TOXICOLOGICAL REVIEW

OF

DECABROMODIPHENYL ETHER (BDE-209)

(CASRN 1163-19-5)

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

December 2006

NOTICE

This document is an external review draft. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency position on this chemical. It is being circulated for review of its technical accuracy and science policy implications.

U.S. Environmental Protection Agency
Washington, DC

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LIST OF ABBREVIATIONS AND ACRONYMS

AhR	aryl hydrocarbon receptor
AIC	Akaike Information Criterion
ALH	amplitude of the lateral head displacement
AUC	area under the curve
BDE-209	decabromodiphenyl ether
BMD	benchmark dose
BMDL	BMD low
BMDS	benchmark dose software
BMR	benchmark response
CALUX	Chemical-Activated LUciferase eXpression
decaBDE	decabromodiphenyl ether
DRE	dioxin-responsive element
ED	effective dose
ER	estrogen receptor
EROD	ethoxyresorufin O-deethylase
FOB	functional observational battery
fw	fresh weight
GC/MS	gas chromatography/mass spectrometry
i.v.	intravenous
IRIS	Integrated Risk Information System
LED	95% confidence limit for the effective dose
LOAEL	lowest-observed-adverse-effect level
lw	lipid weight
MMP	mitochondrial membrane potential
MRL	minimal risk level
NOAEL	no-observed-adverse-effect level
nonaBDE	nonabromodiphenyl ether
NTP	National Toxicology Program
octaBDE	octabromodiphenyl ether
PBDE	polybrominated diphenyl ether
PBPK	physiologically based pharmacokinetic
PND	postnatal day

PROD	pentoxyresorufin O-deethylase
RfC	reference concentration
RfD	reference dose
RSD	risk specific dose
T3	triiodothyronine
T4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
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 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 decabromodiphenyl ether of relatively high purity (≥94% pure) and does not deal with earlier commercial decaBDE mixtures containing lower proportions of decabromodiphenyl ether (e.g., 75% purity).

In Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

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

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This document and the accompanying IRIS Summary have been peer reviewed by EPA scientists and independent scientists external to EPA. Comments from all peer reviewers were evaluated carefully and considered by the Agency during the finalization of this assessment.

During the finalization process, the IRIS Program Director achieved common understanding of the assessment among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and EPA's regional offices.

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of 2,2',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 less-than-lifetime exposure durations, and a carcinogenicity assessment.

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

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

Development of these hazard identification and dose-response assessments for BDE-209 has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and technical panel reports that were used in the development of this assessment include the following: *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996),

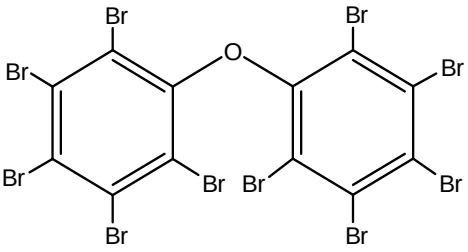
Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for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

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

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 in high-impact polystyrene applications, such as casings for computers and television sets, and in upholstery textile. 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; ACC, 2002; Hardy, 2002). The composition of older decaBDE formulations, which are no longer commercially produced in the United States, 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 1.

Table 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 (2004)
Physical state	Solid	Hardy (2002)
Melting point, °C	290–306	ECB (2003)
Boiling point, °C	decomposes at >320°C	ECB (2002, 2003)
Vapor pressure at 21°C, Pa	4.63×10^{-6}	Hardy (2002)
Henry's Law constant, atm m^3/mole	1.93×10^{-8}	Hardy (2002)
Density, g/cm^3	3.0	NRC (2000)
Water solubility at 25°C, µg/L	<0.1	Hardy (2002)
Henry's Law constant (Pa m^3 mol^{-1}) at 25°C	0.04	Cetin and Odabasi (2005)
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		

3. TOXICOKINETICS

Several studies have been conducted to evaluate the absorption, distribution, metabolism, and elimination of decaBDE after oral or intravenous (i.v.) dosing in rats (Morck et al., 2003; Hakk et al., 2002; El Dareer et al., 1987; NTP, 1986) and mice (Viberg et al., 2003a). The National Toxicology Program (NTP) (1986) conducted four studies to evaluate the absorption and disposition of decaBDE, and the results were reported in 1986 (NTP, 1986) and also in the publication of El Dareer et al. (1987).

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.

Sjodin et al. (1999) investigated the exposure to polybrominated diphenyl ethers (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, a process that 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 lipid weight (lw) with a range of ≤0.3–3.9 pmol/g lw, while the median BDE-209 concentration in computer clerks was <0.7 pmol/g lw with a range of ≤0.3–8.0 pmol/g lw. 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 (range <0.3–9.9 pmol/g lw). 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., 2001a). The presence of BDE-209 in the blood samples from these three groups of workers indicates qualitatively the bioavailability of BDE-209 in humans even though this compound has a 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 BDE-209 in the electronic-dismantling workers decreased during summer vacation, and a 30-day exposure-free period resulted in a 66% decrease in serum

BDE-209 concentration. However, serum half-life was not estimated by the authors.

In a 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 to 20 subjects was 1.6 pmol/g lw in computer technicians, while it was <0.7 pmol/g lw 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 workers engaged in manufacturing decaBDE flame-retarded rubber goods or electric cables. A referent group, abattoir 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 tetraBDEs to heptaBDEs were similar among rubber workers and referents.

Sjodin et al. (2001b) 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. These concentrations of BDE-209 are comparable to blood levels in Swedish cleaners that were collected in the late 1990s (Sjodin et al., 1999). There is no demographic or questionnaire information on the donors, and, therefore, no information is available for assessing exposure sources.

In addition to the observation that decaBDE is present in the serum of the general population, decaBDE is also found in mothers' milk, providing additional evidence of human absorption of decaBDE from the environment. PBDE was found in 47 milk samples from a "milk bank" in Austin, Texas, and from a community women's health clinic in Dallas, Texas, collected in 2002 (Schecter et al., 2003). Mean concentrations of total PBDEs and BDE-209 were 74 ng/g lw and 0.9 ng/g lw, respectively.

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% to 26%. Most researchers acknowledged that 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 orally by gavage with unlabeled decaBDE (purity >98%) in dimethylamide/polyethylene glycol/water 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) following intravenous 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, suggesting rapid absorption kinetics.

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 molecular weight less than 350 (Morck et al., 2003), suggesting that passive diffusion does not play a major role in absorption of decaBDE with a molecular weight of 959. Accordingly, active or facilitated transport and/or uptake with lipids by way of the chylomicrons may provide routes for absorption.

In an oral 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 (>98% purity), prepared by dissolving ^{14}C-decaBDE in toluene, followed by sonication, suspension in Lutrol F127/soy phospholipone/water, and evaporation of the toluene by nitrogen flow. Four noncannulated rats were sacrificed after 3 days and the other 4 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 bile duct-cannulated male Sprague-Dawley rats were also treated similarly with ^{14}C-labeled decaBDE and were sacrificed 3 days later. Bile from these rats was collected and measured 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. Bile collected from the cannulated rats accounted for 10% 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 metabolites. However, interpretation of the data is difficult. The relatively large excretion of metabolites in the feces could be due to a combination of biliary excretion of metabolites, metabolism of decaBDE by the microflora in the GI tract, oxidative 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 (purity >99%) 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 amounts reaching the receptor fluid in the collecting chamber ranged from 0.07 to 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 decaBDE dose applied at 6, 30, and 60 nmol, 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, indicating an inverse relationship between percent dose absorbed and applied dermal 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 that 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 either 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. As indicated by the data that follow, that is not the case. Systemic distribution of more hydrophilic compounds, 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 lower molecular weight, less highly substituted PBDEs.

In a study conducted in 2002 of levels of PBDEs in human milk in the United States, 47 samples from Caucasian, African-American, and Hispanic nursing mothers 20–41 years of age and living in Texas were analyzed for 13 PBDE congeners (Schecter et al., 2003). Mean total concentration of tri- through decaBDEs was 74 ng/g 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), suggesting that some decaBDE is absorbed, distributed to mammary tissue, and secreted in human milk during lactation.

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 orally by gavage with unlabeled decaBDE (purity >98%) in dimethylamide/polyethylene glycol 400/water 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. Results indicated that the mean maximum plasma concentration (C_{max}) was 264 pmol/mL at 6 hours after dosing and area under the curve (AUC) was 12 nmol×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, and 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 of ^{14}C-labeled decaBDE. 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), liver (0.55 and 0.20 nmol/g

fw), kidney (0.17 and 0.07 nmol/g fw), and heart (0.14 and 0.05 nmol/g fw), respectively. On a lipid weight basis, the plasma and liver had the highest concentrations, respectively, of 22 and 14.9 nmol/g lw on day 3 and 8.8 and 5.3 nmol/g lw on day 7, whereas the adipose tissue, testis, thymus, spleen, small intestine wall, skin muscle, lung, kidney, adrenal, and heart had low concentrations. These results indicated that decaBDE was not readily distributed to adipose tissue. Rather, it was found in plasma and blood-rich tissues, including liver, kidney, heart, and intestinal wall.

Viberg et al. (2003a) studied the distribution of decaBDE in mice. NMRI male mice (n = 6 to 8) received a single oral dose (2.22 mg/kg) of [^{14}C]decaBDE by gavage on postnatal day (PND) 3, 10, or 19. One day and 7 days after administration of decaBDE, animals were sacrificed and radioactivity in the brain, heart, and liver was measured. 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.

The amount of radioactivity (the authors did not state whether the radioactivity was that of the parent compound or its metabolites) in the heart was 0.28%, 0.31%, and 0.22% of the total dose 24 hours after administration of radiolabeled decaBDE to mice on PNDs 3, 10, and 19, respectively. After 7 days of administration, the 3-day- 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), but the amount of radioactivity detected in the19-day-old mice had decreased significantly to 0.08%.

One day after administration, the mean radioactivity (the authors did not state whether the radioactivity was that of the parent compound or its metabolites) in the liver of mice treated with radiolabeled decaBDE on PNDs 3, 10, and 19 was 12.6%, 9.4%, or 5.8%, respectively, whereas 7 days after administration, the radioactivity decreased significantly to 4.8%, 4.6%, and 0.3%, respectively. Based on this study, significant age-dependent differences in distribution to the undeveloped brain occurred. Neonatal exposure to [^{14}C]decaBDE in mice on PNDs 3, 10, or 19 resulted in the absorption and distribution of labeled substance in the body, especially in the brain during the period of rapid brain growth (i.e., day 3 and 10 pups versus day 19 pups). The highest concentrations as a percentage of the administered dose were seen in the liver, followed by the brain and heart.

In a study by El Dareer et al. (1987; NTP, 1986) the liver of rats fed two diets containing low (0.0277%) or high (4.8%) amounts of ^{14}C-labeled decaBDE contained 0.45%, 0.21%, and 0.11% of the administered dose on day 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 results

suggest saturation in uptake or distribution. High-pressure liquid chromatography and ultraviolet spectrum analyses indicated that 81% of the radioactivity in the liver was decaBDE rather than metabolites. The maximum percent of dose in the organs and tissues, which was obtained from the rats fed the lower dose (0.0277%) of decaBDE, 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 (purity >97%) in Lutrol F127/soya phospholipid/water as a single oral dose of 3 μmol/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 from 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 uncannulated 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

Animal studies indicated that decaBDE appeared to have low enzyme-inducing potency for both phase I and phase II xenobiotic metabolizing enzymes (Darnerud et al., 2001). Several in vivo and in vitro studies have investigated the potential for decaBDE to affect liver metabolism. DecaBDE was found to have a very low potency to induce hepatic monooxygenase enzymes or induce aryl hydrocarbon receptor (AhR)-dependent DNA binding and gene expression (Brown et al., 2004; Peters et al., 2004; Pullen et al., 2003; Villeneuve et al., 2002; Zhou et al., 2001; Chen 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 P450 1A1 [CYP1A1]; pentoxyresorufin O-deethylase [PROD], a marker for CYP2B1) were measured. PROD activity increased only at 1 and 30 mg/kg-day. The same lack of a 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 CYP1A1 or CYP2B1. 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 CYP1A1 and/or CYP2B1.

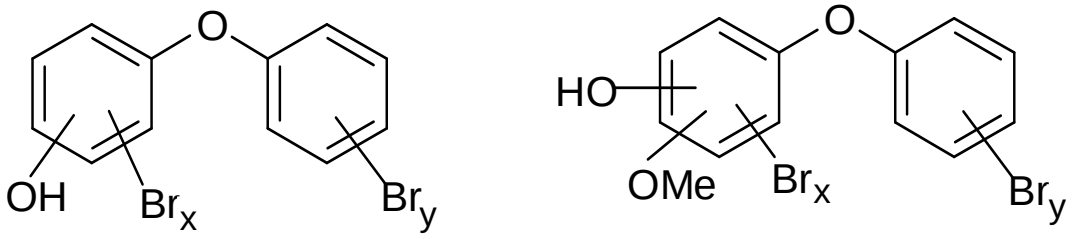
Morck et al. (2003) studied decaBDE metabolism in rats given a single oral dose by gavage. Results indicated that in the uncannulated rats about 90% of the dose was excreted in the feces within 3 days after a single oral dose of ^{14}C-labeled decaBDE and the majority of this radioactivity (65%) represented decaBDE metabolites. Measurement of bile radioactivity indicates 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 lipid-bound metabolites, whereas 30% were unconjugated metabolites (4% and 26% of which were phenolic and neutral metabolites, respectively), 27% was tissue bound, and only 1% was water-soluble. A larger percentage (61%) of the radioactivity in the small intestine wall was tissue-bound, while 7%, 11%, and 20% were lipid-bound metabolites, water-soluble compounds, and unconjugated compounds, respectively. The percentage of the radiolabel in the intestinal wall found as water-soluble compounds 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 unconjugated; 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.

Sandholm et al. (2003) evaluated the bioavailability 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 analysis. These metabolites were characterized as a hydroxy-octaBDE, a hydroxy-nonaBDE, and a hydroxy/methoxy hexaBDE (Figure 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 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 and dihydrodiol and further re-aromatized or via two oxidation steps followed by a methylation reaction.

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 1), indicating debromination may have been the first step in decaBDE metabolism. A small proportion of monohydroxylated metabolites was also found in tissues and feces, indicating a role for reductive dehalogenation followed by an oxidation step or direct oxidative dehalogenation reactions.



x = 4, y = 4 or 5 x = 2, y = 4

Figure 1. DecaBDE metabolites identified.

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 the small fraction as 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 for GC/MS analysis.

Intravenous administration of decaBDE to rats (El Dareer et al., 1987; NTP, 1986) resulted in the major part of the dose being excreted as metabolites in feces (74% of the dose). About 63% of the excreted material in the feces was metabolites, and the remaining 37% was the 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.

Oral and i.v. studies suggested that decaBDE is extensively metabolized through oxidative dehalogenation reactions to form mostly phenolic metabolites. 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 track. Alternatively, there may be pre-absorption metabolism of decaBDE by the intestinal microflora. 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.

3.4. ELIMINATION

As has been mentioned previously, the excretion of decaBDE and its metabolites 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; biliary excretion has accounted for about 10% of the label found in the feces.

In one of the early toxicokinetic studies, the fate of decaBDE in male F344 rats was investigated (El Dareer et al.,1987; NTP, 1986). The study consisted of four sub-studies. In sub-study one, groups of 2-month-old male F344 rats (three animals/group) were fed a standard diet containing unlabeled decaBDE (92% pure) on days 1–7 and 9–11 and the test diet, containing ^{14}C-labeled decaBDE (>98% purity), on day 8. Rats were fed a diet containing 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 27, 55, 272, 512, 2396 and 4577 mg/kg. Radioactivity was determined in feces collected daily on study days 9–12.

In sub-study two, rats were fed two diets containing low (0.0277% or 25 mg/kg body weight) or high (4.80% or 4400 mg/kg body weight) amounts of decaBDE or similar amounts of ^{14}C-labeled decaBDE using the same protocol as in the first sub-study, 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. The extent of the metabolism of labeled decaBDE by rats fed the compound was also evaluated.

In sub-study three, a group of rats (n = 3) were injected intravenously via the tail vein with 1.07 mg/kg of ^{14}C-labeled decaBDE, and urine and feces were collected daily for 3 days and analyzed for radioactivity 72 hours after dosing. In the fourth sub-study, 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. Rats were then sacrificed at the end of 4 hours, and radioactivity was determined in each pooled bile sample.

The recovery of radioactivity in the feces ranged from 91% to 101% of the ingested dose in the first feeding study and 83% to 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 sub-study (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 intravenous dosing of ^{14}C-labeled decaBDE (sub-study 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 radiolabeled decaBDE as a single 3 μmol/kg dose in soya 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% during the 24–48 hours, and a further 2% between 48 hours 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 indicated 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 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 inbound 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, 24–4 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 hours 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

The half-life of BDE-209 is estimated to be in the range of a week in computer technicians (Sjodin et al., 2003; Jakobsson et al., 2002). This indicates that decaBDE is likely to undergo rapid metabolism. 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. The authors did not present any half-life estimate in their publication.

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 at 6 hours after dosing and AUC was 12 nmol×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.

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 later value (51 hours or about 2 days) was the estimate for the oral terminal half-life and was

determined from the slope of the total concentration-time curve by linear regression. 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).

The half-lives of hepta to decaBDE in human serum were estimated using data from occupationally exposed workers sampled before, during, and after a vacation period (Thuresson et al., 2006). The half-lives were found to increase with decreasing degree of bromination. The half-life of heptaBDE-183 was 94 days, while that for BDE-209 was 15 days. This value is longer than that indicated by the data from male rats (Sandholm et al., 2003).

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. LESS-THAN-LIFETIME 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. Acute, Short-term, and Subchronic Studies

The acute, short-term, and subchronic studies are summarized in Table 2.

Table 2. 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[a]	LOAEL[a]	Comments
Rats						
Zhou et al. (2001)	Rat, Long-Evans female weanling rats, 8/dose group	Oral gavage (>98% pure), 0, 0.3, 1, 3, 10, 30, 60, or 100 mg/kg-day, 4 days	Non dose-related increase in PROD activity at 1 and 30 mg/kg-day and 1.8-fold induction in EROD activity at 1 mg/kg-day only	100 mg/kg-day	Not identified	
Carlson (1980)	Rat, Sprague-Dawley male, 4/dose	Oral gavage (purity not given), 0 or 96 mg/kg-day, 14 days	Statistically significant increase in liver to body weight ratio in treated animals	96 mg/kg-day	Not identified	Increased relative liver weight but no change in serum sorbitol dehydrogenase activity; inadequate description of dosing regimen.
NTP (1986)	Rat, F344/N, male and female, 5/sex/dose group	Diet (99% pure), 0, 472, 928, 1846, 4569, or 9326 mg/kg-day in males; 0, 538, 1061, 2137, 5323, or 10,853 mg/kg-day in females; 14 days	None	9326 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 pathologic effects not noted.

Table 2. 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[a]	LOAEL[a]	Comments
NTP (1986)	Rat, F344/N, male and female, 10/sex/dose group	Diet (97–99% pure), 0, 191, 372, 781, 1536, or 3066 mg/kg-day in males; 0, 238, 504, 967, 1955, or 3944 mg/kg-day in females; 13 weeks	None	3066 mg/kg-day in males; 3994 mg/kg-day in females	Not identified	Survival, final mean body weights not adversely affected. Clinical signs or gross pathologic effects not noted
Mice						
NTP (1986)	Mouse, B6C3F$_1$ male and female, 5/sex/dose group	Diet (99% pure), 0, 1027, 2143, 4246, 10,536, or 20,994 mg/kg-day in males; 0, 1146, 2286, 4627, 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, B6C3F$_1$ male and female, 10/sex/dose group	Diet (97–99% pure), 0, 666, 1355, 2659, 5278, or 10,233 mg/kg-day in males; 0, 702, 1437, 2899, 5687, 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 pathologic examination did not show any effect.

[a]NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level.

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 (8 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 one 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 cytochrome P450 1A1 [CYP1A1]; PROD, a marker for CYP2B1; and T_4-uridine diphosphate glucuronyl transferase [T_4-UDPGT]) were measured. 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. PROD activity increased only at 1 and 30 mg/kg-day. The same lack of a 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 these activities. 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 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." (The dose was equivalent to 96 mg/kg-day, based on a molecular weight of 959). 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. Detoxification of O-ethyl O-p-nitrophenyl phenylphosphonothioate, methylation of p-nitroanisole, levels of NADPH cytochrome c reductase and cytochrome P450, 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, resulting in 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. The only dose tested of 96 mg/kg-day used in this study is a NOAEL. Based on limitations in the study design, these results are not adequate for use in the health assessment of decaBDE.

The NTP (1986) conducted a 14-day study in rats exposed to decaBDE. F344/N rats (five animals/sex/dose) were fed diets containing 0, 5000, 10,000, 20,000, 50,000, or 100,000 ppm

decaBDE (99% pure). 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, 1846, 4569, or 9326 mg/kg-day in male rats and 0, 538, 1061, 2137, 5323, 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 9326 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 animals/sex/dose) were administered decaBDE in the diet at concentrations of 0, 3100, 6200, 12,500, 25,000, or 50,000 ppm for 13 weeks. Based on body weight information in the report (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, 191, 372, 781, 1536, or 3066 mg/kg-day in male rats and 0, 238, 504, 967, 1955, or 3944 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. Animals found moribund were killed. 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 3066 mg/kg-day in male rats and 3944 mg/kg-day in female rats.

4.2.1.2. *Studies in Mice*

A 14-day study was also conducted in mice (NTP, 1986). $B6C3F_1$ mice (five animals/sex/dose) were fed diets containing 0, 5000, 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 $B6C3F_1$ male (0.0057 kg food/day) and female (0.0048 kg food/day) mice, the estimated average daily doses were 0, 1027, 2143, 4246, 10,536, or 20994 mg/kg-day in male mice and 0, 1146, 2286, 4627, 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.

$B6C3F_1$ mice (10 animals/sex/species/dose group) were fed diets containing 0, 3100, 6300, 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 $B6C3F_1$ male (0.0057 kg food/day) and female (0.0048 kg food/day) mice, the corresponding estimated average daily doses were 0, 666, 1355, 2659, 5278, or 10,233 mg/kg-day in males and 0, 702, 1437, 2899, 5687, 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 several 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

The NTP (1986) investigated the relationship between ingestion of decaBDE in rats and mice and tumor development. These chronic oral studies are summarized in Table 3. 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.

Table 3. Oral chronic toxicity studies for decaBDE in laboratory animals

Study	Species, sex, and sample size	Route, dose, and duration	Observed effects	NOAEL	LOAEL	Comments
Rats						
NTP (1986)	Rat, F344/N, male and female, 50/sex/dose group	Diet (94–97% pure), 0, 1120, or 2240 mg/kg-day for males; 0, 1200, or 2550 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 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 for both males and females.	1120 mg/kg-day in males 2550 mg/kg-day in females	2240 mg/kg-day in males Not identified	Males, but not females, were adversely affected.
Mice						
NTP (1986)	Mouse, $B6C3F_1$, male and female, 50/sex/dose group	Diet (94–97% pure), 0, 3200, or 6650 mg/kg-day for males; 0, 3760, or 7780 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 males; marginally but not statistically significant increase in the thyroid gland follicular cell adenomas or carcinomas (combined) in both sexes.	N/A[a] in males 3760 mg/kg-day in females	3200 mg/kg-day in males 7780 mg/kg-day in females	

[a]NA = Not available.

4.2.2.1. *Study in Rats*

Groups of 7–8-weeks-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, 1120, or 2240 mg/kg-day for male rats and 0, 1200, or 2550 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 or 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 non-neoplastic 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 statistically insignificant, 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. 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), but the response reached significant level only at the high dose. 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.

Histopathological 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. A slight increase in mononuclear cell leukemia in treated male rats was observed with incidence of 30/50, 33/50, and 35/50 at control, low, and high doses, respectively, and in female rats in 14/50, 21/50, and 18/50 animals 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. There were increases in the incidence of hepatocellular carcinoma in male and female rats; however, these changes were not statistically significant in either sex. Other tumors observed included splenic sarcomas, pancreatic acinar cell adenomas, carcinomas in the Zymbal 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 non-neoplastic 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. Neoplastic changes observed included dose-dependent increases in the incidence of neoplastic nodules in liver of males and females, indicating some evidence of carcinogenicity of decaBDE in rats. Based on these results a NOAEL for systemic toxicity was 1120 mg/kg-day and the lowest-observed-adverse-effect level (LOAEL) was 2240 mg/kg-day for males 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 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 2550 mg/kg-day, with no LOAEL established. The dose-related increase in nonmalignant liver tumors

(neoplastic nodules) in both males and females provide some evidence of carcinogenicity of decaBDE in rats.

4.2.2.2. *Study in Mice*

Groups of 9-week-old male and female B6C3F_1 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 decaDBE as reported in the study were 0, 3200, or 6650 mg/kg-day in male mice and 0, 3760, or 7780 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 non-neoplastic 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 to 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, statistically nonsignificant increases 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 vs. 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. Female mice exhibited an increased incidence of hepatocellular adenomas or carcinomas (combined) which occurred at an incidence of 8/50, 13/50, and 13/50 in control, low-, and high-dose mice, respectively; however, this increase was not statistically significant. 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 increased incidence of hepatocellular adenomas and carcinomas at low dose but not at high doses. Based on these results, no NOAEL for systemic effects for males was established based 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 3200 mg/kg-day or higher. Female mice were less sensitive to the systemic toxicity of decaBDE at the doses used in this study; however, the study identified a NOAEL for a portal-

of-entry effect for females of 3760 mg/kg-day and a LOAEL of 7780 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.

Table 4. 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	Oral gavage (97.34% pure); 0, 100, 30,0 or 1000 mg/kg-day; gestation days 0–19	No statistically significant maternal or developmental treatment-related effects	1000 mg/kg-day	Not identified	
Viberg et al. (2006)	Rat, Sprague-Dawley, male, 20 rats from 3–5 litters/treatment group	Single oral gavage (>98% pure 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% pure in 20% fat emulsion); 0, 2.22, or 20.1 mg/kg (PND 3 and PND 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% pure in corn oil); 0, 10, 100, 500, or 1500 mg/kg-day; PNDs 21to 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	

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 commercial material produced by three manufacturers. In this study, Sprague-Dawley rats (25 mated females/dose group) were administered decaBDE (97.34% decaBDE and 2.66% nonaBDE and octaBDE) in corn oil by gavage at doses of 0, 100, 300, or 1000 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 1000 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. 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 1000 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 1000 mg/kg-day, respectively), lack of a statistically significant positive trend associated with the effect, and the high incidence of this effect historically (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 1000 mg/kg-day, respectively, compared to 4% in controls and at 100 mg/kg-day. However, this effect was not associated with a statistically significant positive trend. A slight, but not statistically significant, decrease in the percentage of viable fetuses per implant was seen (96%, 96%, 93%, and 91% in the control, 100, 300, and 1000 mg/kg-day groups, respectively). Fetal body weights, crown-rump ratio, and fetal sex ratio were not different between treatment and control groups. There were no adverse treatment-related effects with decaBDE 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 1000 mg/kg-day.

The NOAEL for maternal and developmental toxicity in this study was 1000 mg/kg-day, the highest dose tested.

BDE-209 (purity >98%) in a fat emulsion was administered by gavage to three-day-old Sprague-Dawley rats at 0, 6.7, or 20.1 mg/kg (Viberg et al., 2006). A total of 20 rats were picked from three to five different litters in each treatment group. Randomly selecting animals from at least three different litters will have the same statistical effect and power compared to the use of litter-based studies to evaluate developmental neurotoxicity in neonatal animals (Eriksson et al., 2005). 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, 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 period, and significantly higher total activity during the first and second 20-minute period 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 would include effects on the cholinergic system and thereby would alter the response in the adult animal to the cholinergic agent nicotine (Viberg et al., 2006). 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 was investigated in adult animals exposed to a single oral dose as neonates. Radioactivity in the brain of the neonatal mice treated on PND 3, 10, or 19 (i.e., at different stages of neonatal mouse brain development) was also measured to determine whether the tissue and/or a defined critical phase of brain development is the underlying cause of developmental effects. 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 20% (w/w) 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 (4–7 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. Habituation, defined as a decrease in locomotion, rearing, and total activity variables in response to the diminishing novelty of the test chamber over the 60-minute test period (divided into three 20-minute periods), was also determined in the three age groups.

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 treated and vehicle-treated mice in the three different age groups. Control mice treated on PND 3, 10, or 19 exhibited normal habituation profile. 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 only. While this lower dose did not elicit any significant differences

in these three variables compared with controls at 4 months of age, lower activity was observed during the first 20-minute period for the rearing variable at 6 months of age compared with controls. 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. The authors 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 behavioral disturbances.

In order to study time-dependent changes in habituation, an habituation ratio was calculated between the performance period 40–60 minutes and 0–20 minutes for the three spontaneous motor behavior variables (locomotion, rearing, and total activity). The habituation ratio from 2-, 4- and 6-month-old mice within each treatment group were then compared. The habituation ratio calculated from these behavior variables (locomotion, rearing, and total activity) significantly decreased after 2, 4, and 6 months of age in mice exposed on PND 3 to 20.1 mg/kg but not to 2.22 mg/kg. The decrease observed in the disruption of habituation 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 in mice on PNDs 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 decreased from 12.6% for day 3 to 9.5% for day 10 and 5.8% for day 19. These results suggest that gastrointestinal absorption decreased as the gastrointestinal tract matured over the first 19 days after birth. In addition, 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). Results indicated that the amount of radioactivity increased significantly in the brain over a 7-day period in animals exposed on PND 3 and 10. In contrast, animals exposed on PND 19 had low and unchanged amount of radioactivity in the brain over the 7-day period. The radioactivity in the liver and heart was the same or decreased during the 7-day period following oral administration on PND 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 (before the postnatal brain growth spurt) caused a more significant impact on habituation than administration on PND 10, which research by Ankarberg (2003) demonstrated fell in the most active period for development of the nicotinic receptors in the cholinergic system. The cholinergic system plays an important role in movement, learning, and memory (Ankarberg, 2003). Radiolabel data

showed that the amount of decaBDE in the brain 7 days (day 10) after administration on day 3 is about double that observed when the same dose is administered on day 10.

Tseng et al. (2006) studied the effects of BDE-209 on mice sperm function, DNA content, and histopathology of the testes. CD-1 male mice (50/dose group) were fed by gavage from PND 21 to PND 70 decaBDE (purity 98%) at 0, 10, 100, 500, or 1500 mg/kg-day. 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 between 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 1500 mg/kg-day. Significant increase in the generation of hydrogen peroxide in the sperm of sexually mature mice occurred at 500 and 1500 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 1500 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 sperms of adult mice.

4.4. OTHER STUDIES

4.4.1. 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 histologic changes in lymphoid organs, including the spleen (fibrosis) and mandibular lymph nodes (hyperplasia) in rats (NTP, 1986). Although these histopathological changes may play an important role in flagging decaBDE for immunotoxicity, lack of studies to investigate the functional effects on immune cells from exposure to decaBDE preclude 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.

4.4.2. 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]). The decaBDE used in this study was 85.5% pure, and the identity of the other components was 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%) compared to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). DecaBDE demonstrated some activity in the CALUX. However, 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 due to 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 cytochrome P450 (CYP) enzymes 1A1 and 1B1 were studied in human breast carcinoma (MCF7) cells, using EROD activity as a marker for CYP 1A1 and 1B1 activity. DecaBDE (>98% pure) 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 CYP1A1 or 1B1, indicating no effect of decaBDE on AhR gene expression directly.

Villeneuve et al. (2002) evaluated the dioxin-like potency of decaBDE (99% pure) 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 cytochrome P450 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. Morever, 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 cytochrome P450 activity in mouse hepatocytes. Incubation of decaBDE (of unspecified purity) with hepatocytes isolated from C57BL/6 mice for 24 hours did not induce CYP1A1 (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 as do many of the polycyclic aromatic hydrocarbons. The study of Zhou et al. (2001) also indicated that decaBDE did not affect hepatic phase I and phase II enzyme activities (including EROD, PROD, and hepatic UDPGT activity) at doses up to 100 mg/kg-day (see Section 4.2.1).

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, p. 47-48).

4.4.3. Estrogen Receptor Studies

Estrogen receptor (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 of 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.

4.4.4. 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. In another study, decaBDE (98% pure) was tested for mutagenic activity in the presence and absence of an exogenous metabolic system from Aroclor 1254-induced male rat liver S9 (Chemical Manufacturers Association, 1998). Results also indicated that decaBDE was not mutagenic to *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2 *uvr*A in the presence or absence of S9 activation.

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 of 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 either gene mutations, DNA damage, or chromosomal effects.

4.5. SYNTHESIS AND EVALUATION 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 the liver and thyroid gland as the primary target organs for cancer on exposure to decaBDE, as evidenced by the neoplastic nodules in the liver of both sexes of rats and liver and thyroid tumors in male mice (NTP, 1984). In these studies, there are also non-neoplastic 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. Other effects

observed on administration of decaBDE to animals included neurobehavioral developmental effects.

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

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, these in vivo and in vitro studies suggest that decaBDE has very low or no potential to bind the Ah receptor. Therefore, unlike dioxins and PCBs, decaBDE has a limited ability, if any, to activate the AhR signal transduction pathway and thus differs from some of the structurally similar chlorinated dioxins and polychlorinated biphenyl compounds.

The inability of decaBDE to elicit cytochrome P-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(s) 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 showing tissue binding are, therefore, not adequate to elucidate impact on mode of action.

DecaBDE also 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 organohalogenated 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 these is the induction of liver enzymes of different enzyme families, including cytochrome P450 (CYP) 1A1 (i.e., Ah-receptor- or dioxin-like induction) and CYP 2B (i.e., phenobarbital-like induction) (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.

Despite these mechanistic hypotheses for thyroid effects, the available toxicokinetic studies have not detected decaBDE or its metabolites in the thyroid. The only in vivo study that evaluated the interference of decaBDE with thyroid hormone homeostasis was conducted by Zhou et al. (2001) 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 (including EROD and PROD, markers for CYP 1A1 and 2B activity, respectively) and serum T_4, T_3, or TSH or hepatic UDPGT activity at doses up to 100 mg/kg-day. Several studies examining induction of hepatic enzymes both in vivo and in vitro have demonstrated a very limited capacity, if any, of decaBDE to induce such enzymes (Brown et al., 2004; Pullen et al., 2003; Villeneuve et al., 2002; Chen et al., 2001; Zhou et al., 2001). Together these data suggest that decaBDE acts on the thyroid through mechanisms other than altered thyroid hormone kinetics.

Zhou et al. (2001) suggested that the lack of alterations in these hepatic enzyme activities and serum levels of T_4, T_3, and TSH is likely due to the limited absorption of decaBDE, a highly brominated mixture. While some studies have demonstrated relatively poor absorption of decaBDE from the GI tract, oral bioavailability appears to be in the range of 7 to 26% among various oral dosing studies (Morck et al., 2003; Sandholm et al., 2003; El Dareer et al., 1987; NTP, 1986). Furthermore, in some occupational cohorts, increased plasma levels of decaBDE have been observed (Thuresson et al., 2005; Jakobsson et al., 2002; Sjodin et al., 2001a,b, 1999). Since animal studies demonstrated limited absorption and rapid elimination of decaBDE, the absence of an effect in Zhou et al. (2001) could simply reflect an insufficient dose at the target tissues. On the other hand, this difference in response could reflect species differences in sensitivity (rats versus mice). In a 30-day dietary feeding study using a commercial decaBDE mixture containing 77% decaBDE, thyroid hyperplasia was seen in rats at 80 mg/kg-day (Norris et al.,1973), which would seem an apparent contradiction to the work of Zhou et al. (2001). However, the Norris et al. (1973) study used a lower purity test material and impurities in this older formulation may have resulted in the observed thyroid effects at relatively low doses. The existing data are not adequate to identify the specific mode of action for the observed thyroid hyperplasia and tumors induced in rats or mice.

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 estrogen receptor pathways. An in vitro ER-mediated reporter gene assay using stably transfected human breast cancer cells (ER-CALUX assay) has been used to determine the (anti-) estrogenic potency of a number of PBDEs, hydroxylated PBDEs, and brominated bisphenols. Villeneuve et al. (2002) examined ER-mediated (estrogenic) potency of decaBDE (99% pure) by using in vitro assays with MVLN cells. Additionally, the authors performed a competitive hormone displacement assay to examine the ability of decaBDE at concentrations of up to 20 µM to displace estradiol or testosterone from serum proteins. Results showed that decaBDE did not induce a significant estrogenic response in these assays.

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 histopathological changes may play an important role in flagging 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.

The behavioral disturbances observed in the mature rats and mice exposed to decaBDE as neonates (Viberg et al., 2006, 2003a) raise concern about possible developmental neurotoxicity in children. In the study in mice (Viberg et al., 2003a), neonatal mice were given a single dose of decaBDE (up to 20.1 mg/kg) in a fat emulsion on days 3, 10, or 19 after birth 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, habituation (defined as a decrease in locomotion, rearing, and total activity variables in response to the diminishing novelty of the test chamber over the 60-minute test period) was also determined 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., 2006), 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.

Other studies by the same laboratory using PBDE compounds with 4, 5, and 6 bromines have shown effects on habituation that are similar to those observed for the deca compound. However the window of greatest vulnerability is higher on days 10–14 than on days 3–7 or days 19–23 (Ankarberg, 2003). This makes the results from the decaBDE study by Viberg et al.

(2003a) somewhat an anomaly. 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 on day 10 is about 2 times higher (0.7% versus 0.4%) if the compound was administered on day 3 than if it was administered on day 10. Less than 0.1% of the label reaches the brain when the compound is administered on day 19.

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 cholinergic agents (Viberg et al., 2003b, 2006). Ankarberg (2003) determined that there appears to be a critical window of vulnerability in development of the cholinergic system during postnatal development. In mice, this period occurs in the first few weeks after birth with a peak at PND 10 (Viberg et al., 2003b). The resulting deficit in cholinergic receptor is irreversible and could cause a hypoactive response to exposure to cholinergic stimulants in adulthood. Viberg et al. (2006) 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 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 to 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 had been prenatally exposed to decaBDE during the critical window for system development.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

4.6.1. Summary of Overall Weight of Evidence

The weight of evidence of human carcinogenicity of decaBDE is based on (1) no studies of cancer in humans exposed to decaBDE; (2) statistically significant increase in incidence of neoplastic nodules (a benign tumor) in the liver of low- and high-dose male rats and high-dose female rats and increased (nonsignificantly) incidence of carcinomas in treated male and female rats; (3) significantly increased incidence of hepatocellular adenoma or carcinoma (combined) in male mice at the low dose and marginally increased incidence at high dose; (4) nonsignificantly increased incidence of hepatocellular adenoma or carcinoma (combined) in female mice; (5) slightly greater (but statistically not significant) incidence of thyroid gland adenomas or

carcinomas (combined) in dosed male and female mice; (6) significantly increased incidence in male mice, at both doses, of follicular cell hyperplasia, considered by many as a precursor to thyroid tumors; and (7) apparent absence of genotoxic potential.

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 5 and 6, with a summary in Table 7. DecaBDE caused substantial dose-related increases in benign liver tumors (neoplastic nodules) in rats with statistical significance at the high dose in both sexes (Table 5). Since neoplastic nodules in the liver are considered equivalent to adenomas (NTP, 1984) and since adenomas can progress to carcinomas, the increases in neoplastic nodules indicate some evidence of carcinogenicity in treated rats). However, 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.

Table 5. 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	1120	2240	0	1200	2550
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							
Hepatocellular carcinoma							
Overall rates		1/50 (2%)	3.5% 1/50 (2%)	1/49 (2%)	0/50 (0%)	2.6% 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 6. Incidence of non-neoplastic or neoplastic lesions in the liver and thyroid gland of mice fed decaBDE for two years

		Males			Females		
Dose groups	ppm	0	25,000	50,000	0	25,000	50,000
	mg/kg-day	0	3200	6650	0	3760	7780
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

Table 6. Incidence of non-neoplastic or neoplastic lesions in the liver and thyroid gland of mice fed decaBDE for two years

	Males			Females		
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).

Table 7. Summary of carcinogenicity data in rats and mice

Organ	Lesion	Species, sex		Dose
Liver	Neoplastic nodules	Rat	Male	Increase from 25,000 ppm (dose-dependent; significant at 25,000 and 50,000 ppm)
			Female	Increase from 25,000 ppm (dose-dependent; significant at 50,000 ppm)
		Mouse	Male	-
			Female	-
	Hepatocellular adenoma	Rat	Male	-
			Female	-
		Mouse	Male	Increase from 25,000 ppm
			Female	-
	Hepatocellular carcinoma	Rat	Male	-
			Female	-
		Mouse	Male	Increase at 25,000 ppm (dose-independent, significant only at low dose)
			Female	-
Thyroid	Follicular cell hyperplasia	Rat	Male	-
			Female	-
		Mouse	Male	Increase from 25,000 ppm (dose-dependent; significant at 25,000 and 50,000 ppm)
			Female	Increase from 25,000 ppm (dose-independent, not significant at any dose level)
	Follicular cell adenoma or carcinoma (combined)	Rat	Male	-
			Female	-
		Mouse	Male	Marginal increase from 25,000 ppm
			Female	Marginal increase from 25,000 ppm

Source: ECB (2002).

In mice, insignificant increases in hepatocellular adenoma occur in male mice at low (3200 mg/kg-day) and high (6650 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. The

changes in combined incidence of hepatocellular adenomas or carcinomas were not in a dose-dependent manner. However, NTP (1986) indicated that the large number of early deaths in control male mice may have led to a lower incidence of tumors in the control group than would have occurred had more animals survived. 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.

4.6.3. Mode-of-Action Information

The increased incidence of neoplastic nodules of the liver observed in male and female rats constitute some evidence of carcinogenicity in treated rats, and this observation is supported by the significantly increased incidence of hepatocellular adenoma and carcinoma (combined) in 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 in the NTP (1986) study, a common target organ, the liver, in two different 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 cell follicular cell carcinogenic process (NRC, 2000; Hard, 1998; U.S. EPA, 1998c; Hill et al., 1989), decaBDE would be considered as eliciting a carcinogenic effect in a second target organ (i.e., the thyroid) in mice.

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 (Chemical Manufacturers Association, 1998; NTP, 1986).

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 non-neoplastic

lesions were observed at increased incidences, the most notable being thyroid gland follicular cell hyperplasia in male mice.

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; 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.

For decaBDE, increased incidence of neoplastic nodules in the liver was observed in male and female rats, and increased incidence of hepatocellular adenomas and carcinomas was observed in male mice. The observation of neoplastic lesions in the same target organ of two different species further indicates a potential for decaBDE to cause cancer in treated animals. Slightly increased incidences of hepatocellular adenoma or carcinoma also occurred in the female mice. Although these changes in female mice were not statistically significant, the similar target organ as observed in the treated rats and male mice supports that decaBDE can cause neoplastic changes in both sexes in these animal species. In addition, slightly increased thyroid tumors accompanied by significant increase in follicular cell hyperplasia in male mice also suggests carcinogenicity of decaBDE in multiple organs. The weight of evidence suggests that decaBDE is carcinogenic for more than one species, sex, and site; therefore, there is "suggestive evidence of carcinogenic potential." This descriptor is consistent with the NTP (1986) weight-of-evidence evaluation.

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.

4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.7.1. Possible Childhood Susceptibility

As discussed in Section 4.3.1, a gavage study in which rats were exposed in the diet to decaBDE (97.34% pure) 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 (Hardy et al., 2002). The NOAEL in this study was 1000 mg/kg-day, the highest dose tested. In a study in male mice (Tseng et al., 2006) exposed to BDE-209 from PND 21 to PND 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., 2006), exposure of the animals to the lowest dose tested of 6.7 mg/kg-day of BDE-209 on PND 3 resulted in significant changes in spontaneous motor behavior (locomotion, rearing, and total activity) in 2-month-old rats.

Neurobehavioral alterations were observed in mature mice when these animals 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, appears to suggest 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, age-dependent kinetic differences are unclear. Although no studies were identified that investigated placental transfer, decaBDE has been found in human breast milk, suggesting that newborns and infants may have a potential source of exposure through 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 non-neoplastic 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 an increased incidence of liver neoplastic nodules was observed in both sexes of rats (NTP, 1986),

hepatocellular adenomas or carcinomas were only observed in male mice; female mice or rats of both sexes were not affected. No gender-specific effects on reproductive organs were identified. 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

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 (NTP, 1986; Carlson, 1980) 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 developmental studies (Tseng et al., 2006; Viberg et al., 2006, 2003a; Hardy et al., 2002). The results from these studies are summarized in Tables 2, 3, and 4. Among the available studies, three potential principal studies for deriving an RfD were identified: the 2-year chronic studies in rats and mice (NTP, 1986), and a neurodevelopmental study in mice (Viberg et al., 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 (2240 mg/kg-day). A dose-dependent but not statistically 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 1120 mg/kg-day and the LOAEL was 2240 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 2550 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 to 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 3200 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 for portal-of-entry effects for females of 3760 mg/kg-day and a LOAEL of 7780 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 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 appear to suggest that there is a critical window for the induction of behavioral disturbances. The decrease observed in the disruption of habituation 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 20.1 mg/kg.

Since the reported effect levels are substantially lower for the Viberg et al. (2003a) study than the other potential principal studies, several considerations were weighed in determining whether the Viberg et al. (2003a) study should be selected as the principal study for deriving the RfD. 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, 1998b); only single doses were given. The protocol was unique and did not conform to health effects test guidelines for neurotoxicity screening battery or developmental neurotoxicity studies (U.S. EPA, 1998b). 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 was used for the behavioral testing (10 mice were randomly selected from three to five different litters in each treatment group). Increasing the number of samples from each litter may bias the analyses towards false positives, and the observed neurobehavioral effects may be attributable to nontreatment related differences in pups born to a single dam.[1] 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 functional observational battery (FOB) that evaluates neurologic and behavioral signs limits the ability to correlate the reported effects with other FOB parameters. This would be helpful in gauging the reliability of the limited parameters that were measured. As indicated in the *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), it is assumed that an agent that produces 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. Therefore, even chronic exposures may lead to developmental neurotoxicity via disruption of developmental events that take place during a short critical window of development (Rice and Barone, 2000).

The concept that exposure during critical periods of development can induce functional neurological effects later in development has been demonstrated with structurally related PBDE congeners, including tetra-, 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). Therefore, the observed neurobehavioral effects in the Viberg et al. (2003a) study in mice and Viberg et al. (2006) 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

[1] Eriksson et al. (2005) evaluated in 18 different litters the use of the litter (n = 9) or randomly selected individuals (n = 9, three mice randomly selected from 3 different litters) as a statistical unit in development toxicology in the neonate. In this study of mice neonatally exposed to BDE-99, there was no statistical difference whether the litter or the randomly selected individuals are used as the statistical unit, indicating that multiple sampling from the same litter is unlikely to affect the LOAEL.

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 PND 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 only after the day 3 window of vulnerability or 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

Potential critical effect levels based on study NOAELs or LOAELs included a NOAEL of 1120 mg/kg-day for liver, spleen, and lymphoid effects in male rats (NTP, 1986), a LOAEL of 3200 mg/kg-day for liver and thyroid effects in male mice (NTP, 1986), a LOAEL of 6.7 mg/kg for neurobehavioral effects in adult rats exposed on PND 3 to decaBDE (Viberg et al., 2006), and a NOAEL of 2.22 mg/kg for neurobehavioral effects in adult mice exposed as neonates (Viberg et al., 2003a).

The published information presented by Viberg et al. (2006, 2003a) could not be used for benchmark dose 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 displayed graphically only 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), and its half-life in the plasma is less than 2–3 days in rats (Sandholm et al., 2003). Furthermore, the half-life in humans is estimated to be short (Sjodin et al., 2003; Jakobsson et al., 2002). The limited deposition in the adipose tissue and relatively short half-life suggest that the chronic accumulation may not be a significant issue. 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.

Details of the benchmark dose modeling results for the 2-year NTP (1986) studies are presented in Appendix A and summarized in this section.

5.1.2.1. *Benchmark Dose Modeling*

The following data sets from the NTP 2-year rat and mouse studies (1986) were selected for benchmark dose 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.

Benchmark dose (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, 2000c). 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. Benchmark dose model fit was evaluated based on the goodness-of-fit *p*-value (indicating global model fit), Akaike Information Criterion (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 model(s) providing best model fit to the data were selected to estimate benchmark dose (BMD_{10}) and benchmark dose low ($BMDL_{10}$).

5.1.2.2. *Comparison of Benchmark Dose Modeling Results*

For oral exposure to decaBDE, the data sets from the NTP 2-year studies in rats and mice were selected for benchmark dose modeling (NTP, 1986). These endpoints include 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 analyses results are summarized in Table 8. To facilitate BMD comparison, a NOAEL for neurobehavioral effects from another potential critical study (Viberg et al., 2003a) is also listed.

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 published information presented by Viberg et al. (2003a) 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 8. Benchmark doses 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)	1120	2240	2125	1738
Liver degeneration in male rats	NTP (1986)	2240	N/A[a]	765	406
Splenic fibrosis in male rats	NTP (1986)	1120	2240	1446	1001
Lymphoid hyperplasia in male rats	NTP (1986)	1120	2240	1538	1165
Centrilobular hypertrophy in the liver of male mice	NTP (1986)	N/A	3200	No model fit	
Follicular cell hyperplasia in the thyroid of male mice	NTP (1986)	N/A	3200	1670	1190
Neurobehavioral change in male mice	Viberg et al. (2003a)	2.22	20.1	N/A	N/A

[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 subchronic to chronic 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 humans more sensitive than rodents in the absence of specific data. Based on these

considerations the default UF_A value of 10 was used in the absence of sufficient data to move away from defaults.

A default UF_H of 10 was applied to account for variations in susceptibility within the human population (intraspecies or interhuman variability). This factor accounts for humans who may be more sensitive than the general population to exposure to BDE-209.

A UF_S of 3 was used for extrapolating effects seen in a single exposure neurodevelopmental study to a lifetime exposure. Exposure on PND 3 occurred during the period of rapid brain development in mice. Brain development does not continue at an equivalent rate across the lifespan and is more quiescent during adult life stages. Accordingly, it is not necessary to make a 10-fold adjustment for exposure duration.

The UF_S of 3 is needed despite the existence of chronic data since rats and mice in the NTP (1986) study were not exposed to decaBDE until 7–8 weeks of age or 9 weeks of age, respectively. Accordingly the animals were past the critical period of brain development. In addition, the NTP protocol did not include evaluation of neurobehavioral toxicity.

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 (NTP, 1986; Carlson, 1980) and mice (NTP, 1986), 13-week studies in rats and mice (NTP, 1986), and 2-year studies in rats and mice (NTP, 1986). A 7-week study of sperm functions in mice was also available (Tseng et al., 2006). In addition, a standard developmental toxicity study in rats was identified for decaBDE (Hardy et al., 2002). 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 1000 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. Together, these data suggest that a UF_D of 1 is considered adequate.

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 decaBDE (NTP, 1986). However, neither such changes occurred in female rats nor in either sex in 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 histopathological 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:

RfD = NOAEL ÷ UF
= 2.22 mg/kg ÷ 300
= 0.007 mg/kg-day or 7 μg/kg-day.

5.1.4. Previous RfD Assessment

An IRIS health assessment of decabromodiphenyl ether 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 an 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]). This IRIS RfD was not based on the 1986 NTP study because the final NTP report was not available at the time.

The National Research Council of the National Academies of Science (NRC, 2000) derived an 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 an 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 oral intermediate minimal risk level (MRL) for decaBDE based on the Hardy et al. (2002b) developmental toxicity study. In this study, no effects on any maternal or fetal endpoints were

observed at the highest dose tested of 1000 mg/kg-day. Applying a UF of 10 for extrapolation from animals to humans and a UF of 10 for human variability, gives an oral intermediate MRL of 10 mg/kg-day.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

No data are available for deriving a reference concentration for decaBDE.

5.3. CANCER ASSESSMENT

5.3.1. Choice of Study/Data

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) significantly increased incidence of neoplastic nodules and nonsignificantly increased incidence of carcinomas in the liver of male and female rats; (3) significantly increased incidence of hepatocellular adenoma or carcinoma (combined) in male mice at low dose, and marginally increased incidence at high dose; (4) nonsignificantly increased incidence of hepatocellular adenoma or carcinoma (combined) in female mice; (5) slight (but statistically not significant) increase in incidence of thyroid tumors in both male and female mice; (6) significantly increased incidence in male mice of follicular cell hyperplasia, considered by many as a precursor to thyroid tumors; (7) apparent absence of genotoxic potential. All of the data supporting carcinogenicity were obtained from two 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 5), indicating some evidence of carcinogenicity in treated rats. However, there were no significant changes in hepatocellular carcinoma in either sexes. In mice, insignificant increases in hepatocellular adenoma or carcinoma occur in male mice at low (3200 mg/kg-day) and high (6650 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 Conversion

For the chronic rat study (NTP, 1986), the original oral doses were reported as 0, 1120, and 2240 mg/kg-day for males, and 0, 1200, and 2550 mg/kg-day for females. Since there is 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 were calculated by scaling animal daily applied doses experienced for a lifetime in proportion to body weight raised to the 0.75 power.

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. Based on the calculated average lifetime rat body weight and a default human body weight of 70 kg, corresponding human equivalent doses 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 these calculated body weight and default human body weight of 70 kg, corresponding human equivalent doses 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, 3200, and 6650 mg/kg-day. Since there is 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 life-time 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 human equivalent doses 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 human equivalent doses are 0, 486.5, and 1008.6 mg/kg-day, respectively.

5.3.3. Extrapolation Method(s)

U.S. EPA benchmark dose software (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 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 benchmark response 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 the 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 B. The estimated cancer slope factor based on estimated effective doses and their 95% lower confidence limits using the multistage model for each cancer endpoint is summarized in Table 9.

Table 9. Cancer slope factors derived from benchmark doses 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 mice	0.0005
Adenomas or carcinomas (combined) in the liver	male mice	0.0005

Source: NTP (1986).

Neoplastic nodules and carcinomas were also modeled together because it is likely that these two lesions have a similar mode of action. Based on the estimated effective dose for each cancer endpoint, the most sensitive response is neoplastic nodule or carcinoma (combined) in the liver of male rats. The effective dose (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 or uncensored data resulted in higher LED than for this effect in male rats; therefore, the LED in females was not used as 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.

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 adequate data to support alternative mode-of-action hypotheses. In the absence of such data, extrapolation from the point of departure to lower doses is conducted by using a linear approach.

Based on a comparison of estimated effective doses for all the cancer endpoints observed in rat and mouse chronic studies (NTP, 1986), the neoplastic nodule or carcinomas (combined) in the liver of treated male 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 linear extrapolation, a straight line is drawn from the point of departure expressed as a human equivalent dose 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. 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
RSD at 10^{-4} is 100 μg/kg-day.

Doses for excess cancer risks of approximately $5x10^{-6}$ 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).

5.3.5. Previous Cancer Assessment

The carcinogenicity of decabromodiphenyl ether 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, 1986). 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 report of 1986 was not available at the time.

The potential carcinogenicity of decaBDE was evaluated by NRC (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), NRC 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 relatively rapid clearance (on the order of days), largely as a result of metabolism in the liver and excretion in the bile.

Short-term and subchronic studies demonstrated low toxicity for oral exposure to decaBDE with NOAELs of 3000 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 (2240 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 in the incidence of fibrosis was observed in males, which 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. 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 to 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 spontaneous motor behavior changes in one study in male mice. 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 is 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) statistically significant increase in incidence of neoplastic nodules (a benign tumor) and statistically not significant increase in incidence of carcinomas in the liver of low- and high-dose male rats and high-dose female rats; (3) 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) nonsignificantly increased incidence of hepatocellular adenoma or carcinoma (combined) in female mice; (5) slightly greater (but statistically not significant) incidence of thyroid gland adenomas or carcinomas (combined) in dosed male and female mice; (6) significantly increased incidence in male mice, at both doses, of follicular cell hyperplasia, considered by many as a precursor to thyroid tumors; and (7) 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

Benchmark dose 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, centrilobular hypertrophy in the livers, and follicular cell hyperplasia in the thyroid of male mice. The data on neurobehavioral effects could not be modeled because the original data were not available for the 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), subchronic to chronic extrapolation (threefold), 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. Additionally, 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 only employed a single daily dosing schedule. In addition, only limited tests on motor activity were conducted in the one neurobehavioral study of Viberg et al. (2003a). This study could be significantly strengthened if tests on other behavioral activities with repeated dosing protocols were conducted. The latter 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. While decaBDE was not mutagenic or genotoxic in various in vitro studies, there are no adequate data to support alternative mode-of-action hypotheses. 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 effective doses 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. 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.

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APPENDIX A:
BENCHMARK DOSE ANALYSIS OF NONCANCER ENDPOINTS

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

Benchmark dose (BMD) modeling was conducted by using EPA's BMD software (BMDS) version 1.3.2. All the benchmark dose analyses were conducted with the benchmark response (BMR) set to a 10% response (U.S. EPA, 2000c). 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. Benchmark dose 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 model(s) providing best model fit to the data were selected to estimate benchmark dose (BMD_{10}) and benchmark dose low ($BMDL_{10}$). 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, 1120, and 2240 mg/kg-day, respectively. The data were modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table A-1.

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

Model	Goodness of fit *p*-value	AIC	Local chi-square residual	BMD_{10}	$BMDL_{10}$
Gamma	0.31	61.9754	0.01	**2047**	**1717**
Log-logistic	0.32	61.9384	<0.01	**2161**	**1741**
Logistic	0.04	65.0449	0.29	1909	1597
Multistage	0.07	66.1380	0.30	1898	1449.97
Log-probit	NA[a]	63.9383	0	2130	1691.4
Probit	0.04	65.8039	0.46	1869	1513.96
Quantal-linear	0.02	69.4028	1.33	2030	1162.71
Quantal-quadratic	0.07	66.1380	0.74	1898	1449.97
Weibull	0.32	61.9384	<0.01	**2166**	**1757**

[a]NA = Not applicable.

Source: NTP (1986).

Based on these results, the best model 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, low AIC values, as well as relatively small chi-square residuals, indicating satisfactory fit at the data point close to 10% BMR. 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 2125 mg/kg-day and the average $BMDL_{10}$ is 1738 mg/kg-day.

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, 1120, and 2240 mg/kg-day, respectively. The data were modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table A-2.

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

Model	Goodness of fit *p*-value	AIC	Local chi-square residual	BMD_{10}	$BMDL_{10}$
Gamma	0.83	195.178	0.18	**779**	**422**
Log-logistic	0.89	195.148	0.11	**707**	**344**
Logistic	0.71	195.269	0.30	929	600
Multistage	0.83	195.178	0.18	**779**	**422**
Log-probit	0.51	195.555	0.53	1161	718
Probit	0.72	195.259	0.29	914	584
Quantal-linear	0.83	195.178	0.18	**779**	**422**
Quantal-quadratic	0.42	195.779	0.65	1363	962
Weibull	0.83	195.178	0.18	**779**	**422**

Source: NTP (1986).

Based on these results, the best model fit for the data were obtained from five models (shown in bold in the table): gamma, log-logistic, multistage, quantal-linear and Weibull models. These models provided high goodness-of-fit *p*-values, low AIC values, as well as small chi-square residuals. 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 Scaled

Dose	Est._Prob	Expected	Observed	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


Log-Logistic Model with 0.95 Confidence Level
Log-Logistic
BMD Lower Bound
Fraction Affected
0.6
0.5
0.4
0.3
0.2
BMDL
BMD
0
500
1000
1500
2000
dose
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, 1120 and 2240 mg/kg-day, respectively. The data were modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table A-3.

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

Model	Goodness of fit *p*-value	AIC	Local chi-square residual	BMD_{10}	$BMDL_{10}$
Gamma	NA[a]	139.172	≤0.01	1464	700
Log-logistic	NA	139.172	≤0.01	1463	644
Logistic	0.92	137.183	–0.08	**1427**	**1018**
Multistage	0.69	137.336	–0.12	1236	690
Log-probit	0.87	137.197	0.13	**1519**	**1017**
Probit	0.88	137.195	–0.12	**1393**	**968**
Quantal-linear	0.69	137.336	–0.33	1236	690
Quantal-quadratic	0.80	137.233	0.20	1612	1176
Weibull	NA	139.172	0	1470	700

[a]N/A = Not applicable.

Source: NTP (1986).

Based on these results, the best model fit for the data were obtained from three models: logistic, log-probit and probit models (shown in bold in the table). These models provided highest goodness-of-fit *p*-values, low AIC values, as well as small chi-square residuals. 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 1446 mg/kg-day and the average $BMDL_{10}$ is 1001 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, 1120, and 2240 mg/kg-day, respectively. The data were

modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table A-4.

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

Model	Goodness of fit *p*-value	AIC	Local chi-square residual	BMD_{10}	$BMDL_{10}$
Gamma	NA[a]	127.266	<0.01	1601	765
Log-logistic	NA	127.266	≤0.01	1608	725
Logistic	0.62	125.512	–0.39	1404	1063
Multistage	0.36	126.122	–0.29	1207	707
Log-probit	NA	127.266	≤0.01	1575	1032
Probit	0.57	125.59	–0.45	1364	1007
Quantal-linear	0.36	126.122	–0.74	1207	707
Quantal-quadratic	0.86	125.298	–0.15	**1538**	**1165**
Weibull	NA	127.266	0	1620	765

[a]NA = Not applicable.

Source: NTP (1986).

Based on these results, the best model fit for the data were obtained from the quantal-quadratic model (shown in bold in the table). This model provided a relatively high goodness-of-fit *p*-value, a low AIC value, as well as a small chi-square residual. Based on these considerations, the BMD and BMDL estimates from this model are selected. For lymphoid hyperplasia of male rats, the BMD_{10} is 1538 mg/kg-day and the $BMDL_{10}$ is 1165 mg/kg-day.

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, 3200, and 6650 mg/kg-day, respectively. The data were modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table A-5.

Table A-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	1286	1046
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	1253	1036
Quantal-linear	0.005	140.455	2.48	479	389
Quantal-quadratic	0	164.89	5.08	1612	1451
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, 3200, and 6650 mg/kg-day, respectively. The data were modeled with all the dichotomous models available in the BMDS, and modeling results are summarized in Table A-6.

Table A-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	NA	139.241	0	2040	1196
Log-logistic	NA	139.241	<0.01	2089	1008
Logistic	0.34	138.147	0.70	2989	2430
Multistage	0.76	137.337	–0.09	**1670**	**1190**
Log-probit	0.66	137.436	0.34	2561	1977
Probit	0.44	137.846	0.59	2792	2265.1
Quantal-linear	0.76	137.337	–0.25	**1670**	**1190**
Quantal-quadratic	0.26	138.466	0.96	3135	2598
Weibull	NA	139.241	0.00002	2022	1196

[a]NA = Not applicable.

Source: NTP (1986).

Based on these results, the best model fit for the data was obtained from the multistage and quantal-linear models. These models provided highest goodness-of-fit *p*-values, lowest AIC values, as well as small chi-square residuals, indicating good fit at the data point closest to 10% BMR. Since both models provided 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 1670 mg/kg-day and the $BMDL_{10}$ is 1190 mg/kg-day.

APPENDIX B:
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 benchmark dose (BMD) modeling results are summarized below. All the cancer endpoints are modeled with cancer model in the BMD software (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, 1120, and 2240 mg/kg-day, and the corresponding human equivalent doses 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 B-1.

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

Model	Goodness of fit *p*-value	AIC[a]	Local chi-square residual	$ED_{12.2}$[b]	$LED_{12.2}$[c]
Multistage	0.58	114.935	-0.44	**250**	**174**

[a]AIC = Akaike Information Criterion.
[b]ED = Effective dose.
[c]ED = Lower confidence limit for ED.

Source: NTP (1986).

The low-dose group resulted in an incidence of 7/50, which corresponds to 12.2% extra risk. Therefore, the cancer effective dose at an extra risk of 12.2% was estimated to be 250 mg/kg-day (ED), 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. Limit   Upper Conf. 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 d.f.	P-value
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

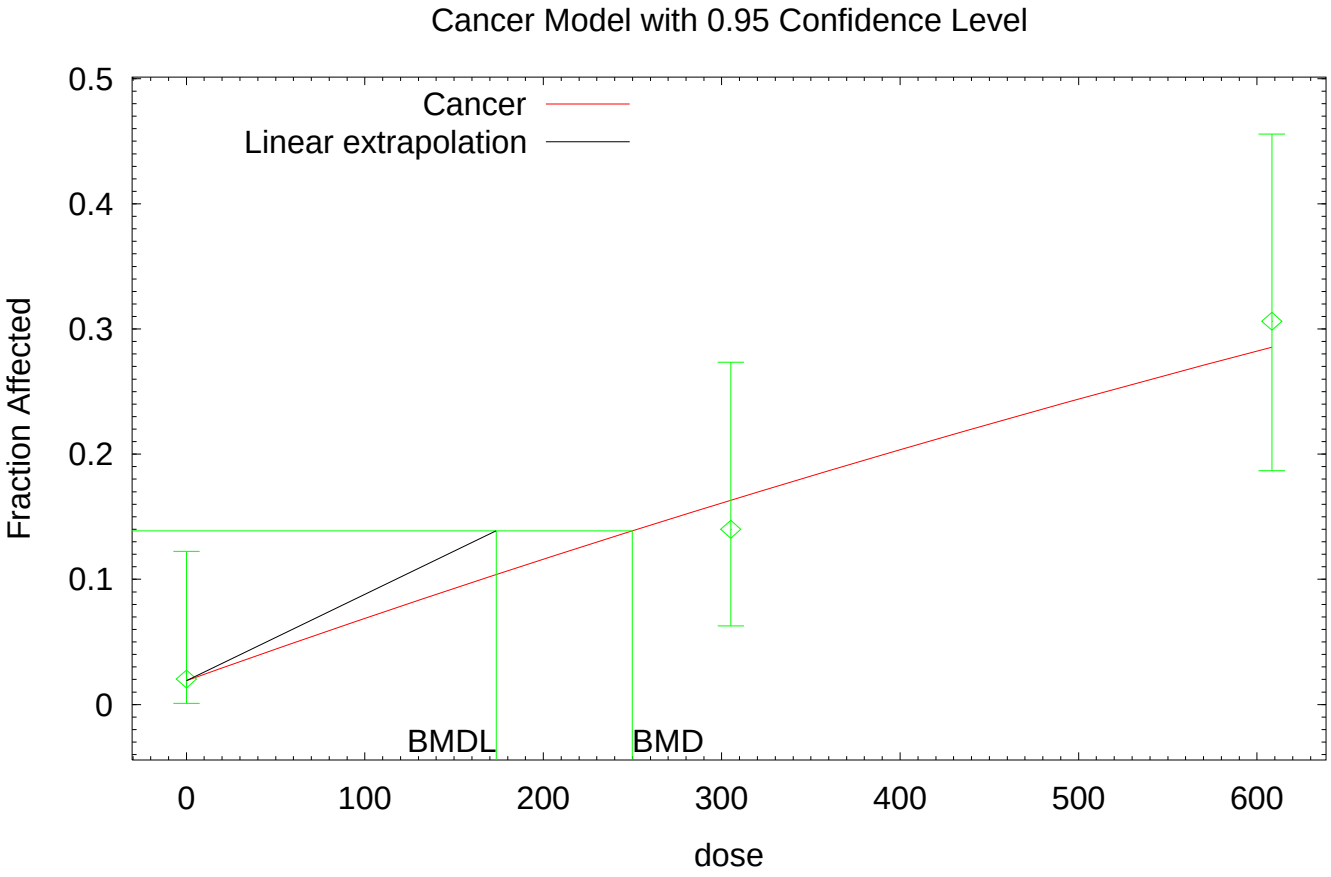
BMD = 249.822

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


Cancer Model with 0.95 Confidence Level
Cancer
Linear extrapolation
Fraction Affected
0.5
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
100
200
300
400
500
600
dose
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 B-2.

Table B-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	Local chi-square residual	$ED_{12.4}$	$LED_{12.4}$
Multistage	0.71	125.18	–0.30	**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 effective dose at an extra risk of 12.4% was estimated to be 263 mg/kg-day (ED), 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(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-60.5216	3			
Fitted model	-60.5898	2	0.136394	1	0.7119
Reduced model	-67.2168	1	13.3904	2	0.001237

AIC: 125.18

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	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

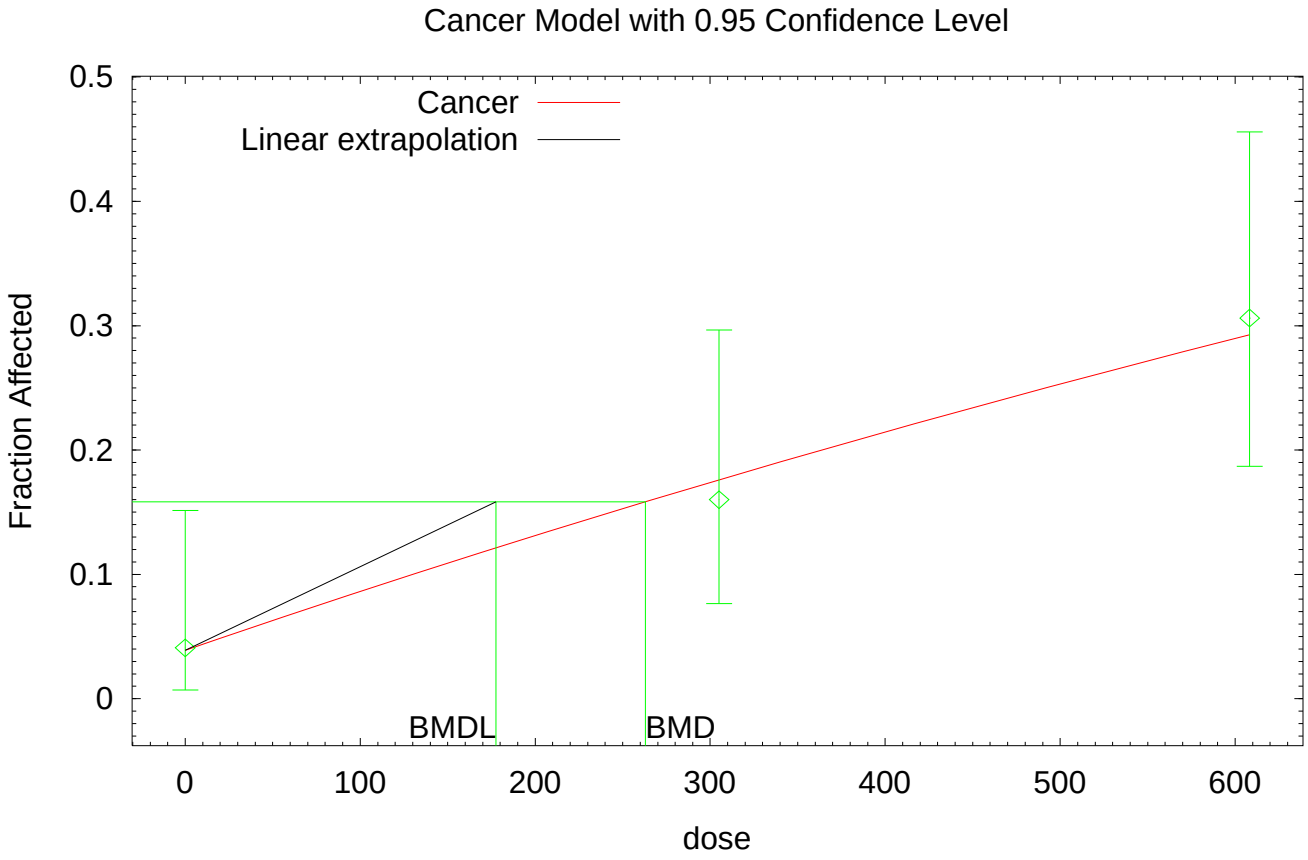
BMD = 262.883

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


Cancer Model with 0.95 Confidence Level
Cancer
Linear extrapolation
Fraction Affected
0.5
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
100
200
300
400
500
600
dose
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, 1200, and 2550 mg/kg-day, and the corresponding human equivalent doses are 0, 292.2, and 618.4 mg/kg-day, respectively. The modeling results are summarized in Table B-3.

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

Model	Goodness of fit *p*-value	AIC	Local chi-square residual	$ED_{4.2}$	$LED_{4.2}$
Multistage	0.44	84.15	-0.64	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 effective dose at an extra risk of 4.2% was estimated to be 171 mg/kg-day (ED), 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)	0.000250587	0.000317551	-0.000371802	0.000872976

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
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

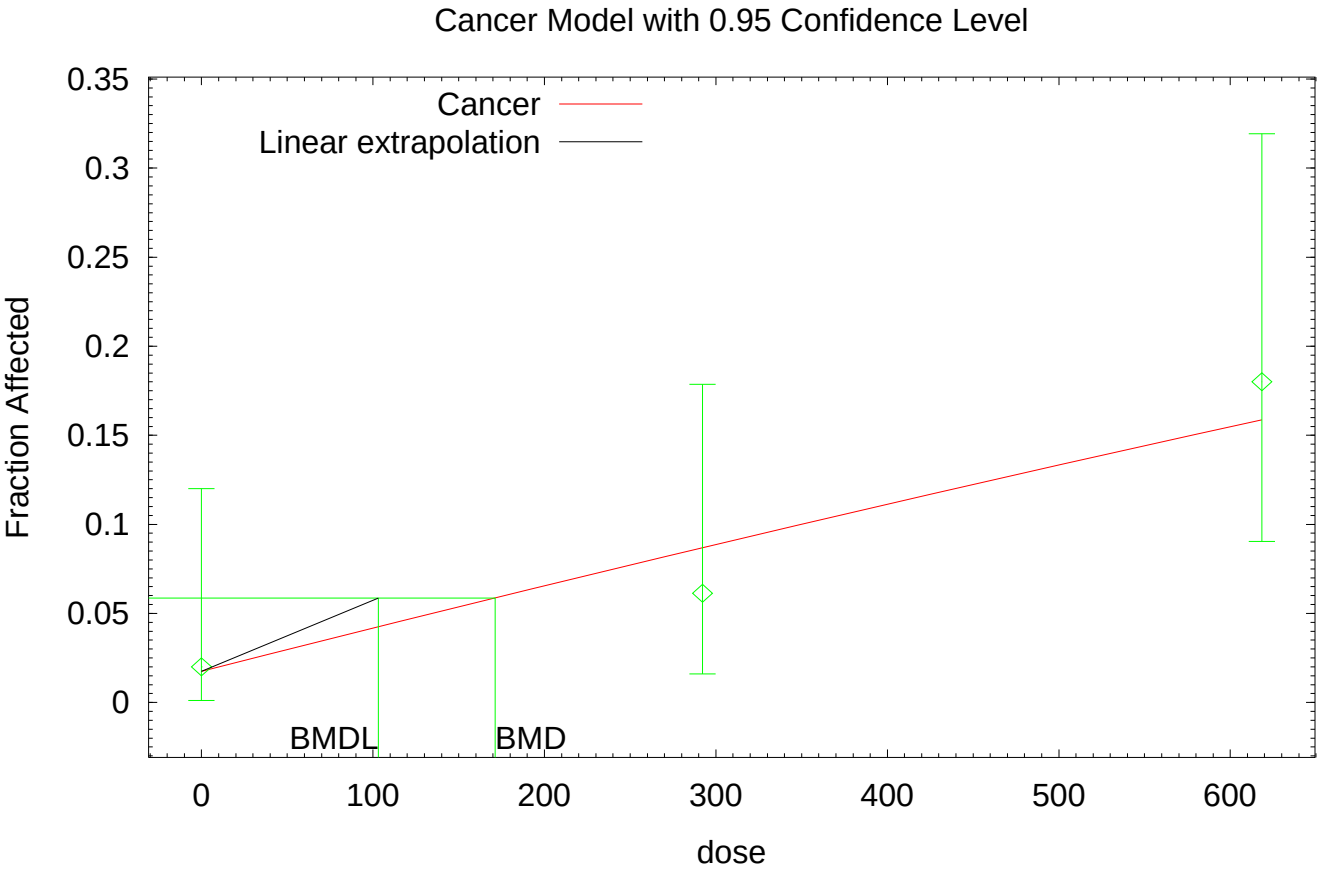
Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0174	0.868	1	50	0.143
292.2000	0.0867	4.250	3	49	-0.635
618.4000	0.1584	7.921	9	50	0.418

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


Cancer Model with 0.95 Confidence Level
Cancer
Linear extrapolation
Fraction Affected
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
BMDL
BMD
0
100
200
300
400
500
600
dose
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, 1200, and 2550 mg/kg-day, and the corresponding human equivalent doses are 0, 292.2, and 618.4 mg/kg-day, respectively. The modeling results are summarized in Table B-4.

Table B-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	Local chi-square residual	$ED_{8.4}$	$LED_{8.4}$
Multistage	0.96	93.24	0.05	**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 effective dose at an extra risk of 8.4% was estimated to be 301 mg/kg-day (ED), and 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 d.f.	P-value
Full model	-44.6193	3			
Fitted model	-44.6208	2	0.00302891	1	0.9561
Reduced model	-48.6567	1	8.07484	2	0.01764

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

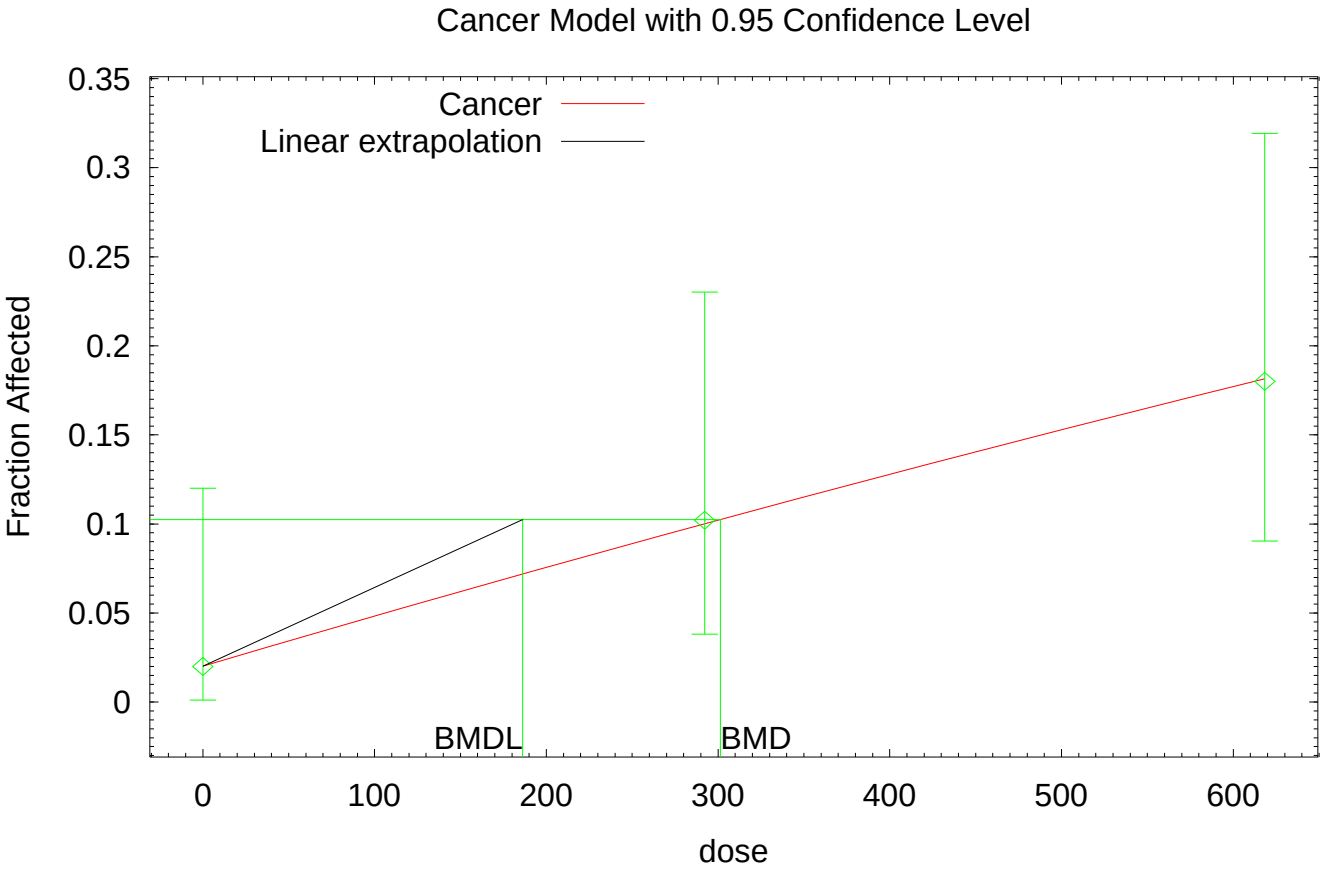
BMD = 301.382

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


Cancer Model with 0.95 Confidence Level
Cancer
Linear extrapolation
Fraction Affected
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
BMDL
BMD
0
100
200
300
400
500
600
dose
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, 3200, and 6650 mg/kg-day, and the corresponding human equivalent doses are 0, 486.5, and 1008.6 mg/kg-day, respectively. The modeling results are summarized in Table B-5.

Table B-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	Local chi-square residual	$ED_{16.7}$	$LED_{16.7}$
Multistage	0.75	137.34	-0.25	**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 effective dose at an extra risk of 16.7% was estimated to be 440 mg/kg-day (ED), 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)          0.000415702    0.000222182     -1.97658e-005         0.00085117

Analysis of Deviance Table
```

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

AIC: 137.34

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	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

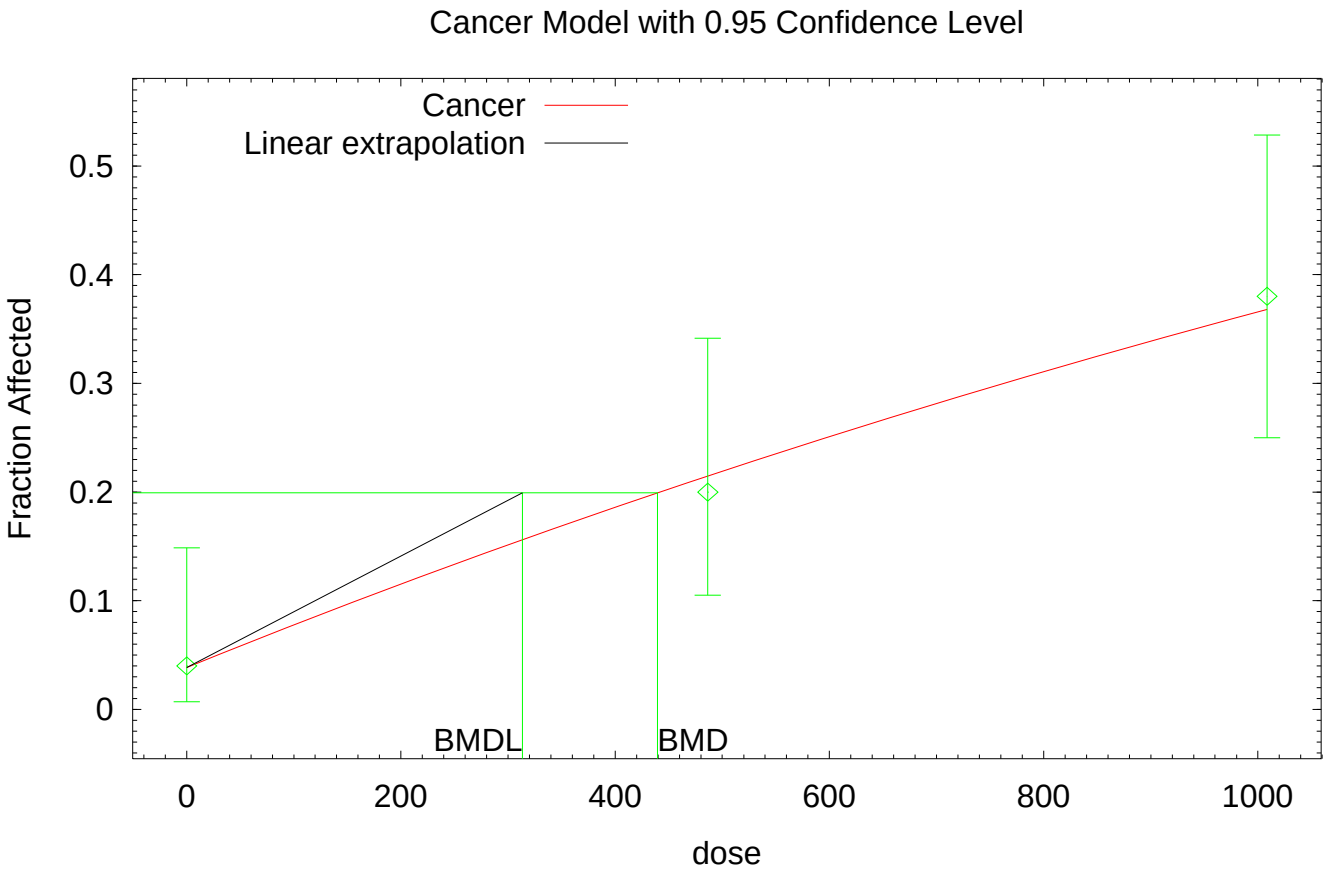
BMD = 439.549

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


Cancer Model with 0.95 Confidence Level
Cancer
Linear extrapolation
Fraction Affected
0.5
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
200
400
600
800
1000
dose
01:32 12/01 2005

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 human equivalent doses are 0, 486.5, and 1008.6 mg/kg-day, respectively. The modeling results are summarized in Table B-6.

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

Model	Goodness of fit *p*-value	AIC	Local chi-square residual	$ED_{33.3}$	$LED_{33.3}$
Multistage	0.03	186.5	1.77	**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 effective dose at an extra risk of 33.3% was estimated to be 1154 mg/kg-day (ED), 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 d.f.	P-value
Full model	-88.9509	3			
Fitted model	-91.2692	2	4.63657	1	0.0313
Reduced model	-94.0304	1	10.159	2	0.006223

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

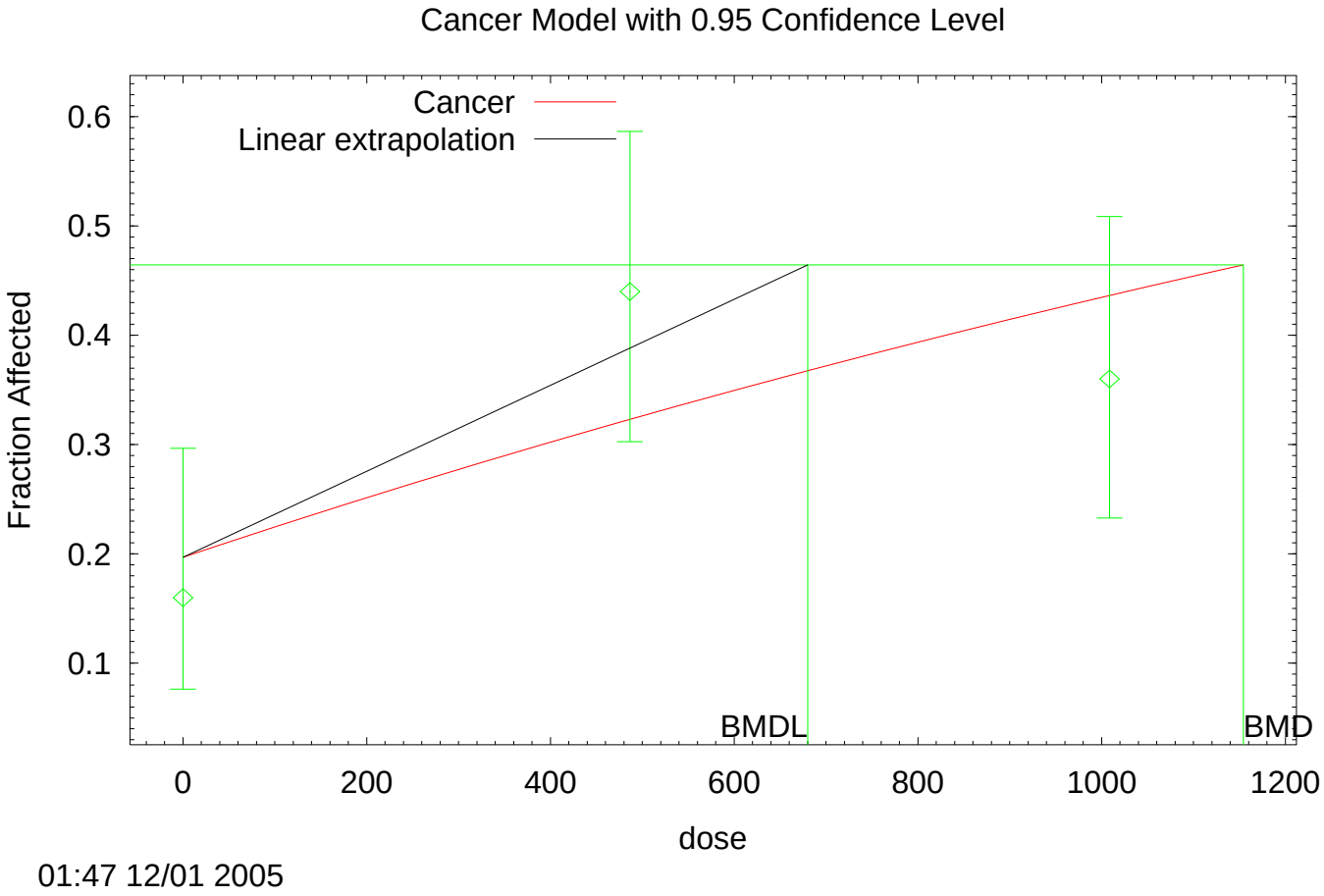
Benchmark Dose Computation

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


Cancer Model with 0.95 Confidence Level
Cancer
Linear extrapolation
Fraction Affected
0.6
0.5
0.4
0.3
0.2
0.1
BMDL
BMD
0
200
400
600
800
1000
1200
dose
01:47 12/01 2005