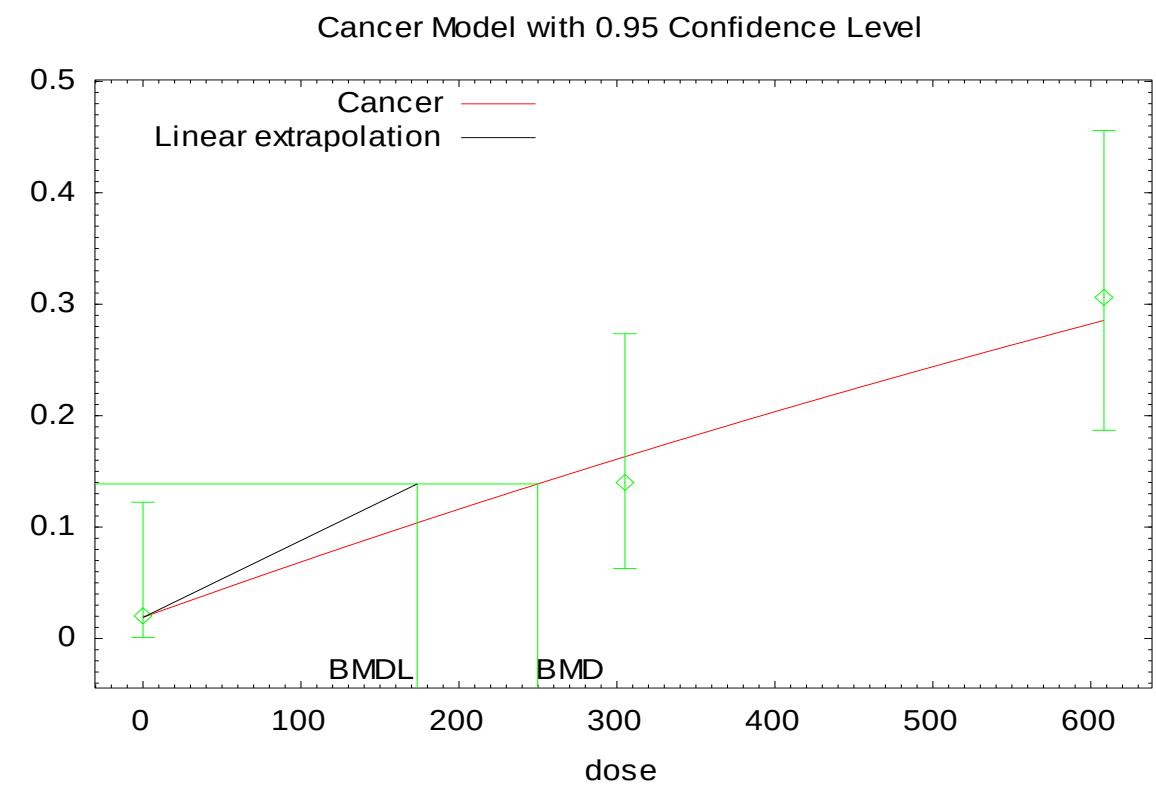
BMD = 249.822

BMDL = 173.541

BMDU = 401.462

Taken together, (173.541, 401.462) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 0.000703004



00:38 12/01 2005

Neoplastic Nodules or Carcinomas (Combined) in the Liver of Male Rats

The data (2/50, 8/50, and 15/49 for control, low dose, and high dose, respectively) on neoplastic nodules or carcinomas (combined) in the liver of male rats treated with decaBDE (NTP, 1986) were also modeled with BMDS. The modeling results are summarized in Table C-2.

Table C-2. Summary of BMD modeling results for increases in neoplastic nodules or carcinomas (combined) in the liver of male rats

Model	Goodness-of-fit *p* value	AIC	$ED_{12.4}$	$LED_{12.4}$
Multistage	0.71	125.18	**263**	**178**

Source: NTP (1986).

The low-dose group resulted in an incidence of 8/50, which corresponds to 12.4% extra risk. Therefore, the cancer ED at an extra risk of 12.4% was estimated to be 263 mg/kg-day, and the corresponding LED is 178 mg/kg-day. The slope factor at the 12.4% extra risk is 0.0007 per mg/kg-day.

Neoplastic Nodules or Carcinomas (Combined) in the Liver of Male Rats (NTP, 1986)
BMR=12.4% extra risk

```
 ======================================================================
              Cancer Model. (Version: 1.2;  Date: 10/20/2005)
              Input Data File: C:\BMDS140B\UNSAVED1.(d)
              Gnuplot Plotting File:  C:\BMDS140B\UNSAVED1.plt
                                                Thu Dec 01 00:51:35 2005
 ======================================================================
 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = response
Independent variable = dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

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
Background  =     0.0312973
Beta(1)     =     0.00053218

Asymptotic Correlation Matrix of Parameter Estimates
            Background  Beta(1)
Background  1           -0.73
Beta(1)     -0.73       1

Parameter Estimates
                                 95.0% Wald Confidence Interval
 Variable      Estimate      Std. Err.     Lower Conf. Limit  Upper Conf.
                                                             Limit
 Background    0.039023      0.127636      -0.21114           0.289186
 Beta(1)       0.000503605   0.000358293   -0.000198637       0.00120585

Analysis of Deviance Table
 Model         Log(likelihoo   #          Deviance     Test d.f.    P-value
               d)              Param's
 Full model    -60.5216        3
 Fitted        -60.5898        2          0.136394     1            0.7119
 model
 Reduced       -67.2168        1          13.3904      2             0.001237
 model

           AIC:          125.18
```

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0390	1.912	2	49	0.065
305.1000	0.1759	8.795	8	50	-0.295
608.3000	0.2926	14.337	15	49	0.208

Chi^2 = 0.13 d.f. = 1 P-value = 0.7136

Benchmark Dose Computation

Specified effect = 0.124
Risk Type = Extra risk
Confidence level = 0.95

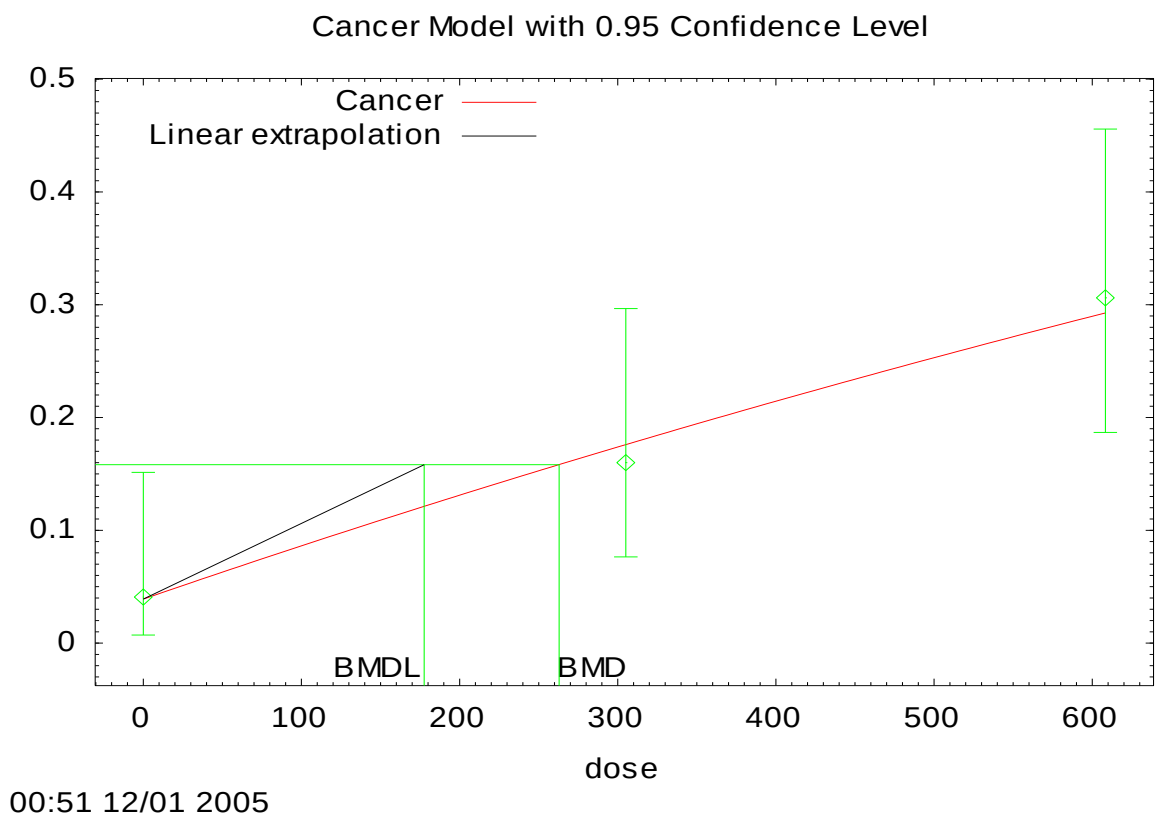
BMD = 262.883

BMDL = 177.57

BMDU = 464.193

Taken together, (177.57 , 464.193) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 0.000698317



00:51 12/01 2005

Neoplastic Nodules in the Liver of Female Rats

In the rat chronic study (NTP, 1986), there was also a dose-dependent increase in the incidences of neoplastic nodules in the livers of female rats. The incidences of the lesion were 1/50, 3/49, and 9/50 for control, low dose, and high dose, respectively. The original oral doses were 0, 1,200, and 2,550 mg/kg-day, and the corresponding HEDs are 0, 292.2, and 618.4 mg/kg-day, respectively. The modeling results are summarized in Table C-3.

Table C-3. Summary of BMD modeling results for increases in neoplastic nodules in the liver of female rats

Model	Goodness-of-fit *p* value	AIC	$ED_{4.2}$	$LED_{4.2}$
Multistage	0.44	84.15	**171**	**103**

Source: NTP (1986).

The low-dose group resulted in an incidence of 3/49, which corresponds to 4.2% extra risk. Therefore, the cancer ED at an extra risk of 4.2% was estimated to be 171 mg/kg-day, and the corresponding LED is 103 mg/kg-day. The slope factor at the 4.2% extra risk is 0.0004 per mg/kg-day.

Neoplastic Nodules in the Liver of Female Rats (NTP, 1986)
BMR=4.2% extra risk

```
 ======================================================================
              Cancer Model. (Version: 1.2;  Date: 10/20/2005)
              Input Data File: C:\BMDS140B\UNSAVED1.(d)
              Gnuplot Plotting File:  C:\BMDS140B\UNSAVED1.plt
                                          Thu Dec 01 01:12:25 2005
 ======================================================================
 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = response
Independent variable = dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

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
Background  =     0.00569581
Beta(1)     =     0.000290683

Asymptotic Correlation Matrix of Parameter Estimates
            Background  Beta(1)
Background  1           -0.73
Beta(1)     -0.73       1

Parameter Estimates
                                                        95.0% Wald
Confidence Interval
 Variable     Estimate     Std. Err.    Lower Conf. Limit  Upper Conf.
                                                           Limit
 Background   0.0173602    0.122542     -0.222817          0.257538
 Beta(1)                   0.000317551  -0.000371802        0.000872976
              0.000250587

                              Analysis of Deviance Table
 Model            Log(likelihood   #          Deviance    Test       P-value
                  )                Param's                d.f.
 Full model       -39.7575         3
 Fitted model     -40.074          2          0.633004    1           0.4263
 Reduced model    -44.1226         1          8.73022     2          0.01271

           AIC:          84.148
```

Goodness of Fit

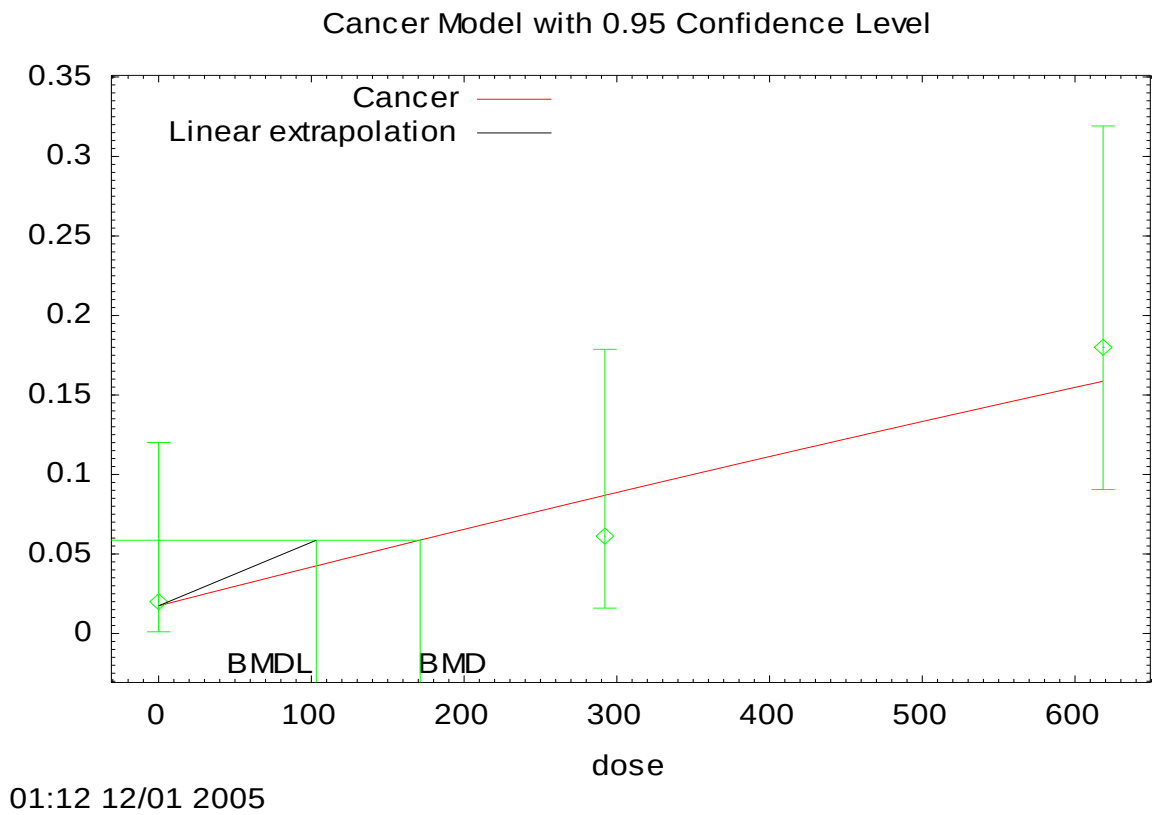
Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
0.0000	0.0174	0.868	1	50	0.143
292.2000	0.0867	4.250	3	49	-0.635
618.4000	0.1584	7.921	9	50	0.418

Chi^2 = 0.60 d.f. = 1 P-value = 0.4394

Benchmark Dose Computation

Specified effect = 0.042
Risk Type = Extra risk
Confidence level = 0.95
BMD = 171.228
BMDL = 103.205
BMDU = 388.232

Taken together, (103.205, 388.232) is a 90% two-sided confidence interval for the BMD
Cancer Slope Factor = 0.000406959



01:12 12/01 2005

Neoplastic Nodules and Carcinomas (Combined) in the Liver of Female Rats

The data (1/50, 5/49, and 9/50 for control, low dose, and high dose, respectively) on neoplastic nodules or carcinomas (combined) in the liver of female rats treated with decaBDE (NTP, 1986) were also modeled. The original oral doses were 0, 1,200, and 2,550 mg/kg-day, and the corresponding HEDs are 0, 292.2, and 618.4 mg/kg-day, respectively. The modeling results are summarized in Table C-4.

Table C-4. Summary of BMD modeling results for increases in neoplastic nodules and carcinomas (combined) in the liver of female rats

Model	Goodness-of-fit *p* value	AIC	$ED_{8.4}$	$LED_{8.4}$
Multistage	0.96	93.24	**301**	**186**

Source: NTP (1986).

The low-dose group resulted in an incidence of 5/49, which corresponds to 8.4% extra risk. Therefore, the cancer ED at an extra risk of 8.4% was estimated to be 301 mg/kg-day, and the corresponding LED is 186 mg/kg-day. The slope factor at the 8.4% extra risk is 0.0005 per mg/kg-day.

Neoplastic Nodules and Carcinomas (Combined) in the Liver of Female Rats (NTP, 1986)
BMR=8.4% extra risk

```
 ======================================================================
              Cancer Model. (Version: 1.2;  Date: 10/20/2005)
              Input Data File: C:\BMDS140B\UNSAVED1.(d)
              Gnuplot Plotting File:  C:\BMDS140B\UNSAVED1.plt
                                                Thu Dec 01 01:23:38 2005
 ======================================================================
 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = response
Independent variable = dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

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
Background  =     0.0211024
Beta(1)     =     0.000288051

Asymptotic Correlation Matrix of Parameter Estimates

            Background      Beta(1)
Background  1           -0.75
Beta(1)     -0.75       1

Parameter Estimates
                        95.0% Wald Confidence Interval
 Variable      Estimate      Std. Err.     Lower Conf. Limit  Upper Conf.
                                                             Limit
 Background    0.0202022     0.128079      -0.230828          0.271232
 Beta(1)       0.000291122   0.000344031   -0.000383167       0.00096541

                          Analysis of Deviance Table
 Model          Log(likelihood)    # Param's  Deviance    Test      P-value
                                                           d.f.
 Full model     -44.6193           3
 Fitted model   -44.6208           2          0.0030289   1         0.9561
                                              1
 Reduced        -48.6567           1          8.07484     2         0.01764
 model
          AIC:         93.2416
```

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
0.0000	0.0202	1.010	1	50	-0.010
292.2000	0.1001	4.905	5	49	0.045
618.4000	0.1816	9.081	9	50	-0.030

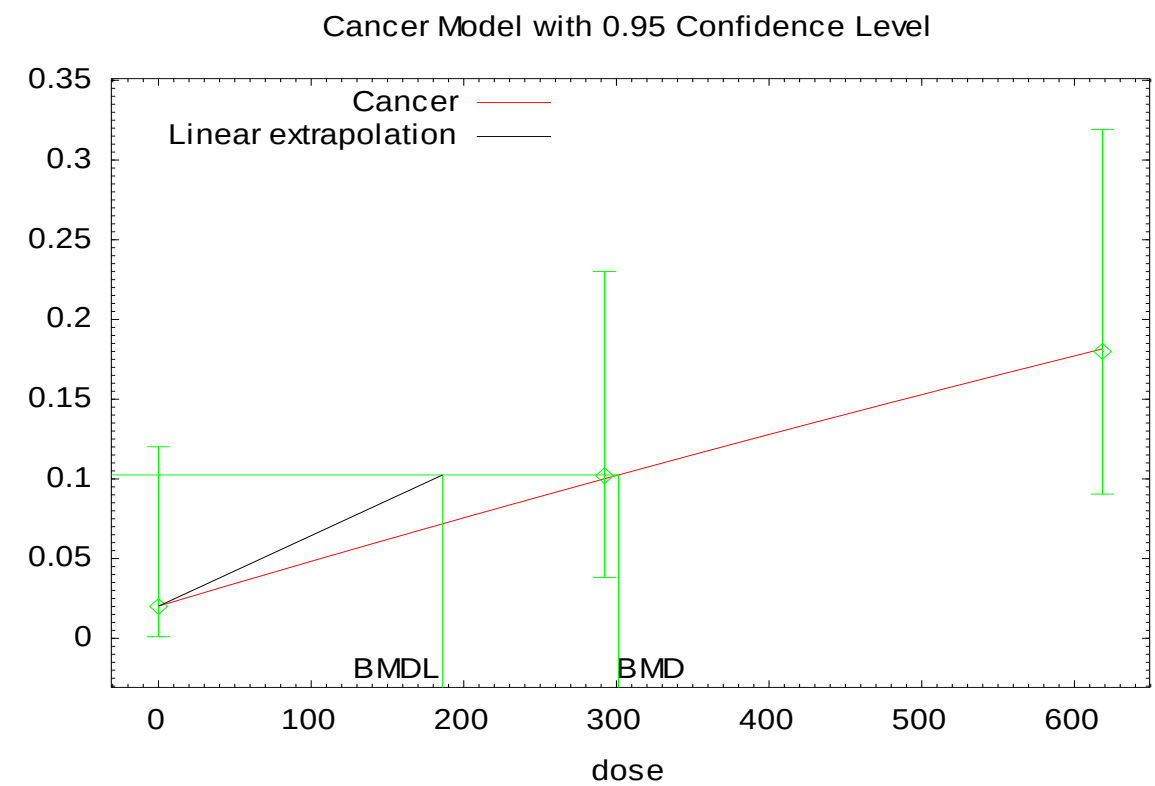
Chi^2 = 0.00 d.f. = 1 P-value = 0.9560

Benchmark Dose Computation
Specified effect = 0.084
Risk Type = Extra risk
Confidence level = 0.95

BMD = 301.382
BMDL = 186.131
BMDU = 676.695

Taken together, (186.131, 676.695) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 0.000451296



01:23 12/01 2005

Thyroid Follicular Cell Hyperplasia in Male Mice

In the mouse chronic study (NTP, 1986), there were slight increases in follicular cell adenomas or carcinomas (combined) accompanied by significant increase in follicular cell hyperplasia in male mice. Because the follicular cell hyperplasia is considered a stage of the thyroid cell carcinogenic process, this endpoint was also modeled as a cancer endpoint. Therefore, the data (2/50, 10/50, and 19/50 for control, low dose, and high dose, respectively) on follicular cell hyperplasia in the thyroid of male mice treated with decaBDE (NTP, 1986) were modeled. The original oral doses were 0, 3,200, and 6,650 mg/kg-day, and the corresponding HEDs are 0, 486.5, and 1,008.6 mg/kg-day, respectively. The modeling results are summarized in Table C-5.

Table C-5. Summary of BMD modeling results for increases in thyroid follicular cell hyperplasia as a key event of thyroid tumor in male mice

Model	Goodness-of-fit *p* value	AIC	$ED_{16.7}$	$LED_{16.7}$
Multistage	0.75	137.34	**440**	**313**

Source: NTP (1986).

The low-dose group resulted in an incidence of 10/50, which corresponds to 16.7% extra risk. Therefore, the cancer ED at an extra risk of 16.7% was estimated to be 440 mg/kg-day, and the corresponding LED is 313 mg/kg-day. The slope factor at the 16.7% extra risk is 0.0005 per mg/kg-day.

Thyroid Follicular Cell Hyperplasia in Male Mice (NTP, 1986)
BMD:16.7%

```
 ======================================================================
              Cancer Model. (Version: 1.2;  Date: 10/20/2005)
              Input Data File: C:\BMDS140B\UNSAVED1.(d)
              Gnuplot Plotting File:  C:\BMDS140B\UNSAVED1.plt
                                          Thu Dec 01 01:32:16 2005
 ======================================================================
 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
The parameter betas are restricted to be positive

Dependent variable = response
Independent variable = dose
Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

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
Background   =    0.0304923
Beta(1)     =     0.000434152

Asymptotic Correlation Matrix of Parameter Estimates

            Background  Beta(1)
Background  1           -0.72
Beta(1)     -0.72       1

Parameter Estimates
                        95.0% Wald Confidence Interval
 Variable      Estimate      Std. Err.     Lower Conf. Limit  Upper Conf.
                                                             Limit
 Background    0.0386341     0.126668      -0.209631           0.2869
 Beta(1)                     0.000222182   -1.97658e-005      0.00085117
               0.000415702

                          Analysis of Deviance Table
 Model          Log(likelihood)    # Param's  Deviance    Test      P-value
                                                           d.f.
 Full model     -66.6205           3
 Fitted model   -66.6699           2          0.0987661   1         0.7533
 Reduced        -76.426            1          19.6109     2         <.0001
 model
           AIC:          137.34
```

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0386	1.932	2	50	0.050
486.5000	0.2147	10.733	10	50	-0.252
1008.6000	0.3679	18.394	19	50	0.178

Chi^2 = 0.10 d.f. = 1 P-value = 0.7544

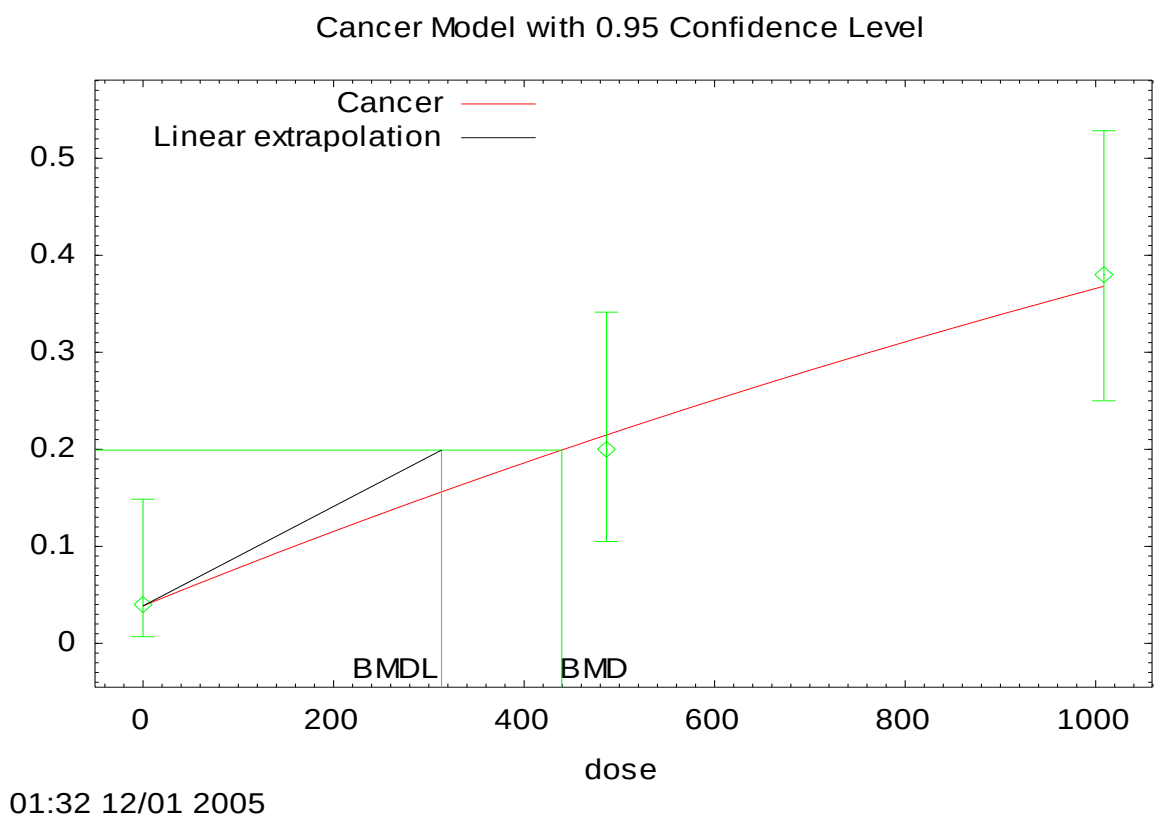
Benchmark Dose Computation

Specified effect = 0.167
Risk Type = Extra risk
Confidence level = 0.95

BMD = 439.549
BMDL = 313.299
BMDU = 685.676

Taken together, (313.299, 685.676) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 0.000533037



Combined Incidence of Hepatocellular Adenomas or Carcinomas in Male Mice

In the mouse chronic study (NTP, 1986), there were significant increases in combined hepatocellular adenomas or carcinomas in male mice. Therefore, the data (8/50, 22/50, and 18/50 for control, low dose, and high dose, respectively) on combined hepatocellular adenomas or carcinomas of male mice treated with decaBDE (NTP, 1986) were modeled. The original oral doses were 0, 3200, and 6650 mg/kg-day, and the corresponding HEDs are 0, 486.5, and 1008.6 mg/kg-day, respectively. The modeling results are summarized in Table C-6.

Table C-6. Summary of BMD modeling results for increases in combined hepatocellular adenomas or carcinomas in male mice

Model	Goodness-of-fit *p* value	AIC	$ED_{33.3}$	$LED_{33.3}$
Multistage	0.03	186.5	**1154**	**680**

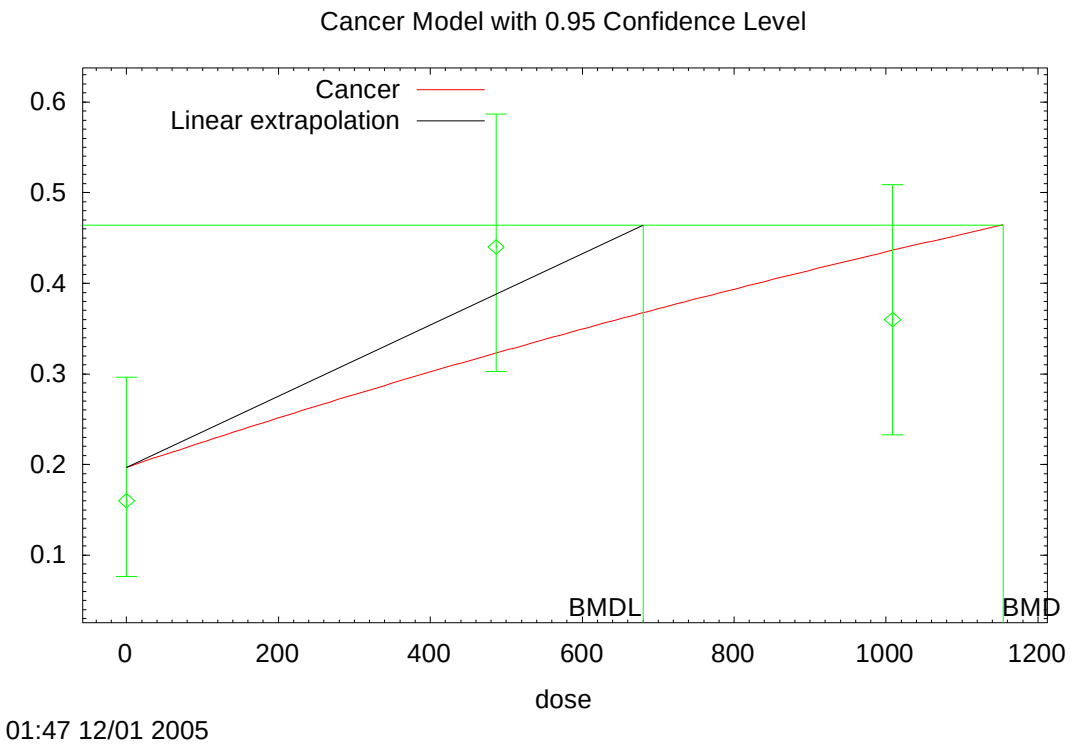
Source: NTP (1986).

The low-dose group resulted in an incidence of 22/50, which corresponds to 33.3% extra risk. Therefore, the cancer ED at an extra risk of 33.3% was estimated to be 1,154 mg/kg-day, and the corresponding LED is 680 mg/kg-day. The slope factor at the 33.3% extra risk is 0.0005 per mg/kg-day.

Combined Incidence of Hepatocellular Adenomas or Carcinomas in Male Mice (NTP, 1986)
BMR=33.3% extra risk

```
 ======================================================================
              Cancer Model. (Version: 1.2;  Date: 10/20/2005)
              Input Data File: C:\BMDS140B\UNSAVED1.(d)
              Gnuplot Plotting File:  C:\BMDS140B\UNSAVED1.plt
                                                Thu Dec 01 01:47:16 2005
 ======================================================================
 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]

The parameter betas are restricted to be positive

Dependent variable = response
Independent variable = dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

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
                     Background =     0.235837
                        Beta(1) =  0.000263218


Asymptotic Correlation Matrix of Parameter Estimates

            Background  Beta(1)
Background  1           -0.77
Beta(1)     -0.77       1

Parameter Estimates
                        95.0% Wald Confidence Interval
 Variable      Estimate      Std. Err.     Lower Conf. Limit   Upper Conf.
                                                               Limit
 Background    0.19695       0.125592      -0.0492049          0.443105
 Beta(1)       0.000350822    0.00026836   -0.000175153        0.000876797

                           Analysis of Deviance Table
 Model           Log(likelihood)    # Param's  Deviance    Test      P-value
                                                           d.f.
 Full model      -88.9509           3
 Fitted model    -91.2692           2          4.63657     1         0.0313
 Reduced                 -94.0304   1          10.159      2         0.006223
 model


Goodness of Fit
                                       Scaled
 Dose          Est._Prob.   Expected     Observed     Size         Residual
 0.0000        0.1970       9.848        8            50           -0.657
 486.5000      0.3230       16.148       22           50           1.770
 1008.6000     0.4363       21.813       18           50           -1.087
```

```
Chi^2 = 4.75      d.f. = 1        P-value = 0.0293
```

Benchmark Dose Computation

```
Specified effect  =     0.333
Risk Type         =     Extra risk
Confidence level  =     0.95

BMD         =     1154.33
BMDL        =     680.241
BMDU        =     3796.73

Taken together, (680.241, 3796.73) is a 90% two-sided confidence interval for
the BMD

Cancer Slope Factor =   0.000489532
```