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TOXICOLOGICAL REVIEW

OF

BROMOBENZENE

(CAS No. 108-86-1)

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

June 2007

NOTICE

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U.S. Environmental Protection Agency
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LIST OF ABBREVIATIONS AND ACRONYMS

AIC	Akaike's Information Criteria
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BB	Bromobenzene
BCF	Bioconcentration factor
BMC	Benchmark concentration
BMD	Benchmark dose
BMDS	Benchmark Dose Software
BMR	Benchmark response
BUN	Blood urea nitrogen
CASRN	Chemical Abstract Service Registry Number
DENA	Diethylnitrosamine
EH	Epoxide hydrolase
EPA	Environmental Protection Agency
GC-MS	Gas chromatography-mass spectrometry
GGT	γ-Glutamyltranspeptidase-positive
H&E	Hematoxylin and eosin
HEC	Human equivalent concentration
IRIS	Integrated Risk Information System
LOAEL	Lowest-observed-adverse-effect level
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin content
MCV	Mean corpuscular volume
NOAEL	No-observed-adverse-effect level
NTP	National Toxicology Program
PAS	Periodic acid-Schiff
PBPK	Physiologially based pharmacokinetic
PBTK	Physiologically based toxicokinetic
RfC	Inhalation reference concentration
RfD	Oral reference dose
SDH	Sorbitol dehydrogenase
UF	Uncertainty factor
VHC	Volatile hydrocarbon

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 bromobenzene. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of bromobenzene.

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 bromobenzene. 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 (=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 bromobenzene has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). U.S. Environmental Protection Agency (EPA) guidelines and Risk Assessment Forum 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), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a, 2005c), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

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

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Bromobenzene is a heavy, colorless liquid with a pungent odor (Lewis, 1997). Synonyms include monobromobenzene and phenyl bromide (Budavari, 2001). Selected chemical and physical properties of bromobenzene are listed below:

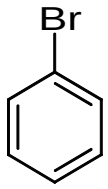

Figure 2-1. Chemical structure of bromobenzene

CASRN:	108-86-1 (Lide, 2000)
Molecular weight:	157.01 (Budavari, 2001)
Chemical formula:	C_6H_5Br (Budavari, 2001)
Boiling point:	156.0°C (Lide, 2000)
Melting point:	-30.6°C (Lide, 2000)
Vapor pressure:	4.18 mm Hg at 25°C (Riddick et al., 1986)
Density:	1.4950 g/mL at 20°C (Lide, 2000)
Vapor density:	2.46 (air = 1) (Budavari, 2001)
Water solubility:	4.46×10^2 mg/L at 30°C (Chiou et al., 1977)
Other solubility:	Miscible with chloroform, benzene, and petroleum hydrocarbons. Solubility in alcohol (0.045 g/100 g at 25°C), in ether (71.3 g/100 g at 25°C) (Budavari, 2001)
Partition coefficient:	log K_{ow} = 2.99 (Hansch et al., 1995)
Flash point:	51°C (Budavari, 2001)
Heat of combustion:	-1.98×10^7 J/kg (HSDB, 2003)
Heat of vaporization:	44.54 kJ/mol at 25°C (Lide, 2000)
Critical temperature:	397°C (Budavari, 2001)
Critical pressure:	33,912 mm Hg (Budavari, 2001)
Viscosity:	1.124 cp at 20°C (Budavari, 2001)
Vapor density (air=1):	5.41 (Budavari, 2001)
Surface tension:	0.036 N/m at 20°C (HSDB, 2003)
Soil sorption constant:	Koc = 150
Air pollution factors:	1 mg/m^3 = 0.15 ppm, 1 ppm = 6.53 mg/m^3 (Verschueren, 2001)
Henry's Law constant:	2.47×10^{-3} atm m^3/mol at 25°C (Shiu and Mackay, 1997)
OH reaction rate constant:	7.70×10^{13} cm^3/molecule sec at 25°C (Atkinson, 1989)

Bromobenzene is prepared commercially by the action of bromide on benzene in the presence of iron powder (Budavari, 2001). An alternate procedure uses pyridine as a halogen

carrier. Bromobenzene was produced in quantities less than 10,000 pounds ($4.5x10^3$ kg) in 1986, 1990, 1994, 1998, and 2002 (U.S. EPA, 2002). U.S. imports of bromobenzene were $2.00x10^3$ kg in 1984 (HSDB, 2003). Bromobenzene is used for organic synthesis, especially in the production of the synthetic intermediate phenyl magnesium bromide (Budavari, 2001; Lewis, 1997). Bromobenzene is also used as an additive to motor oils and a crystallizing solvent.

Release of bromobenzene to the environment may occur during its production and the production of phenyl magnesium bromide as well as in its use as a solvent and as an additive in motor oil (HSDB, 2003). It has been detected at low frequencies and at low concentrations in samples of food, ambient air, and finished water.

If released to air, bromobenzene will exist solely as a vapor in the ambient atmosphere, based on its vapor pressure of 4.18 mm Hg at 25°C (Bidleman, 1988; Riddick et al., 1986). Reaction of vapor-phase bromobenzene with photochemically-produced hydroxyl radicals will result in degradation with an estimated half-life of 21 days (HSDB, 2003).

Bromobenzene is expected to have moderate to high mobility in soil, based on a soil sorption constant (Koc) of 150 and an octanol/water partition coefficient (log K_{ow}) of 2.99 (Hansch et al., 1995; U.S. EPA, 1987; Swann et al., 1983). Volatilization of bromobenzene from moist soil surfaces may be significant, based on its Henry's Law constant of $2.47x10^{-3}$ atm m^3/mol at 25°C (Shiu and Mackay, 1997; Lyman et al., 1990).

If released to water, bromobenzene is not expected to adsorb to suspended solids or sediment, based on its Koc and water solubility (Swann et al., 1983). Bromobenzene will volatilize from water surfaces, based on its Henry's Law constant (Lyman et al., 1990). Hydrolysis of bromobenzene should be very slow because halogenated aromatics are generally resistant to hydrolysis (Lyman et al., 1990). Experimental bioconcentration factor (BCF) values ranging from 8.8 in carp to 190 in algae (*Chlorella fusca*) suggest that bioconcentration in aquatic organisms is low to moderately high (HSDB, 2003; CITI, 1992; Freitag et al., 1985).

Bromobenzene does not appear to be degraded rapidly by aquatic microorganisms (U.S. EPA, 1987). It was not degraded at an initial concentration of 30 mg/L after 4 weeks of inoculation in 100 mg/L activated sludge during a screening test (CITI, 1992).

Bromobenzene has been detected in water samples from the Delaware River basin, the Mississippi River, the Hudson River, and Lake Michigan (U.S. EPA, 1987). The average concentration of bromobenzene from eight observations in stream water reported in 1976 was 12.75 μg/L, with a range of 3-38 μg/L, according to the STORET database (U.S. EPA, 1987). Bromobenzene was identified with a maximum concentration of 10 ng/L in a contaminated plume of groundwater near Falmouth, MA over 3500 meters long (Barber et al., 1988). The plume resulted from the long-term disposal of secondary treated sewage effluent into a shallow,

unconfined aquifer since 1936. The concentration of 10 ng/L was the lowest concentration reported for approximately 50 volatile organic compounds that were detected.

Bromobenzene can be formed in small quantities during water chlorination (HSDB, 2003). For example, it has been detected (albeit infrequently) at low concentrations in finished water in the lower Mississippi River area. During a groundwater supply survey (Westrick et al., 1984), finished water samples were collected from public water systems located across the United States that serve both greater than 10,000 persons and fewer than 10,000 persons. Bromobenzene was detected above 0.5 μg/L (quantitation limit) in 3 out of 280 random sample sites serving fewer than 10,000 persons with a median of positives of 1.9 μg/L and a maximum value of 5.8 μg/L. It was also detected in 1 out of 186 random sample sites serving greater than 10,000 persons at 1.7 μg/L. In 2 of 321 nonrandom sample sites serving fewer than 10,000 persons, bromobenzene was detected with a median of positives of 0.97 μg/L and a maximum value of 1.2 μg/L. Bromobenzene was not detected above the quantitation limit in 158 nonrandom sample sites serving more than 10,000 persons. In 0.13% of 24,125 public water systems tested in a 20-state cross-section survey conducted for the U.S. EPA Office of Water between 1993 and 1997 (U.S. EPA, 2003), bromobenzene was detected. The overall median concentration of the detections was 0.5 μg/L. Detection frequency was higher in public water systems using surface water (0.23% of 2664 surface water systems) than those using groundwater (0.12% of 21,461 groundwater systems).

Bromobenzene has been detected at low concentrations in air samples collected near unidentified emission sources (U.S. EPA, 1987; Brodzinsky and Singh, 1982). In 35 air samples from El Dorado, AR collected from 1976 to 1978, bromobenzene concentrations ranged from 0.83 to 2100 ppt, with a mean concentration of 210 ppt. In 28 air samples from Magnolia, AR collected in 1977, bromobenzene concentrations ranged from 0 to 8.3 ppt, with a mean concentration of 1.5 ppt. Bromobenzene was not detected in seven air samples from Grand Canyon, AZ or in one air sample from Edison, NJ.

Heikes et al. (1995) detected bromobenzene in 2 of 234 table foods above the limit of quantitation (1.83 ppb) using EPA Method 524.2. Concentrations were 4.69 ppb in sandwich cookies and 9.06 ppb in cake doughnuts. The authors stated that volatile halocarbons (VHCs) are frequently encountered in table-ready foods as contaminant residues and that foods high in fat had more elevated levels (>1000 ppb).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

Data on absorption of bromobenzene by the gastrointestinal tract, respiratory tract, or skin in humans are not available. Findings of systemic effects following oral (Casini et al., 1984, 1985; Kluwe et al., 1984) or inhalation (Dahl et al., 1990; Brondeau et al., 1986) exposure of animals serve as an indication that bromobenzene is absorbed through the gastrointestinal tract and lungs. Quantitative data on absorption of orally-administered bromobenzene are limited. However, bromobenzene is readily absorbed by the gastrointestinal tract, as evidenced by the appearance of metabolites of bromobenzene (detected by gas chromatography-mass spectrometry [GC-MS]) in the urine of rats, mice, and rabbits that had been administered single oral doses (3–30 mg/kg-day) of bromobenzene (Ogino, 1984a). The urinary metabolites accounted for 60–70% of the administered dose, most of which had been recovered in the first 8 hours following dosing. Absorption of bromobenzene across the lungs was demonstrated by the appearance of parent compound (determined by head-space GC) in the blood of laboratory animals immediately following a single 4-hour inhalation exposure to bromobenzene vapors (Aarstad et al., 1990). At 1000 ppm, measured bromobenzene blood concentrations were 153, 102, and 47 mg/mL for rats, mice, and rabbits, respectively. *In vitro* experiments with rat blood indicated a blood/air partition coefficient of approximately 200 (Aarstad et al., 1990). A blood/air partition coefficient for bromobenzene in humans was not found.

3.2. DISTRIBUTION

Results of parenteral injection studies in animals indicate that, following absorption, bromobenzene and its metabolites are widely distributed, with highest levels found in adipose tissue (Ogino, 1984b; Zampaglione et al., 1973; Reid et al., 1971).

The distribution of bromobenzene following intraperitoneal injection of a 750 mg/kg-day dose of bromobenzene (in sesame oil) was studied in male Sprague-Dawley rats (Reid et al., 1971). Levels of bromobenzene in tissues obtained 4 and 24 hours after administration were determined by gas-liquid chromatography of tissue extracts for all tissues except fat. Levels of bromobenzene in fat were calculated from detected levels of ^{3}H and the specific activity of the applied ^{3}H-bromobenzene. At 4 hours post-injection, the highest levels of bromobenzene were found in fat (5600 μg/g tissue), followed by liver (282 μg/g), kidney (235 μg/g), brain (206 μg/g), heart (146 μg/g), lung (142 μg/g), stomach (132 μg/g), and blood plasma (34 μg/g). After 24 hours, measured concentrations were: fat (400 μg/g), kidney (19 μg/g), stomach (17 μg/g), liver (11 μg/g), brain (7.0 μg/g), lung (6.2 μg/g), heart (5.0 μg/g), and blood plasma (2 μg/g).

In another study, concentrations of bromobenzene in tissues from rats 10 hours after intraperitoneal injection of 5 mg of bromobenzene were highest in adipose tissue (3.38 μg/g), followed by liver (0.18 μg/g), seminal fluid (0.15 μg/g), blood (0.12 μg/g), brain (0.08 μg/g), and pectoral muscle (0.04 μg/g). Levels of bromobenzene in kidney, spleen, heart, and lung tissues were below the detection limit of 0.01 μg/g. Levels of phenolic metabolites (m-bromophenol and p-bromophenol) were highest in the kidney (0.43 μg/g), lungs (0.27 μg/g), and blood (0.19 μg/g), with lesser amounts in seminal fluid, brain, heart, liver, and pectoral muscle; proportions of the individual phenols (m-bromophenol and p-bromophenol) were approximately equal in each of the tissues examined (Ogino, 1984b). The phenols were below the level of detection (0.01 μg/g) in spleen and adipose tissues. Concentrations of bromobenzene were reported to show a pattern of peaking within 10 hours after dosing, followed by rapidly decreasing concentrations, but collected data to show this pattern were not reported (Ogino, 1984b).

In order to monitor tissue distribution immediately following exposure, male Sprague-Dawley rats were administered ^{14}C-bromobenzene intravenously at a dose of 10 μmol/kg and plasma levels of radioactivity were monitored (Zampaglione et al., 1973). Plasma levels dropped triphasically during 70 minutes following administration. During the first 5 minutes following dosing, radioactivity in the liver increased to a peak, at which time measured radioactivity was highest in the liver, followed by adipose tissue and plasma in decreasing order. Levels in the liver subsequently dropped in a manner similar to that of plasma radioactivity, although measured levels in the liver remained higher than those in the plasma. Adipose tissue levels reached a peak within 20 minutes after dosing and remained high throughout the 70-minute observation period.

Monks et al. (1982) assessed distribution by monitoring covalent binding to the protein fraction in various tissues following intraperitoneal injection of 3 mmol/kg (471 mg/kg-day) of ^{14}C-bromobenzene in male Sprague-Dawley rats. Covalent binding to proteins was most prominent in the liver, followed by the kidney, small intestine, lung, and muscle.

3.3. METABOLISM

The metabolism of bromobenzene has been extensively studied in *in vivo* and *in vitro* mammalian systems (see Lau and Monks, 1997a,b; Lertratanangkoon et al., 1993; Lau and Monks, 1988). Based on available data, a proposed metabolic scheme for bromobenzene is illustrated in Figure 3-1. There are two initial competing steps involving conversion of bromobenzene to either the 3,4-oxide derivative catalyzed by phenobarbital-induced cytochrome isozymes CYP 450 1A2, 2A6, 2B6, and 3A4 or the 2,3-oxide derivative catalyzed by 3-methylcholanthrene and β-naphthoflavone-induced CYP isozymes, CYP 450 1A1, 1A2, and

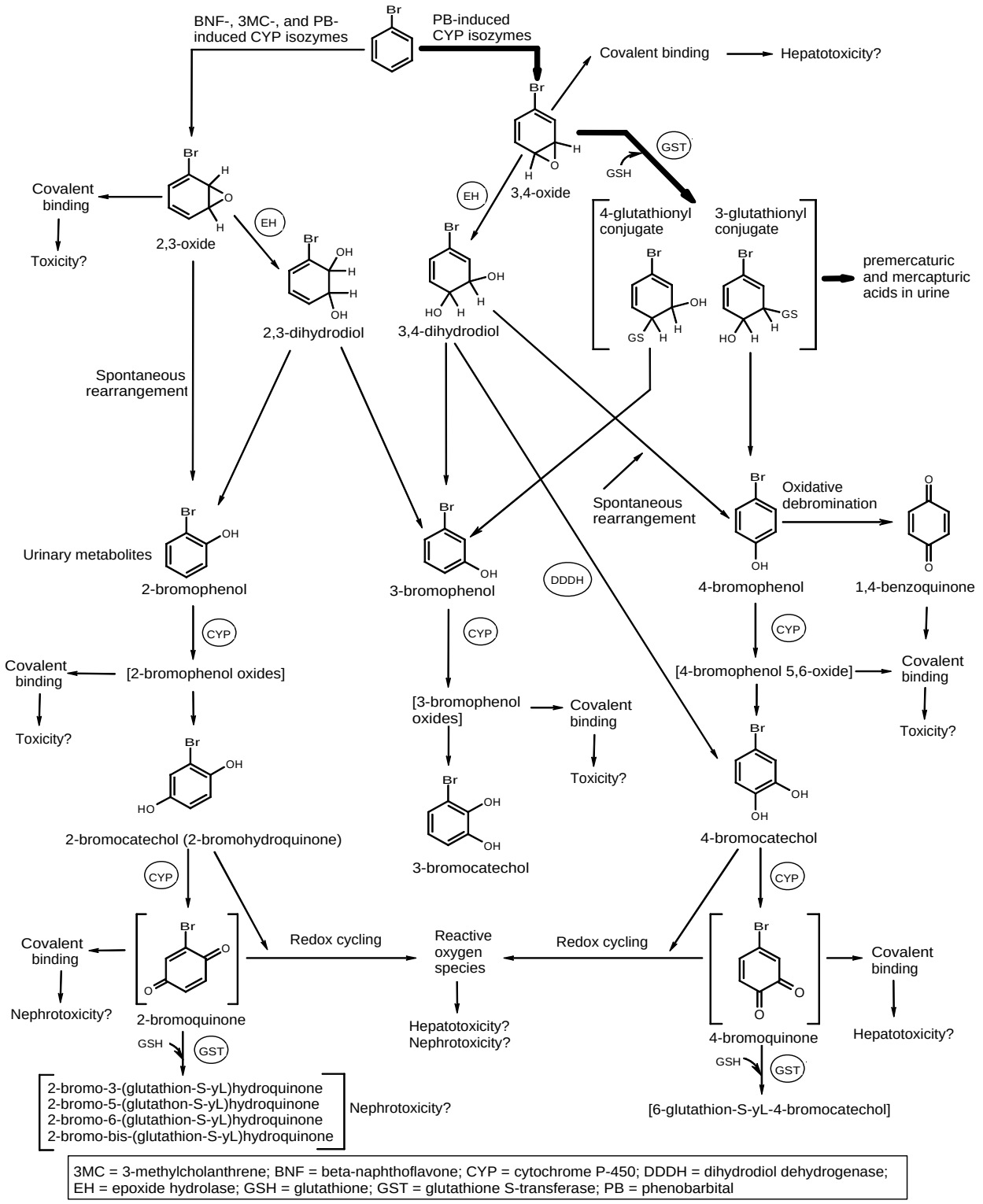

Figure 3-1. Proposed metabolic scheme for bromobenzene in mammals (adapted from Lertratanangkoon et al., 1993; Lau and Monks, 1988)

1B1, as well as phenobarbital-induced CYP isozymes (Girault et al., 2005; Krusekopf et al., 2003; Lau and Zannoni, 1979, 1981a; Reid et al., 1971).

The predominant metabolic pathway in the rat liver leads to enzymatic (glutathione-S-transferase) conjugation of the 3,4-oxide derivative with glutathione, followed by urinary excretion as premercapturic and mercapturic acids, as evidenced by the recovery of approximately 70% of the radioactivity as mercapturic acids in the urine of male Sprague-Dawley rats that had been injected intravenously with 0.05 mmol/kg (7.9 mg/kg-day) of ^{14}C-bromobenzene (Zampaglione et al., 1973). Glutathione conjugation is thought to be a protective mechanism for acute bromobenzene hepatotoxicity (see Section 4.5.3). The 2,3-oxide derivative has not been observed to undergo glutathione conjugation.

Both the 3,4- and 2,3-oxide derivatives may be converted to the corresponding dihydrodiols by epoxide hydrolase (EH). The subsequent formation of bromophenols (2-, 3-, and 4-bromophenol) from the oxide derivatives includes several proposed pathways (Lertratanangkoon et al., 1993; Lau and Monks, 1988). The chemical instability of the 2,3-oxide derivative and its relatively short biological half-life indicate that spontaneous rearrangement is the predominant pathway to the formation of 2-bromophenol in the rat and guinea pig *in vivo* (Lertratanangkoon et al., 1993), although it has been suggested that both 2- and 3-bromophenol may also be formed by rearrangement of the 2,3-dihydrodiol (Lertratanangkoon et al., 1987, 1993; see also Figure 3-1). Other pathways to the formation of 3-bromophenol may include rearrangement of the 3,4-dihydrodiol or the 4-S-glutathione conjugate of the 3,4-oxide derivative (Lertratanangkoon et al., 1987, 1993). Spontaneous rearrangement of the 3,4-dihydrodiol is thought to be the major pathway leading to the formation of 4-bromophenol in the rat, whereas the pathway leading through the 3-S-glutathione conjugate of the 3,4-oxide derivative is thought to predominate in the guinea pig (Lertratanangkoon et al., 1987, 1993).

The bromophenol metabolites may be subsequently oxidized by CYP to their respective bromocatechols (2-, 3-, or 4-bromocatechol, Figure 3-1), likely involving bromophenol oxide intermediates. The 4-bromocatechol may also be formed via dihydrodiol dehydrogenase (DDDH)-catalyzed conversion of the 3,4-dihydrodiol, the pathway that appears to predominate in the rat *in vivo* (Miller et al., 1990). The 4-bromophenol may undergo oxidative debromination to form 1,4-benzoquinone (Slaughter and Hanzlik, 1991; Zheng and Hanzlik, 1992). Redox cycling of 2- and 4-bromocatechol and conjugation by glutathione S-transferase (GT) produce 2-bromo-3-(glutathion-S-yL)hydroquinone and 6-glutathion-S-yL-4-bromocatechol, respectively (Lau and Monks, 1988).

Mercapturic acids are the predominant urinary metabolites of bromobenzene in laboratory animals, indicating that glutathione conjugation of the 3,4-epoxide is the primary metabolic pathway for bromobenzene. Approximately 60-70% of the administered dose was

detected (using GC-MS) as mercapturic acids, derived from the 3,4-oxide pathway, in the 24-hour urine of rats given bromobenzene parenterally at doses ranging from 7.9 to 158 mg/kg-day (Chakrabarti and Brodeur, 1984; Zampaglione et al., 1973). Following oral administration of bromobenzene (10 mg/rat, 1 mg/mouse, 10 mg/rabbit), approximately 50-60% of the 96-hour urinary recovery of bromobenzene metabolites was in the form of 4-bromophenylmercapturic acid (Ogino, 1984a). Other metabolites that have been measured in the urine of rats include the phenolic compounds, dihydrodiols, catechols, and hydroquinones (Miller et al., 1990; Lertratanangkoon and Horning, 1987; Chakrabarti and Brodeur, 1984; Lau et al., 1984a; Monks et al., 1984a,b; Jollow et al., 1974; Zampaglione et al., 1973).

Animal studies have elucidated species-specific differences in urinary excretion of the bromophenols (2-, 3-, and 4-bromophenol) following exposure to bromobenzene. For example, in the 96-hour urine of mice that had been administered a nontoxic oral dose of bromobenzene (1 mg/mouse; approximately 33 mg/kg-day), 2-bromophenol accounted for 12.1% of the dose, 3-bromophenol accounted for 8.8%, and 4-bromophenol accounted for 3.1% (Ogino, 1984a). In similarly-treated rats (10 mg/rat; approximately 56 mg/kg-day), however, 2-bromophenol accounted for only 2.6% of the dose, while 3-bromophenol accounted for 19.2% and 4-bromophenol accounted for 13.1%. In the urine of the mice, 2-bromophenol was 4 times more prevalent than 4-bromophenol, whereas 4-bromophenol was 5 times more prevalent than 2-bromophenol in the urine of the rats. This metabolic difference between rats and mice has been associated with a difference in susceptibility to bromobenzene acute nephrotoxicity (Reid, 1973; see also Section 4.5.3).

Metabolism of bromobenzene in the liver appears to be capacity-limited. For example, approximately 79% of the radioactivity from an intraperitoneally-injected nonhepatotoxic (130 mg/kg-day) dose of ^{14}C-bromobenzene was recovered in the urine of rats within 24 hours following administration, whereas only 47% was recovered following a hepatotoxic (1200 mg/kg-day) dose (Lertratanangkoon and Horning, 1987). Section 4.5.3 discusses relationships between glutathione depletion and hepatotoxicity in more detail.

Although liver tissue has been shown to be capable of producing all of the major metabolites depicted in Figure 3-1, as demonstrated by numerous *in vivo* and *in vitro* animal studies, bromobenzene can be metabolized at sites other than the liver. *In vitro* studies in rats and mice have demonstrated that lung (Monks et al., 1982; Reid et al., 1973) and kidney (Monks et al., 1982) tissues are capable of metabolizing bromobenzene, although the extent to which extrahepatic tissues metabolize bromobenzene *in vivo* is not known.

Following oral exposure, a first-pass metabolic effect is expected to occur due to the extensive metabolic capacity of the liver; however, the extent of the first-pass effect as a function

of administered dose has not been empirically characterized. Likewise, the extent of first-pass metabolism in the lung has not been demonstrated following inhalation exposure.

Recent studies have noted that intraperitoneal injection of bromobenzene into rats can induce many different types of enzymes. In a toxicogenomics approach, Heijne et al. (2005, 2004, 2003) noted induction of more than 20 liver proteins (including γ-glutamylcysteine synthetase, a key enzyme in glutathione biosynthesis) and transient changes in the transcriptional expression of numerous genes involved in drug metabolism, oxidative stress, glutathione depletion, the acute phase response, metabolism, and intracellular signaling following intraperitoneal administration of bromobenzene to rats. Other studies (Minami et al., 2005; Stierum et al., 2005; Waters et al., 2006) have utilized toxicogenomics to characterize the relationship between bromobenzene hepatotoxicity and hepatic gene expression profiles.

3.4. ELIMINATION

Results of animal studies indicate that urinary excretion of metabolites is the principal route of elimination of absorbed bromobenzene (Lertratanangkoon and Horning, 1987; Merrick et al., 1986; Ogino, 1984a; Zampaglione et al., 1973; Reid et al., 1971), although biliary excretion of the 3- and 4-glutathionyl conjugates formed from the 3,4-oxide derivative has been demonstrated in bile-cannulated rats (Sipes et al., 1974).

In rats, mice, and rabbits given bromobenzene in single oral doses of approximately 3-30 mg/kg-day, detection of metabolites in urine collected for 4 days accounted for 60-70% of the administered dose, most of which had been recovered within 8 hours following administration (Ogino, 1984a). Small amounts of parent compound were detected in the urine and feces of all three species. Approximately 85% of an intraperitoneally injected dose (250 mg/kg-day) of ^{14}C-bromobenzene was excreted within 24 hours as metabolites in the urine of rats (Reid et al., 1971). In other rat studies, metabolites detected in the urine collected for 48 hours accounted for more than 90% of administered doses of 8 mg/kg-day (intravenous) or 1570 mg/kg-day (intraperitoneal) (Zampaglione et al., 1973).

Biliary excretion of bromobenzene-glutathione conjugate has been demonstrated in rats; the rate of biliary excretion can be used as an index of *in vivo* activation of bromobenzene (Madhu and Klaassen, 1992). Additional information regarding biliary excretion of bromobenzene metabolites was demonstrated in bile-cannulated rats that were administered a non-hepatotoxic dose (20 mg/kg-day) of ^{14}C-bromobenzene in the femoral vein (Sipes et al., 1974). Cumulative excretion of radioactivity in the bile was 56% of administered radioactivity during 3 hours after dosing. Combined with demonstrations that, in normal non-cannulated rats, elimination of bromobenzene predominantly occurs via urinary excretion of metabolites (Ogino, 1984a; Zampaglione et al., 1973; Reid et al., 1971) and not via fecal excretion (Ogino, 1984a), it

appears that most of the metabolites in the bile are reabsorbed from the intestine by enterohepatic circulation and subsequently excreted by the kidneys.

The biological half-life of bromobenzene in laboratory animals is relatively short. Using a two-phase model, Ogino (1984a) calculated a half-life of 4.65 hours for the first phase (0-16 hours) and 26.8 hours for the second phase (24-96 hours), based on total excretion of brominated compounds in the urine of mice given a single oral dose of approximately 33 mg/kg-day. A first-order elimination half-life of 5.87 hours was calculated for clearance of radioactivity from the blood of rats given a relatively high (1178 mg/kg-day) dose of ^{14}C-bromobenzene by intraperitoneal injection (Merrick et al., 1986). A much shorter first-phase half-life (approximately 10 minutes) was reported for the elimination of radioactivity from the whole body of rats that had been injected intravenously with a nontoxic (8 mg/kg-day) dose of radiolabeled bromobenzene (Zampaglione et al., 1973). In this study, a second-phase half-life was not calculated.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS (PBTK)

No PBTK models have been developed for bromobenzene.

4. HAZARD IDENTIFICATION

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

Studies on health effects in humans exposed to bromobenzene were not identified in literature searches.

4.2. LESS-THAN-LIFETIME AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Exposure

4.2.1.1. Subchronic Toxicity

The National Toxicology Program (NTP) conducted subchronic gavage studies of bromobenzene in rats (NTP, 1985a) and mice (NTP, 1985b). These studies have not been officially released by NTP, but unpublished reports, including the review comments and conclusions of NTP's Pathology Working Group (NTP, 1986a), were obtained from NTP. The unpublished NTP studies are available by calling EPA's IRIS Hotline at (202)566-1676, by fax at (202)566-1749 or by email at iris@epa.gov.

Groups of 10 male and 10 female Fischer 344/N rats were given 0, 50, 100, 200, 400, or 600 mg/kg-day of bromobenzene (>99% purity) by gavage in corn oil 5 days/week for 90 days in the basic study. In a supplementary study designed to evaluate clinical pathologic effects of bromobenzene, groups of five rats/sex were similarly treated with 0, 50, 200, or 600 mg/kg-day and housed individually in metabolism cages throughout the study; urine samples were collected from these rats on days 1, 3, 23, and 94 for detailed urinalysis. Blood samples were collected on days 2, 4, 24, and 95 for hematology and clinical chemistry. Rats from both the basic and supplementary studies were observed twice daily for morbidity and mortality. Clinical observations and body weight measurements were performed weekly. Blood samples for hematologic and clinical pathologic examinations were collected from all surviving rats at terminal sacrifice. Terminal body and organ (liver, brain, testis, kidney, lung, heart, and thymus) weights were recorded; organ-to-body weight and organ-to-brain weight ratios were calculated for each sex. Complete gross necropsy was performed on all rats. Complete histopathologic examinations of all major tissues and organs (including liver, kidney, urinary bladder, spleen, pancreas, brain, spinal cord, sciatic nerve [if neurologic signs were present], heart, lung, trachea, nasal cavity, esophagus, stomach, small intestine, cecum, colon, uterus, ovaries, preputial or clitoral glands, testes, prostate, seminal vesicles, sternebrae, adrenals, pituitary, thyroid, parathyroids, salivary gland, mandibular and mesenteric lymph nodes, thymus, mammary gland,

blood, gross lesions, and tissue masses) were performed on all control rats and all rats from the 400- and 600-mg/kg-day dose groups.

In the basic study, all rats of the 50- and 100-mg/kg-day groups were subjected to histopathologic examination of liver and kidney. Furthermore, sections of livers from all control and bromobenzene-treated rats were examined following hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining for glycogen. In the supplementary study, liver and kidney tissues from all rats and any gross lesions were examined histologically. Serum of rats in the supplementary study was assessed for blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), glucose, and aspartate aminotransferase (AST). Parameters assessed in urinalysis included volume, color, specific gravity, pH, hemoglobin, glucose, creatinine, and protein. Hematologic evaluations of blood collected at terminal sacrifice from all surviving rats included erythrocyte and leukocyte counts and morphology; hemoglobin concentration; volume of packed cells; measures of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin content (MCHC); qualitative estimates of leukocyte differential count; and platelet and reticulocyte counts. Serum was analyzed for BUN, creatinine, ALT, SDH, total protein, albumin, albumin/globulin ratio, glucose, and AST.

In the basic study, treatment-related clinical signs were observed only at the 600 mg/kg-day dose level and included ruffled fur (9/10 rats of each sex), emaciation (9/10 rats of each sex), tremors (2/10 males and 1/10 females), ataxia (1/10 of each sex), hypoactivity (5/10 males and 7/10 females), and ocular discharge (2/10 of each sex). Observations of similar clinical signs were made in rats of the supplementary study, but distinguishing between treatment-related clinical signs and symptoms that may have resulted from repeated anesthesia, blood sample collection, and prolonged housing in metabolism cages was difficult.

Treatment-related mortality was observed in male and female rats at 600 mg/kg-day (9/10 males and 8/10 females in the basic study and 3/5 males and 1/5 females in the supplementary study). By the end of week 7, mortality rates in high-dose male and female rats were 7/10 and 3/10, respectively. Occasional deaths at lower doses were attributed to gavage error. Statistically significantly reduced mean body weight (approximately 7-11% lower than controls) was observed in 400-mg/kg-day male rats from week 5 until study end. At 600 mg/kg-day, both male and female rats were visibly emaciated. Table 4-1 presents terminal body and liver weights and serum levels of selected liver enzymes in male and female rats of the basic study. Dose-related statistically significantly increased mean liver and kidney weights (absolute, relative-to-body weight) were observed at doses $\geq$100 mg/kg-day in male rats and at all dose levels (including 50 mg/kg-day) in female rats. Changes in the 600 mg/kg-day males were similar in magnitude to changes in the 400 mg/kg-day males, but could not be assessed for statistical

Table 4-1. Effects of bromobenzene on terminal body and liver weights and serum liver enzymes of male and female Fischer 344/N rats exposed by oral gavage 5 days/week for 90 days in the basic study (mean +/- standard deviation)

Male rats						
Dose (mg/kg-day)	Controls	50	100	200	400	600
Number of rats	10	10	9	8	10	1[a]
Body weight (g)	343.0 ± 12.9	330.3 ± 12.2	342.3 ± 18.5	331.3 ± 20.0	293.0[b] ± 11.9	203.1[c]
Liver weight (g) Difference (%)[d]	9.16 ± 0.66 --	Not available	10.64[b] ± 0.76 +16.2	11.29[b] ± 0.69 +23.3	11.87[b] ± 0.80 +29.6	10.50 +14.6
Ratio liver/body weight Difference (%)[d]	26.72 ± 1.88 --	Not available	31.08[b] ± 1.18 +16.4	34.10[b] ± 0.68 +27.7	40.56[b] ± 3.16 +51.9	51.70[c] +93.6
Serum AST (IU/L) Serum ALT (IU/L) Serum SDH (IU/L)	83.70 ± 10.97 41.90 ± 9.33 3.90 ± 2.59	93.40 ± 18.39 41.30 ± 6.66 3.68 ± 1.85	82.56 ± 17.63 38.67 ± 9.45 3.56 ± 0.96	87.88 ± 10.64 39.50 ± 7.28 5.25 ± 1.64	820.10[b] ± 694.95 893.20[b] ± 727.39 311.90[b] ± 228.19	268.00 403.00 80.00
Female rats						
Dose (mg/kg-day)	Controls	50	100	200	400	600
Number of rats	10	10	10	10	10	3[a]
Body weight (g)	192.8 ± 9.0	197.1 ± 11.9	193.5 ± 9.1	187.6 ± 8.2	182.3[b] ± 10.5	167.4[b] ± 9.8
Liver weight (g) Difference (%)[d]	4.68 ± 0.35 --	5.23[b] ± 0.37 +11.6	5.55[b] ± 0.36 +18.6	6.28[b] ± 0.40 +34.2	7.85[b] ± 0.49 +67.7	9.11[b] ± 0.57 +94.7
Ratio liver/body weight Difference (%)[d]	24.25 ± 1.13 --	26.55[b] ± 1.23 +9.5	28.69[b] ± 1.20 +18.3	33.48[b] ± 1.37 +38.1	43.11[b] ± 2.38 +77.8	54.78[b] ± 6.64 +125.9
Serum AST (IU/L) Serum ALT (IU/L) Serum SDH (IU/L)	88.50 ± 23.69 41.70 ± 10.83 3.80 ± 0.98	83.50 ± 5.35 37.50 ± 5.16 4.00 ± 1.26	74.30 ± 12.92 30.70 ± 6.17 6.20[b] ± 1.47	72.60 ± 10.24 27.80 ± 4.71 3.78 ± 0.98	215.20 ± 339.55 265.38 ± 596.73 61.60 ± 143.07	119.00 ± 48.00 111.00 ± 59.00 23.00 ± 17.00

[a]High rates of early mortality at the 600 mg/kg-day dose level (9/10 males and 7/10 females) preclude meaningful statistical analysis of terminal body and organ weight data or serum enzyme changes

[b]Statistically significantly increased from controls ($p<0.05$) based on student's two-tailed t-test

[c]Outside 3 standard deviations from the control mean

[d]Change relative to controls

Source: NTP (1985a)

significance because only one survivor remained in this group at study termination. Significant increases in serum enzymes indicative of hepatotoxicity (ALT, AST, SDH) were found in 400 mg/kg-day male rats, but not males of lower dose levels. Serum SDH was significantly increased in 100 mg/kg-day female rats (approximately 60% greater than that of controls), but was not increased at the next higher dose level (200 mg/kg-day). Female rats of the 400 mg/kg-day dose level exhibited mean serum levels of ALT, AST, and SDH that were markedly increased over controls, but the large variance precluded using the t-test for statistical analysis (see Table 4-1). Significant increases in serum creatinine (males and females) and BUN (males only) were also observed at doses $\geq$400 mg/kg-day. Effects on the hematopoietic system were generally unremarkable. Significantly increased mean relative (but not absolute) testis weight was noted in male rats of the 400 and 600 mg/kg treatment groups (increased by 10 and 35%, respectively, over controls). There were no indications of treatment-related effects on reproductive organ weights in female rats.

As shown in Table 4-2, histopathologic examinations revealed treatment-related significantly increased incidences of rats exhibiting cytomegaly (doses $\geq$200 mg/kg-day in males and $\geq$400 mg/kg-day in females), inflammation (doses $\geq$200 mg/kg-day in males), and necrosis (doses $\geq$400 mg/kg-day in males and females). Cytomegaly was characterized by an increase in the size of the nucleus and cytoplasm of individual hepatocytes and was more common in the central parts of the hepatic lobule. Liver necrosis was primarily coagulative in nature and considered a direct result of bromobenzene treatment. Inflammation was principally centrilobular and consisted of focal infiltrates of macrophages, lymphocytes, and occasional neutrophils. The incidences and severity of each of these liver lesions generally increased with increasing dose. Centrilobular mineralization was observed in 2/10 and 1/10 high-dose males and females, respectively, and was considered to be the result of hepatocellular necrosis. Other histological findings in the liver included cytoplasmic alterations, infiltration, and pigmentation, which were generally of low incidence and did not exhibit consistent dose-response characteristics.

There is some evidence to suggest a common mechanism of action for bromobenzene-induced cytomegaly, necrosis, inflammation, and mineralization. All four lesions were principally observed in the central part of the hepatic lobules. Significantly increased incidences of hepatocellular necrosis or inflammation were observed only at doses equal to or greater than those eliciting significantly increased incidences of cytomegaly. In the NTP report, inflammation and mineralization were considered to be direct results of hepatocellular necrosis (NTP, 1985a). Based on these observations, incidences of rats with one or more of these liver lesions (cytomegaly, necrosis, inflammation, mineralization) were combined for each sex (as shown in Table 4-2).

Table 4-2. Incidences of male and female Fischer 344/N rats with liver and kidney lesions following administration of bromobenzene by gavage 5 days/week for 90 days in the basic study

Endpoint	Dose (mg/kg-day)[a]											
	0		50		100		200		400		600[b]	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Males												
Liver, centrilobular												
Inflammation	2/10	1.0	2/10	1.0	2/10	1.0	7/10[d]	1.6	9/10[d]	2.1	7/10[d]	2.1
Cytomegaly	0/10		0/10		0/10		4/10[d]	1.5	10/10[d]	2.0	9/10[d]	2.4
Necrosis	0/10		0/10		0/10		3/10	1.3	9/10[d]	2.0	9/10[d]	2.4
Mineralization	0/10		0/10		0/10		0/10		0/10		2/10	2.5
Combined[c]	2/10		2/10		2/10		7/10[d]		10/10[d]		10/10[d]	
Kidney, tubule												
Necrosis	0/10		0/10		0/10	2.0	0/10		0/10		6/10[d]	2.2
Degeneration	2/10	1.0	1/10	1.0	2/10		4/10	1.0	1/10	2.0	7/10[d]	2.6
Casts	0/10		0/10		0/10		1/10	1.0	3/10	2.0	7/10[d]	2.6
Mineralization	0/10		0/10		0/10		0/10		0/10		3/10	2.3
Pigment	0/10		0/10		0/10		0/10		7/10[d]	1.9	0/10	
Females												
Liver, centrilobular												
Inflammation	2/10	1.5	2/10	1.0	4/10	1.5	3/10	1.0	6/10	1.7	5/10	2.8
Cytomegaly	0/10		0/10		0/10		3/10	1.0	10/10[d]	2.4	10/10[d]	2.6
Necrosis	0/10		0/10		0/10		0/10		7/10[d]	2.0	9/10[d]	2.7
Mineralization	0/10		0/10		0/10		0/10		0/10		1/10	3.0
Combined[c]	2/10		2/10		4/10		5/10		10/10[d]		10/10[d]	
Kidney, tubule												
Necrosis	0/10		0/10		0/10		0/10		0/10		6/10[d]	2.3
Degeneration	0/10		0/10		0/10		0/10		1/10	2.0	8/10[d]	3.0
Casts	0/10		0/10		0/10		0/10		0/10		6/10[d]	2.5
Mineralization	0/10		0/10		0/10		1/10	2.0	0/10		3/10	2.0
Pigment	0/10		0/10		0/10		0/10		8/10[d]	2.1	2/10	2.0

[a]Incidence = number of animals in which lesion was found/number of animals in which organ was examined.
[b]Most male and female rats of the 600 mg/kg-day dose level died during the study, which may have affected incidences of selected lesions.
[c]Incidences of rats with one or more of the liver lesion types (cytomegaly, necrosis, inflammation, mineralization), extracted from individual animal histopathologic results provided to Syracuse Research Corporation by NTP.
[d]Statistically significantly different from control groups according to Fisher's exact test ($p<0.05$), performed by Syracuse Research Corporation.
Severity: Average severity score: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.
Source: NTP (1985a)

Observed kidney effects included a brown staining pigment of the cytoplasm (presumed to be bile pigment) in the convoluted tubules of 400-mg/kg-day male and female rats and degeneration of the convoluted tubules and necrosis in 600-mg/kg-day males and females, in the absence of indications of tubular regeneration. It was noted that the reduced incidence of the tubular (brown-staining) pigment in the 600-mg/kg-day rats (0/10 males and 2/10 females) might be related to high rates of early mortality at this dose level, in which case there may not have been enough time for this lesion to appear. Other histopathologic effects (hyperkeratosis, ulceration, and hemorrhage in the stomach; brain mineralization and necrosis; thymus atrophy; and bone marrow atrophy) were observed only in the high-dose groups of male and female rats. The effects in the stomach were probably associated with bolus gavage dosing. Atrophy or necrosis of the thymus was observed in six male and six female rats treated in the 600 mg/kg dose group. These effects were only noted in rats that died or were euthanized while moribund and were considered to be the result of stress. Testicular degeneration of moderate severity was noted in a single high-dose male rat. Gross and histopathologic examinations of female reproductive tissues did not reveal treatment-related effects.

The NTP Pathology Working Group (NTP, 1986a) reviewed the pathology results from the subchronic gavage studies in rats and mice (NTP, 1985a). This group designated the brain as an organ susceptible to chemically-related lesions based on cerebellar necrosis (granular layer) in 1 of 10 males and 3 of 10 females in the 600 mg/kg dose group; however, some members of the group (2 of 6) thought that degeneration, rather than necrosis, was a more appropriate descriptor of the lesion in some animals. The Pathology Working Group (NTP, 1986a) noted that bone marrow atrophy was either absent or only minimally present in the 400 mg/kg group, but was recorded in 3 of 10 males and 6 of 10 females in the 600 mg/kg group. It was also noted that most of the rats in this dose group died or were sacrificed in a moribund state and were emaciated, raising the possibility of marrow atrophy as a secondary rather than a direct effect. The Pathology Working Group (NTP, 1986a) indicated that testicular degeneration was apparent in a number of high-dose male rats, but suggested that this effect may have been secondary to emaciation. .

The most prominent toxicological effects observed in Fischer 344/N rats treated with bromobenzene by oral gavage for 90 days (NTP, 1985a) were observed in the liver. Significantly increased incidences of hepatocellular necrosis (a clear indicator of an adverse effect) were observed at doses of 400 and 600 mg/kg-day in both male and female rats. Significantly increased incidences of cytomegaly were noted at doses $\geq$200 mg/kg-day in male rats and at doses $\geq$400 mg/kg-day in female rats. Statistically significant increases in mean liver weight were observed at doses as low as 50 mg/kg-day in female rats and 100 mg/kg-day in male rats.

Treatment-related increased occurrence of cytomegaly and increased liver weight represent an adaptive liver response to bromobenzene, a known enzyme-inducing agent, and may provide an indication of liver toxicity from higher levels of exposure. By themselves, increased liver weight and increased incidences of cytomegaly can be considered to be of questionable toxicological significance.

The biological significance of the presence of pigments in the convoluted tubules of the kidneys of 400 mg/kg-day male and female rats is unclear. Incidences of other renal tubular effects (necrosis, degeneration, and casts) were statistically significantly increased only in high-dose male and female rats.

In the NTP (1985a) study the LOAEL is considered to be 50 mg/kg-day in female rats for statistically significant increased liver-to-body weight ratios and absolute liver weights. The designation of increased liver weights as an adverse effect is supported by the presence of liver lesions (including inflammation, cytomegaly, and necrosis) and elevated serum enzymes indicative of liver damage at higher doses.

In the mouse study (NTP, 1985b), groups of 10 male and 10 female B6C3F1 mice were administered 0, 50, 100, 200, 400, or 600 mg/kg-day of bromobenzene by gavage in corn oil 5 days/week for 90 days; supplementary groups of 10 mice/sex were similarly treated with 0, 50, 200, or 600 mg/kg-day and housed in pairs in metabolism cages throughout the study. Blood samples were collected on days 2, 4, 24, and 95 for hematology and clinical chemistry. Urine and clinical chemistry samples were collected from these mice on days 1, 3, 17, and 94. Other details of study design were the same as those described for the rats (NTP, 1985a), with the exception of histopathologic examination of kidney tissues, which was not performed in 50 or 100 mg/kg-day mice.

In the basic study of mice, clinical signs of treatment-related effects were minimal and apparent mainly during the first week of treatment and included ruffled fur (8/10 of the 400 mg/kg-day males, 7/10 of the 600 mg/kg-day males, 8/10 of the 600 mg/kg-day females) and hypoactivity (6/10 of the 600 mg/kg-day males). The only reported clinical sign in the supplementary groups of treated mice was that of ruffled fur in 9/10 and 6/10 of the 600 mg/kg-day males and females, respectively.

Deaths that could be attributed to bromobenzene included 5/10 and 2/10 of the 600 mg/kg-day males of the basic and supplementary studies, respectively. The original report included 1/10 and 2/10 deaths in the 400 mg/kg-day males and females, respectively, from the basic study. However, in these cases, results of histologic examinations indicated that gavage error likely contributed to the deaths. Occasional other deaths among control and treated males and females were likely the result of gavage error or anesthesia. At the end of the basic study, body weight was significantly decreased (approximately 9% lower than controls) in 400

mg/kg-day (but not 600 mg/kg-day) males. The 600 mg/kg-day males in the supplemental study exhibited approximately 12% lower terminal body weight, relative to controls. Consistent treatment-related effects on body weight were not seen in female mice. Table 4-3 presents terminal body and liver weights and serum levels of selected liver enzymes in male and female mice of the basic study. In male mice, absolute liver weight was significantly increased at dose levels ≥200 mg/kg-day, while the liver:body weight ratio was significantly increased at dose levels ≥100 mg/kg-day and the liver:brain weight ratio was significantly increased at dose levels ≥400 mg/kg-day. In female mice, all three measures of liver weight were significantly increased at all dose levels, relative to controls. The effect on absolute liver weight increased with dose, ranging from approximately 12% in the 50 mg/kg-day group to greater than 50% in the 600 mg/kg-day group. Statistically significantly increased serum SDH activity (indicative of hepatotoxicity) was observed in both sexes at dose levels ≥200 mg/kg-day, relative to sex-matched controls, but the magnitude only approached a 2-fold increase (a biologically significant level) at ≥200 mg/kg-day in males and ≥400 mg/kg-day in females. Activities of AST or ALT were not elevated in any exposed mouse group, compared with control values. Results of urinalysis and serum chemistry did not indicate clear evidence of bromobenzene-induced effects on the renal system. Hematological results were generally unremarkable.

As shown in Table 4-4, histopathologic examination revealed statistically significant effects on the liver that included cytomegaly in male and female mice at doses ≥200 mg/kg-day, necrosis and mineralization in male mice at doses of 400 and 600 mg/kg-day, and necrosis and inflammation in female mice at the 600 mg/kg-day dose level. The severity of these responses was generally greater in males than females. Cytomegaly was the most common response seen in the livers of treated mice and was characterized by an increase in the size of the nucleus and cytoplasm of individual hepatocytes. Liver necrosis was primarily coagulative in nature and was considered to be a direct result of bromobenzene treatment. Cytomegaly, inflammation, and necrosis occurred primarily in the central part of the hepatic lobules. Significantly increased incidences of hepatocellular necrosis or inflammation were observed only at doses equal to or greater than those eliciting significantly increased incidences of cytomegaly. The study authors considered inflammation and mineralization to be direct responses to hepatocellular necrosis. Based on these observations, incidences of mice with one or more of these liver lesions (cytomegaly, necrosis, inflammation, mineralization) were combined for each sex (as shown in Table 4-4).

Treatment-related statistically significantly increased incidences of renal lesions (casts, tubular degeneration without evidence of regeneration) were observed only in high-dose (600

Table 4-3. Effects of bromobenzene on terminal body and liver weights and levels of selected serum liver enzymes of male and female B6C3F1 mice exposed by oral gavage 5 days/week for 90 days in the basic study (mean +/- standard deviation)

Male mice						
Dose (mg/kg-day)	Controls	50	100	200	400	600
Number of mice	9	9	10	10	9	5
Body weight (g)	31.4 ± 2.5	33.3 ± 2.5	31.1 ± 3.1	33.4 ± 3.5	28.0[a] ± 2.0	30.5 ± 2.5
Liver weight (g) Difference (%)[b]	1.05 ± 0.14 --	1.13 ± 0.15 +7.6	1.12 ± 0.12 +6.7	1.25[a] ± 0.22 +19.1	1.27[a] ± 0.11 +21.0	1.56[a] ± 0.16 +48.6
Ratio liver/body weight Difference (%)[b]	33.4 ± 2.41 --	33.9 ± 3.52 +1.5	36.0[a] ± 1.91 +7.8	37.3[a] ± 4.48 +11.7	45.3[a] ± 1.83 +35.6	51.2[a] ± 3.48 +53.3
Serum AST (IU/L) Serum ALT (IU/L) Serum SDH (IU/L)	100 ± 33.3 144 ± 86.0 25 ± 2.5	90 ± 25.5 57[a] ± 27.5 27 ± 3.1	80 ± 11.6 80 ± 43.0 27 ± 3.2	88 ± 23.2 102 ± 61.5 41[a] ± 19.3	99 ± 17.2 132 ± 41.0 89[a] ± 28.3	70 ± 8.8 115 ± 35.8 101[a] ± 29.0
Female mice						
Dose (mg/kg-day)	Controls	50	100	200	400	600
Number of mice	10	9	9	10	8	10
Body weight (g)	22.7 ± 1.3	23.8 ± 1.1	23.7 ± 1.2	24.3[a] ± 1.0	23.4 ± 0.6	23.6 ± 0.8
Liver weight (g) Difference (%)[b]	0.86 ± 0.06 --	0.96[a] ± 0.08 +11.6	1.01[a] ± 0.08 +17.4	1.08[a] ± 0.06 +25.6	1.12[a] ± 0.07 +30.2	1.30[a] ± 0.06 +51.2
Ratio liver/body weight Difference (%)[b]	38.1 ± 1.42 --	40.2[a] ± 2.02 +5.5	42.5[a] ± 1.62 +11.6	44.4[a] ± 2.12 +16.5	48.0[a] ± 2.13 +26.0	55.2[a] ± 2.56 +44.9
Serum AST (IU/L) Serum ALT (IU/L) Serum SDH (IU/L)	130 ± 72.0 64 ± 43.5 13 ± 1.9	94 ± 27.7 39 ± 18.5 12 ± 1.6	101 ± 21.4 51 ± 28.9 14 ± 1.8	83 ± 11.3 62 ± 21.3 15[a] ± 1.7	91 ± 18.4 73 ± 31.2 23[a] ± 4.6	123 ± 55.4 126 ± 79.0 43[a] ± 18.8

[a]Statistically significantly increased from controls (p<0.05) based on Student's two-tailed t-test

[b]Change relative to controls

Source: NTP (1985b)

Table 4-4. Incidences of male and female B6C3F1 mice with liver and kidney lesions following administration of bromobenzene by gavage 5 days/week for 90 days in the basic study

Endpoint	Dose (mg/kg-day)[a]											
	0		50		100		200		400		600[b]	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Males												
Liver, centrilobular												
Inflammation	1/10	1.0	0/10		1/10	1.0	0/10		4/10	2.0	3/10	1.7
Cytomegaly	0/10		0/10		1/10	1.0	6/10[d]	1.2	4/10[d]	1.5	4/10[d]	2.3
Necrosis	0/10		0/10		0/10		1/10	1.0	4/10[d]	2.5	8/10[d]	3.5
Mineralization	0/10		0/10		0/10		0/10		8/10[d]	2.9	4/10[d]	3.8
Combined[c]	1/10		0/10		2/10		6/10[d]		10/10[d]		10/10[d]	
Kidney, tubule												
Degeneration	0/10		NE		NE		1/10	1.0	1/10	2.0	5/10[d]	2.2
Casts	0/10		NE		NE		0/10		1/10	1.0	5/10[d]	2.0
Mineralization	0/10		NE		NE		0/10		0/10		0/10	
Females												
Liver, centrilobular												
Inflammation	0/10		1/10	1.0	0/10		2/10	1.0	3/10	1.0	9/10[d]	1.6
Cytomegaly	0/10		0/10		1/10	1.0	5/10[d]	1.0	9/10[d]	1.8	10/10[d]	3.0
Necrosis	0/10		0/10		1/10	2.0	0/10		1/10	2.0	7/10[d]	1.6
Mineralization	0/10		0/10		0/10		0/10		0/10		2/10	1.5
Combined[c]	0/10		1/10		2/10		6/10[d]		9/10[d]		10/10[d]	
Kidney, tubule												
Degeneration	0/10		NE		NE		0/10		0/10		2/10	
Casts	0/10		NE		NE		0/10		0/10		2/10	
Mineralization	0/10		NE		NE		0/10		0/10		1/10	

[a]Incidence = number of animals in which lesion was found/number of animals in which organ was examined.
[b]Cytomegaly and mineralization were not diagnosed in 5 high-dose male mice that died on treatment day 1
[c]Incidences of mice with one or more of the liver lesion types (cytomegaly, necrosis, inflammation, mineralization), extracted from individual animal histopathologic results provided to Syracuse Research Corporation by NTP.
[d]Statistically significantly different from control groups according to Fisher's exact test ($p<0.05$), performed by Syracuse Research Corporation.
Severity: Average severity score: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.
NE = Not examined.
Source: NTP (1985b)

mg/kg-day) males. Sporadic lesions in other organs were not considered meaningful by the NTP Pathology Working Group (NTP, 1986a). There was no report of bromobenzene-induced gross or histopathological effects on reproductive tissues of male or female mice.

The most prominent toxicological effects observed in B6C3F1 mice treated with bromobenzene by oral gavage for 90 days (NTP, 1985b) were observed in the liver. Significantly increased incidences of hepatocellular necrosis (a clear indicator of an adverse effect) were observed at doses of 400 and 600 mg/kg-day in male mice and the 600 mg/kg-day dose level in female mice. Significantly increased incidences of cytomegaly were noted at doses $\geq$200 mg/kg-day in male and female mice. Significant increases in mean liver weight were observed at doses as low as 50 mg/kg-day in female mice and 100 mg/kg-day in male mice. Treatment-related increased occurrence of cytomegaly (i.e., hypertrophy) and increased liver weight may provide indication of liver toxicity from higher levels of exposure, but the toxicological significance of these effects by themselves is questionable.

In the NTP (1985b) study the LOAEL is considered to be 50 mg/kg-day in female mice for statistically significant increased absolute liver weight and increased liver-to-body weight ratios. The designation of increased absolute liver weight and increased liver-to-body weight ratios as an adverse effect is supported by the presence of liver lesions (including inflammation, cytomegaly and necrosis) and statistically significantly increased SDH values at higher dose levels. The increased serum enzyme (SDH) levels are indicative of liver damage.

Popper et al. (1952) investigated the hepatotoxic effects of subchronic dietary bromobenzene exposure in rats. Control (n=9) and test (n=8) groups of female Wistar rats were fed for 8 weeks on a synthetic diet that, in the test group, was supplemented with 5% (50,000 ppm) bromobenzene [corresponding to a dose of approximately 5130 mg/kg-day, calculated using reference values for food consumption and body weight from U.S. EPA (1988)]. Histologic examination of the liver revealed mild changes, including distortion of the liver cell plates and clumping and hydropic swelling in the cytoplasm of peripheral zone hepatocytes. Alkaline phosphatase activity was markedly increased in both the liver and the serum. In addition, liver and serum esterase levels were significantly decreased and serum xanthine oxidase activity was increased (albeit not significantly). No other endpoints were monitored.

4.2.1.2. Chronic Toxicity

No studies were located on health effects in animals following chronic oral exposure to bromobenzene.

4.2.2. Inhalation Exposure

4.2.2.1. Subchronic Toxicity

NTP conducted subchronic inhalation studies of bromobenzene in rats (NTP, 1985c) and mice (NTP, 1985d). These studies have not been officially released by NTP, but unpublished reports, including the review comments and conclusions of NTP's Pathology Working Group (NTP, 1986b), were obtained from NTP. The unpublished NTP studies are available by calling EPA's IRIS Hotline at (202)566-1676, by fax at (202)566-1749 or by email at iris@epa.gov.

Groups of 10 male and 10 female Fischer 344/N rats were exposed to bromobenzene vapors through whole body exposure at 0, 10, 30, 100, or 300 ppm (0, 64.2, 192.6, 642, or 1926 mg/m^3) for 6 hours/day, 5 days/week for 13 weeks. Rats were observed twice daily for morbidity and mortality. Clinical observations and body weight measurements were performed weekly. Blood samples for hematologic examination (erythrocyte and leukocyte counts; hemoglobin concentrations; red blood cell indices of MCV, MCH, and MCHC; leukocyte differential counts) were collected from all surviving rats at terminal sacrifice. Terminal body and organ (liver, brain, testis, kidney, lung, heart, and thymus) weights were recorded; organ-to-body weight and organ-to-brain weight ratios were calculated for each sex. Complete gross necropsy was performed on all rats. Complete histopathologic examinations of all major tissues and organs (including liver, kidney, urinary bladder, spleen, pancreas, brain, spinal cord [if neurologic signs were present], heart, lung, trachea, nasal cavity, larynx, esophagus, stomach, small intestine, cecum, colon, skin, uterus, ovaries, preputial or clitoral glands, testes, prostate, sternebrae, adrenals, pituitary, thyroid, parathyroids, salivary gland, mandibular lymph node, thymus, mammary gland, blood, and gross lesions, and tissue masses) were performed on all control rats and all rats from the 300-ppm groups. Kidney tissue was examined histopathologically in all male rats of the lower exposure concentrations (10, 30, and 100 ppm).

No mortality was observed during the study. Clinical signs were unremarkable except for tearing, facial grooming, and listlessness in 300-ppm rats on the first day of exposure. Terminal body weights did not differ significantly from controls. Liver and kidney weights (absolute, relative-to-body weight, and relative-to-brain weight) were significantly increased at concentrations $\geq$100 ppm in both sexes. Liver and kidney weight data are reported in Table 4-5. In males, absolute liver weights increased 13% at 100 ppm and 20% at 300 ppm. In females, absolute liver weights increased 12% at 100 ppm and 22% at 300 ppm. MCH and MCV were statistically significantly decreased in males at concentrations $\geq$10 ppm and in females at 300 ppm, but the changes were small and considered not to be biologically significant. There was no histopathological evidence of bromobenzene-induced liver lesions, although livers were examined only from control rats and rats of the highest exposure level (100 ppm in males and 300 ppm in females) (see Table 4-6).

Table 4-5. Effects of bromobenzene on terminal body, liver, and kidney weights of male and female rats exposed by inhalation 6 hours/day, 5 days/week for 13 weeks (mean +/- standard deviation)

Male rats					
Exposure concentration (ppm)	Controls	10	30	100	300
Number of rats	10	10	10	10	10
Body weight (g)	318 ± 15.5	322.9 ± 14.2	331.1 ± 18.2	312.4 ± 39.1	309.4 ± 18.3
Liver weight (g) Difference (%)[a]	11.58 ± 1.18	12.04 ± 0.4 + 4%	12.13 ± 0.77 + 5%	13.13[b] ± 1.59 + 13%	14.33[c] ± 1.67 + 20%
Ratio liver/body weight x 1000 Difference (%)[a]	33.37 ± 2.86	37.31 ± 1.96 + 10.5%	36.68 ± 2.05 + 9%	42.11[c] ± 2.09 + 21%	46.26[c] ± 3.86 + 28%
Right kidney weight	0.98 ± 0.06	1.04 ± 0.05	1.87 ± 0.05	1.07[b] ± 0.11	1.11[c] ± 0.09
Ratio right kidney/body weight x 1000 Difference (%)[a]	3.09 ±0.06	3.22 ± 0.17 + 4%	3.16 ± 0.16 + 2%	3.43[c] ± 0.19 + 10%	3.60[c] ± 0.11 + 14%
Female rats					
Exposure concentration (ppm)	Controls	10	30	100	300
Number of rats	10	10	10	10	10
Body weight (g)	186.0 ± 11.2	191.4 ± 10.5	182.8 ± 9.1	187.7 ± 8.3	189.9 ± 11.6
Liver weight (g) Difference (%)[a]	6.36 ± 0.65	6.71 ± 0.55 + 7%	6.52 ± 0.60 + 4%	7.23[c] ± 0.30 + 12%	8.22[c] ± 0.63 + 23%
Ratio liver/body weight x 1000 Difference (%)[a]	34.12 ± 1.83	35.05 ± 1.82 + 3%	35.68 ± 2.84 + 4%	38.56[c] ± 1.62 12%	43.54[c] ± 2.53 22%
Right kidney weight	0.62 ± 0.05	0.65 ± 0.03	0.66 ± 0.06	0.66[b] ± 0.03	0.70[c] ± 0.05
Ratio kidney/body weight x 1000 Difference (%)[a]	3.31 ± 0.21	3.39 ± 0.09 + 2%	3.62[b] ± 0.26 + 9%	3.53[b] ± 0.18 + 6%	3.73[c] ± 0.16 + 11%

[a]Change relative to controls

[b]Statistically significantly different from controls ($p<0.05$) based on student's two-tailed t-test

[c]Outside 3 standard deviations from the control mean

Source: NTP (1985d)

Table 4-6. Incidences of male and female Fischer 344/N rats with liver and kidney lesions following repeated exposure to bromobenzene vapors for 13 weeks

Endpoint	Exposure concentration*									
	0		10		30		100		300	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Males										
Liver			NE		NE		NE			
Necrosis	1/10	1.0							0/10	
Inflammation	0/10								0/10	
Kidney, tubule										
Regeneration	10/10	1.0	10/10	1.0	9/10	1.0	10/10	0.9	10/10	1.9
Females										
Liver			NE		NE		NE			
Necrosis	1/10	1							0/10	
Inflammation	2/10	1							3/10	1
Kidney, tubule			NE		NE		NE			
Regeneration	0/10								0/10	

*Incidence = number of animals in which lesion was found/number of animals in which organ was examined

Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe. NE = Not examined.

Source: NTP (1985c)

Histopathologic examination of the kidneys revealed renal cortical tubular regeneration, characterized by basophilic (regenerative) tubules scattered throughout the renal cortex of all control and bromobenzene-exposed male rats (with the exception of a single male in the 30-ppm exposure group; see Table 4-6). The renal tubular regeneration was observed in the absence of convincing evidence of degeneration or necrosis. NTP (1985c) noted that the severity of nephropathy in 300-ppm males could be distinguished from that of controls in blind evaluations. These findings were confirmed upon re-examination of kidney tissues from control and 300-ppm male mice by the Pathology Working Group (NTP, 1986b). The Working Group considered the effect to be mild and not life threatening.

Gross and histopathologic examinations of reproductive tissues of male and female rats did not reveal evidence of bromobenzene-induced effects. No significant treatment-related lesions were found in gross or histopathologic examinations of other tissues in female rats.

Since increased liver weight at the 100 ppm and 300 ppm dose groups in the NTP (1985c) study were not accompanied by bromobenzene induced liver lesions these effects were considered to be of questionable toxicological significance and not considered to be a LOAEL; therefore, the highest dose level tested (300 ppm) is considered to be a NOAEL in this study.

In the mouse study, groups of 10 male and 10 female B6C3F1 mice were exposed to 0, 10, 30, 100, or 300 ppm (females only) and (0, 64.2, 192.6, 642, or 1926 mg/m^3) of bromobenzene 6 hours/day, 5 days/week for 13 weeks (NTP, 1985d). No rationale for excluding a 300-ppm exposure level for the male mice was included in the available study report. Clinical observations and body weight measurements were performed weekly. Blood samples for hematologic examination (erythrocyte and leukocyte counts; hemoglobin concentrations; red blood cell indices of MCV, MCH, and MCHC; leukocyte differential counts) were collected from all surviving mice at terminal sacrifice. Terminal body and organ (liver, brain, testis, kidney, lung, heart, and thymus) weights were recorded; organ-to-body weight and organ-to-brain weight ratios were calculated for each sex. Complete gross necropsy was performed on all mice. Histopathologic examinations of all major tissues and organs (including liver, kidney, urinary bladder, spleen, pancreas, gall bladder, brain, spinal cord [if neurologic signs were present], heart, lung, trachea, nasal cavity, larynx, esophagus, stomach, small intestine, cecum, colon, skin, uterus, ovaries, preputial or clitoral glands, testes, prostate, sternebrae, adrenals, pituitary, thyroid, parathyroids, salivary gland, mandibular lymph node, thymus, mammary gland, blood, gross lesions, and tissue masses) were performed on all control, 100-ppm male and 300-ppm female mice. Liver and kidney tissues were examined histopathologically in all other groups of mice.

There were no deaths during this study and no clinical signs of toxicity were observed. Terminal body weights of treated groups did not differ significantly from controls. In female

mice, liver weights (absolute, relative to body weight, relative to brain weight) were statistically significantly increased in an exposure concentration-related manner. Absolute liver weights were increased approximately 8, 17, and 66% at 30, 100, and 300 ppm, respectively. Liver-to-body weight ratios were increased approximately 6, 5, 14, and 53% at 10, 30, 100, and 300 ppm, respectively. Smaller increases in these parameters were also seen in 100-ppm males. Liver and kidney weight data are reported in Table 4-7. Sporadic changes in hematology parameters, observed in male and female mice of most exposure groups, were not considered to be biologically significant. Females of the 300 ppm exposure level exhibited enlarged, diffusely mottled livers.

Incidences of histopathologic liver lesions are summarized in Table 4-8. In the original study report, histopathologic evidence of hepatic effects was presented. Cytomegaly was diagnosed in the liver of 4/10 and 2/10 male mice of the 30- and 100-ppm exposure groups, respectively, as well as 2/10 and 10/10 female mice of the respective 100- and 300-ppm exposure groups. The Pathology Working Group agreed with the diagnoses of cytomegaly, hepatic necrosis, and mineralization in the 300-ppm female mice, but did not consider observed liver effects to be adverse in female mice at lower exposure levels (NTP, 1986b). Furthermore, the Pathology Working Group considered the reported cytomegaly in 100-ppm male mice to be more appropriately described as centrilobular hepatocellular hypertrophy or enlargement and to be less severe than cytomegaly observed in the female mice (NTP, 1986b). The associated effect in 30-ppm males was not considered by the Pathology Working Group to be indicative of centrilobular hypertrophy, but it was noted that some increased eosinophilic staining of centrilobular hepatocytes suggested an effect typical of hepatocellular enzyme induction.

The NTP study report (NTP, 1985d) also presented histopathological evidence for renal lesions (see Table 4-8). The kidneys of 2/10 and 3/10 of the 30- and 100-ppm male mice exhibited evidence of minimal tubular degeneration, but the Pathology Working Group did not consider this finding to represent an adverse effect since it may have been the result of artifacts of fixation and staining procedures (NTP, 1986b). Gross and histopathologic examinations of reproductive tissues of male and female mice did not reveal evidence of bromobenzene-induced effects.

In the NTP (1985d) inhalation study in mice, the highest dose tested, 300 ppm, is considered to be a LOAEL (Lowest Observed Adverse Effect Level). The 100 ppm dose is considered to be a NOAEL because the increases in absolute liver weight and increases in cytomegally were not considered to be adverse by the Pathology Working Group at exposure levels below 300 ppm. Treatment-related significantly increased liver weights were seen in all exposure groups of female mice, and a significantly increased incidence of cytomegaly was observed in the 300 ppm female mice. Treatment-related increased occurrence of cytomegaly

Table 4-7. Effects of bromobenzene on terminal body, liver, and kidney weights of male and female mice exposed by inhalation 6 hours/day, 5 days/week for 13 weeks (mean +/- standard deviation)

Male mice					
Exposure concentration (ppm)	Controls	10	30	100	300
Number of mice	10	10	10	10	
Body weight (g)	36.3 ± 3.6	33.4 ± 2.0	33.6 ± 3.0	34.4 ± 3.2	
Liver weight (g) Difference (%)[a]	1.84 ± 0.21	1.73 ± 0.14 -6.0	1.73 ± 0.18 -6.0	1.87 ± 0.21 +1.6	
Ratio liver/body weight x 1000 Difference (%)[a]	50.71 ± 3.66	51.86 ± 3.57 +2.2	51.57 ± 2.78 +1.7	54.28[b] ± 2.42 +7.0	
Right kidney weight	0.29 ± 0.02	0.30 ± 0.03	0.30 ± 0.02	0.30 ± 0.02	
Ratio right kidney/body weight x 1000 Difference (%)[a]	8.13 ± 0.66	8.84 ± 0.86 +8.7	8.88[b] ± 0.75 +9.2	8.78 ± 0.90 +8.0	
Female mice					
Exposure concentration (ppm)	Controls	10	30	100	300
Number of mice	10	10	10	10	10
Body weight (g)	27.4 ± 1.4	27.5 ± 1.3	28.3 ± 1.7	28.3 ± 0.9	29.7[c] ± 1.7
Liver weight (g) Difference (%)[a]	1.43 ± 0.15	1.52 ± 0.09 +6.3	1.54[b] ± 0.07 +7.7	1.68[c] ± 0.10 +17.5	2.37[c] ± 0.21 +65.7
Ratio liver/body weight x 1000 Difference (%)[a]	52.0 ± 3.22	55.25[b] ± 3.49 +6.3	54.66[b] ± 1.80 +5.1	59.37[c] ± 3.43 +14.2	79.73[c] ± 5.27 +53.3
Right kidney weight	0.19 ± 0.01	0.20[c] ± 0.01	0.20 ± 0.02	0.20[c] ± 0.01	0.23[c] ± 0.02
Ratio kidney/body weight x 1000 Difference (%)[a]	6.80 ± 0.28	7.38[c] ± 0.25 +8.5	7.04 ± 0.51 +3.5	7.14 ± 0.32 +5.0	7.64 ± 0.45 +12.4

[a]Change relative to controls
[b]Statistically significantly different from controls (p<0.05) based on Student's two-tailed t-test
[c]Outside 3 standard deviations from the control mean
Source: NTP (1985d)

Table 4-8. Incidences of male and female B6C3F1 mice with liver and kidney lesions following repeated exposure to bromobenzene vapors for 13 weeks

Endpoint	Exposure concentration[a]									
	0		10		30		100		300	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Males										
Liver									NG	
Cytomegaly[b]	0/10		0/10		4/10[c]	2.0	2/10	1.5		
Necrosis	0/10		0/10		0/10		2/10	1.0		
Inflammation	1/10	3.0	0/10		0/10		4/10	1.8		
Kidney, tubule									NG	
Degeneration[d]	0/10		0/10		2/10	1.5	3/10	2.0		
Females										
Liver										
Cytomegaly	0/10		0/10		0/10		2/10	1.0	10/10[c]	3.2
Necrosis	2/10	1.0	1/10	1.0	0/10		2/10	1.0	5/10	1.3
Inflammation	4/10	1.5	3/10	1.3	2/10	1.0	2/10	1.5	2/10	1.3
Mineralization[e]	0/10		0/10		0/10		0/10		2/10	2.0
Kidney[f]										

[a]Incidence = number of animals in which lesion was found/number of animals in which organ was examined. Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe. NG = No group (the study did not include a 300 ppm exposure group of male mice)

[b] The Pathology Working Group (NTP, 1986b) considered this diagnosis in 100-ppm male mice to be more appropriately described as centrilobular hepatocellular hypertrophy or enlargement and the results in 30-ppm male mice to be suggestive of hepatocellular enzyme induction, rather than cytomegaly as noted in female mice.

[c]Statistically significantly different from control groups according to Fisher's exact test ($p<0.05$), performed by Syracuse Research Corporation.

[d]Kidney tubular degeneration could not be distinguished from artifacts of fixation or staining.

[e]Mineralization was not reported in male mice.

[f]No histopathologic renal lesions were identified in any group of female mice.

Source: NTP (1985d)

and increased liver weight may provide an early indication of liver toxicity from higher level exposure. Hepatocyte necrosis was noted in 5/10 of the 300-ppm female mice, but the incidence of this lesion was not significantly greater than the incidence in controls (2/10). The 300-ppm exposure level may represent an effect level in female mice that is near the threshold for bromobenzene hepatotoxicity.

Shamilov (1969) exposed rats to 3 or 20 $\mu g/m^3$ of bromobenzene 4 hours daily for 140 days. At 20 $\mu g/m^3$, bromobenzene gradually accumulated in the tissues, producing decreases in body growth, liver sulfhydryl concentration, serum protein levels and leukocyte, platelet, and reticulocyte counts as well as neurological disorders. No effects were seen at 3 $\mu g/m^3$. More detailed study information was not presented in the available abstract thus precluding critical assessment of the study.

4.2.2.2. Chronic Toxicity

No studies were located on health effects in animals following chronic inhalation exposure to bromobenzene.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Reproductive Toxicity Studies

No reproductive toxicity studies were located for bromobenzene.

4.3.2. Developmental Toxicity Studies

No developmental toxicity studies were located for bromobenzene.

4.4. OTHER STUDIES

4.4.1. Acute Toxicity Studies

The toxic effects of bromobenzene following acute exposure have been extensively studied. Liver, kidney, and lung have been identified as the target organs for this chemical by a variety of routes. Histopathologic examinations have revealed necrotic changes in all of these organs following short-term bromobenzene exposure (Szymańska and Piotrowski, 2000; Szymańska, 1998; Becher et al., 1989; Casini et al., 1986; Forkert, 1985; Rush et al., 1984; Kluwe et al., 1984; Roth, 1981; Reid et al., 1973; Patrick and Kennedy, 1964).

The liver is the most sensitive target following acute oral exposure. In rats given single oral doses of bromobenzene by gavage, a dose of 39 mg/kg resulted in reduced hepatic glutathione; a higher dose (157 mg/kg-day) resulted in moderate periportal and midzonal hydropic changes, while increased serum liver enzyme levels and hepatic centrilobular necrosis were observed following dosing at 314 mg/kg-day (Kluwe et al., 1984). In the same study, renal

glutathione was reduced at a dose of 157 mg/kg-day, but no other renal effects were noted at doses up to 628 mg/kg-day. Other acute oral studies reported hepatic necrosis in rats (Heijne et al., 2004) or mice (Patrick and Kennedy, 1964) administered bromobenzene at doses in the range of 500-700 mg/kg; reduced renal glutathione levels, increased BUN levels, and severe tubular necrosis in mice given 2355 mg/kg-day (Casini et al., 1986); extensive vacuolization and necrosis in Clara cells in the lungs of mice given 785 mg/kg-day (Forkert, 1985); and increased LDH levels in lung lavage fluid accompanied by bronchiolar damage in the lungs of mice given 2355 mg/kg-day (Casini et al., 1986).

When rats were exposed to a bromobenzene vapor concentration of 107 ppm for 4 hours, serum liver enzyme changes were noted (Brondeau et al., 1983). Extrahepatic effects observed in other acute inhalation studies included pulmonary effects, seen as moderate vacuolization of pulmonary Clara cells in mice exposed to 250 ppm for 4 hours (Becher et al., 1989) and pulmonary necrosis in mice exposed to 1000 ppm for 4 hours (Becher et al., 1989).

4.4.2. Genotoxicity Studies

Table 4-9 summarizes available results of genotoxicity tests for bromobenzene. Results of gene mutation assay systems did not indicate a mutagenic response in several strains of *Salmonella typhimurium* at bromobenzene concentrations as high as 500 μg/plate with or without S-9 activation (Nakamura et al., 1987; Rosenkranz and Poirier, 1979; Simmon, 1979; Simmon et al., 1979; McCann et al., 1975). Bromobenzene was not mutagenic in an *in vivo* test for nondisjunction in *Drosophila* (Ramel and Magnusson, 1979). Bromobenzene did not induce sister chromatid exchanges in Chinese hamster ovary cells (Galloway et al., 1987) or cell transformation in Syrian hamster embryo cells (Pienta et al., 1977). A weakly positive result was reported for bromobenzene-induced chromosomal aberrations in Chinese hamster ovary cells in the absence, but not the presence, of metabolic S-9 activation (Galloway et al., 1987).

Bromobenzene was observed to increase formation of micronucleated erythrocytes, in femoral polychromatic mouse bone marrow cells *in vivo* (Mohtashamipur et al., 1987) and actively bind to rat and mouse DNA, RNA, and proteins both *in vivo* and *in vitro* (Prodi et al., 1986; Colacci et al., 1985). Following intraperitoneal injection of ^{14}C-bromobenzene (6.35 μmol/kg; lower than a minimally hepatotoxic dose) in rats and mice, the degree of binding in liver, kidney, and lung tissues of both species was RNA > proteins > DNA (Colacci et al., 1985). Mouse kidney exhibited a much greater degree of binding to macromolecules than rat kidney. In both rats and mice, the relative order of binding to DNA in the various organs was liver > kidney > lung. Bromobenzene was second only to 1,2-dibromoethane in its relative *in vivo* reactivity with rat liver DNA, exhibiting higher reactivity than 1,2-dichloroethane, chlorobenzene, epichlorohydrin, and benzene (Prodi et al., 1986). Microsomal enzyme-catalyzed the *in vitro*

Table 4-9. Results of bromobenzene genotoxicity testing

Assay and test system	Dose/ concentration	HID or LED*	Result	Reference
Reverse mutation in *S. typhimurium* strains TA1535, TA1537, TA98, TA100	NS + S9 activation	NS	Negative	McCann et al., 1975
Reverse mutation in *S. typhimurium* strains TA1535, TA1538	10 μg/plate ± S9 activation	10	Negative	Rosenkranz and Poirier, 1979
Reverse mutation in *S. typhimurium* strains TA1535, TA1536, TA1537, TA1538, TA98, TA100	250 μg/plate ± S9 activation	250	Negative	Simmon, 1979
Reverse mutation in *S. typhimurium* strains TA1530, TA1538 (host-mediated assay using mice)	600 mg/kg-day	600	Negative	Simmon et al., 1979
Reverse mutation in *S. typhimurium* strains TA1535, TA1538 (host-mediated assay using mice)	1000 mg/kg-day	1000	Negative	Simmon et al., 1979
SOS-response in *S. typhimurium* strain TA1535/pSK1002	Up to 500 μg/mL ± S9 activation	500	Negative	Nakamura et al., 1987
Nondisjunction in *Drosophila*	1000 ppm	1000	Negative	Ramel and Magnusson, 1979
Sister chromatid exchanges in Chinese hamster ovary cells (CHO-W-B1)	50–500 μg/mL ± S9 activation	500	Negative	Galloway et al., 1987
Cell transformation in Syrian hamster embryo cells	0.0001–0.5 μg/mL	0.5	Negative	Pienta et al., 1977
Chromosomal aberrations in Chinese hamster ovary cells (CHO-W-B1)	50–500 μg/mL ± S9 activation	500	Weakly positive -S9, negative +S9	Galloway et al., 1987
Micronuclei in mouse (NMRI) bone marrow cells	125–500 mg/kg-day (2x62.5–2x250 doses 24 hours apart)	125	Positive	Mohtashamipur et al., 1987
DNA binding in rat and mouse (*in vivo*)	6.35 μmol/kg (intraperitoneal)	6.35	Positive, rat and mouse liver, mouse kidney	Colacci et al., 1985; Prodi et al., 1986
RNA binding in rat and mouse (*in vivo*)	6.35 μmol/kg (intraperitoneal)	6.35	Positive, rat and mouse liver, kidney, and lung	Colacci et al., 1985; Prodi et al., 1986

*HID, highest ineffective dose/concentration for negative tests; LED, lowest effective dose/concentration for positive tests; NS, not stated

binding of ^{14}C-bromobenzene to rat and liver DNA; liver microsomes of mice appeared to be slightly more efficient than those of rats (Colacci et al., 1985). The degree of *in vitro* binding in liver, kidney, and lung tissues of both species was RNA > proteins > DNA. In both rat and mouse microsomal preparations, the relative order of binding to macromolecules was liver > lung > kidney.

Reactive metabolites of bromobenzene are produced *in vivo* as discussed in Section 3.3 and could be expected to interact with DNA. The central pathway for the mammalian metabolism of bromobenzene appears to be the production of bromocatechols via bromophenols, as depicted in Figure 3-1 (Lertratanangkoon et al., 1993; Lau and Monks, 1988). Although reactive metabolites, 2,3-oxide and 3,4-oxide, are formed as precursors in the predominant pathway in bromobenzene's metabolism, 2,3-oxide has a very short biological half-life, indicating spontaneous rearrangement to the formation of 2-bromophenol in the rat and pig (Lertratanangkoon et al., 1993). Another reactive intermediate, 2,3-dihydrodiol, also rapidly rearranges to form both 2-bromophenol and 3-bromophenol in the detoxification bromocatechol pathway (Lertratanangkoon et al., 1987). Furthermore, spontaneous rearrangement of the 3,4-dihydrodiol is considered to be the major pathway in bromobenzene's metabolism, leading to the formation of 4-bromophenol in the rat, while a pathway leading through an S-glutathione conjugate to 4-bromophenol is predominant in the guinea pig (Lertratanangkoon et al., 1987, 1993). The bromophenols are subsequently oxidized by CYP to their respective bromocatechols in a detoxification pathway (Miller et al., 1990; Lau and Monks, 1988). While these toxicokinetic events are expected to elicit a toxicity response from liver tissue, the reactive metabolites generated in the process may be too transient and reactive to elicit measurable responses in Salmonella mutagenicity assays and other genotoxicity assays involving external rat liver S-9 metabolic activation.

In conclusion, the available data from bacterial mutagenicity assays were predominately negative however, clastogenic and mutagenic results in mammalian cell cultures and whole animals studies were positive. Bromobenzene was not mutagenic in the Ames assay and did not consistently produce marked cytogenic effects *in vitro* with mammalian cells, even in the presence of rat liver S-9 preparations. Bromobenzene increased formation of micronucleated polychromatic erythrocytes in bone marrow of mice given acute oral doses of 125 mg/kg and was bound to DNA and RNA following intraperitoneal injection. Results of *in vivo* testing of DNA binding in rat and mouse liver indicate that bromobenzene is greater than 20-fold more reactive to rat liver DNA than benzene (Prodi et al., 1986), the nonhalogenated parental compound known to be carcinogenic and considered a weak tumor initiator. Whereas the extent of DNA binding was similar in other tissues examined such as lung and kidney. However,

bromobenzene has not been tested in tumor initiation assays or long-term carcinogenicity bioassays.

4.4.3. Tumor Promotion Studies

The potential for bromobenzene to promote diethylnitrosamine (DENA)-initiated rat liver foci was investigated in two rat liver assays. Herren-Freund and Pereira (1986) dosed male and female Sprague-Dawley rats by gavage (0.5 mmol/kg of DENA), followed by intraperitoneal injection of bromobenzene (1.0 mmol/kg), 1 and 5 weeks after DENA administration. The rats were sacrificed 2 weeks after the last injection of bromobenzene. Treatment with bromobenzene did not enhance the occurrence of γ-glutamyltranspeptidase-positive (GGT) foci in the liver. Ito et al. (1988) administered a single intraperitoneal injection of DENA to male Fischer rats to initiate hepatocarcinogenesis. Some of these rats were administered bromobenzene (15 mg/kg-day) by intraperitoneal injections (eight injections, initiated 2 weeks following DENA treatment and ending before sacrifice at 8 weeks post-DENA administration). All rats were subjected to 2/3 partial hepatectomy at 3 weeks to maximize any interaction between proliferation and effects of test compound. The number and area per cm^2 of induced glutathione S-transferase placental form-positive ($GST\text{-}P^+$) foci in the liver of bromobenzene-treated rats was assessed and compared with those receiving DENA only. Bromobenzene treatment did not result in statistically significant increases in the number or area per cm^2 of DENA-induced $GST\text{-}P^+$ foci.

4.5. MECHANISTIC STUDIES

4.5.1. Mechanistic Studies of Liver Effects

As discussed in Sections 4.2 and 4.4, animal studies identify the liver as the most sensitive toxicity target of oral or inhalation exposure to bromobenzene. As discussed in detail below, the results of numerous mechanistic studies in animals collectively demonstrate that bromobenzene hepatotoxicity is associated with metabolism of parent compound, cytotoxicity may result from modifications of hepatocellular macromolecules by one or more reactive metabolites, and that these reactive metabolites are formed primarily via the metabolic pathway that involves the 3,4-oxide (rather than the 2,3-oxide) derivative of bromobenzene (see Slaughter and Hanzlik, 1991; Monks et al., 1984a; Jollow et al., 1974; Mitchell et al., 1971). Nephrotoxicity has also been observed in animals following acute-duration exposure to bromobenzene, albeit at higher doses than the lowest hepatotoxic doses. Repeated-dose oral and inhalation studies in rats and mice provide evidence for kidney effects, but only at the highest exposure levels tested, which also resulted in lethality. Nephrotoxicity also appears to result

from modification of macromolecules in cells of the proximal convoluted tubule by one or more reactive metabolites (Reid, 1973).

To demonstrate that hepatotoxic effects are elicited by metabolites of bromobenzene and not bromobenzene itself, one group of rats was administered single intraperitoneal doses (1500 mg/kg-day) of bromobenzene, while another group was administered β-diethylaminoethyl diphenylpropyl acetate (SKF 525A, a CYP inhibitor) before and after administration of the same intraperitoneal dose (1.5 mg/kg-day) of bromobenzene (Mitchell et al., 1971). As shown in Table 4-10, extensive centrilobular necrosis was observed in the group of bromobenzene-treated rats examined 24 hours following dosing. However, the CYP-inhibited rats exhibited no clear signs of the liver lesion, although concentrations of parent compound in plasma and liver of the CYP-inhibited rats were five to six times higher than those in the group not treated with the CYP-inhibitor.

Table 4-10. The effect of CYP inhibition on the hepatotoxicity and metabolism of single intraperitoneal doses of bromobenzene

Treatment	Severity of hepatic centrilobular necrosis	24-Hour bromobenzene concentration	
		Plasma (μg/mL)	Liver (μg/g)
Bromobenzene (1500 mg/kg-day)	Extensive	2.8 ± 0.3*	26 ± 3
Bromobenzene (1500 mg/kg-day) + SKF 525A	No specific lesions	14.4 ± 0.5	149 ± 8

*Mean ± standard error from 5-7 rats/group; CYP = cytochrome P-450 isozymes; SKF 525 = β-diethylaminoethyl diphenylpropyl acetate
Source: Mitchell et al. (1971)

Chemically reactive metabolites of bromobenzene may damage cellular macromolecules, leading to cytotoxicity. These metabolites include the 2,3- and 3,4-oxides of bromobenzene, the oxides of the bromophenols, the 1,4-benzoquinone, and the radicals and quinones derived from redox cycling of the 2- and 4-bromocatechols (Slaughter and Hanzlik, 1991; Lau and Monks, 1988). The 3,4-epoxide binds covalently to microsomal protein at the site of synthesis while the 2,3- epoxide binds to the soluble protein, i.e., hemoglobin β chain (Lau and Zannoni, 1981b). The bromobenzene 3,4-oxide alkylates histidine and lysine side chains in rat liver proteins *in vivo* (Bambal and Hanzlik, 1995). Phenolic metabolites of bromobenzene are activated to toxic metabolites, which deplete cellular glutathione and have caused cell death in isolated hepatocytes (Lau and Monks, 1997a). Hydroquinone metabolites of bromobenzene have been indicated as subcellular targets of nephrotoxicity in the rat, causing changes in proximal tubular brush border,

nuclei, and endoplasmic reticulum (Lau and Monks, 1997b). Slaughter et al. (1993) demonstrated that bromobenzene-derived oxides, quinones, and bromoquinones are capable of alkylating protein sulfhydryl groups, the major adduct arising from the 1,4-benzoquinone electrophilic metabolite. Quinone-derived protein adducts appear to be formed to a greater extent than those derived from the epoxides (Bambal and Hanzlik, 1995; Slaughter and Hanzlik, 1991). Several liver proteins have been identified as targets for reactive metabolites of bromobenzene (Koen and Hanzlik, 2002; Koen et al., 2000; Rombach and Hanzlik, 1997, 1998, 1999; Aniya et al., 1988). While electrophilic metabolites of bromobenzene have the ability to interact with tissue macromolecules, a causal role for this binding in hepatotoxicity has yet to be demonstrated (Koen and Hanzlik, 2002; Lau and Monks, 1997a).

Results of mechanistic studies further indicate that hepatotoxicity is primarily elicited via the metabolic pathway that involves the 3,4-oxide derivative of bromobenzene, and that the toxic effect is likely mediated via covalent binding of one or more reactive metabolites with hepatocellular macromolecules (Monks et al., 1984a; Jollow et al., 1974; Reid and Krishna, 1973; Zampaglione et al., 1973; Brodie et al., 1971). Supporting evidence includes the findings that: (1) induction of β-naphthoflavone- or 3-methylcholanthrene-induced CYP isozymes (possibly cytochrome P-488) results in increased urinary excretion of 2-bromophenol (formed via the 2,3-oxide pathway) and decreased hepatotoxicity (Lau et al., 1980; Lau and Zannoni, 1979; Jollow et al., 1974; Zampaglione et al., 1973), whereas (2) induction of phenobarbital-induced CYP isozymes results in increased urinary excretion of 4-bromophenol (formed via the 3,4-oxide pathway), as well as increases in both severity of hepatocellular necrosis and the extent of covalent binding of radioactivity from ^{14}C-bromobenzene to hepatocellular macromolecules in the region of observed hepatocellular necrosis (Brodie et al., 1971).

The importance of glutathione conjugation as a protective mechanism for bromobenzene acute hepatotoxicity was demonstrated in male Sprague-Dawley rats that were administered a single intraperitoneal dose of ^{14}C-bromobenzene (1570 mg/kg; 236 mg/kg in phenobarbital-pretreated rats) (Jollow et al., 1974). Selected groups of these rats were additionally treated with either phenobarbital (a known CYP inducer), SKF 525A (a known CYP inhibitor), diethyl maleate (which depletes glutathione), or cysteine (a precursor of glutathione). Selected rats from each group were periodically sacrificed during 48 hours following bromobenzene treatment in order to determine rates of liver glutathione depletion. Bromobenzene metabolism was associated with clearance of radioactivity from the whole body over time. All groups of rats were assessed for the severity of centrilobular necrosis. Results are summarized in Table 4-11. Bromobenzene treatment alone resulted in minimal signs of necrosis. In contrast, rats that had been pretreated with phenobarbital exhibited massive necrotic areas, as well as statistically significant (p<0.05) increases in bromobenzene metabolism and rate of glutathione depletion

from the liver. CYP-inhibition (by SKF 525A) significantly retarded bromobenzene metabolism and reduced the rate of glutathione depletion; these rats exhibited no histopathologic signs of

Table 4-11. The influence of various treatments on the metabolism of bromobenzene (BB) and severity of bromobenzene-induced hepatic necrosis in rats administered a single intraperitoneal dose of bromobenzene

Treatment	Severity[a] of centrilobular liver necrosis	Metabolism of bromobenzene (t1/2 in minutes)[b]	Rate of glutathione depletion (t1/2 in minutes)
BB (1570 mg/kg)	Minimal	10.0 ± 0.8	66 ± 8
BB (236 mg/kg) + Phenobarbital	Massive	5.5 ± 0.5[c]	20 ± 3[c]
BB (1570 mg/kg) + SKF 525A	None	15.5 ± 1.8[c]	230 ± 15[c]
BB (1570 mg/kg) + Diethyl maleate	Extensive	10.2 ± 0.7	17 ± 3[c]
BB (1570 mg/kg) + Cysteine	None	9.8 ± 0.8	68 ± 6

[a]Criteria of Brodie et al. (1971) (minimal = a few degenerating parenchymal cells; extensive = central veins surrounded by several rows of dead or degenerating cells; massive = necrosis of extensive liver areas).
[b]Half-time of clearance of radioactivity from the whole body of rats administered ^{14}C-bromobenzene.
[c]Significantly different from the values of rats treated with bromobenzene only; $p<0.05$.
Source: Jollow et al. (1974)

hepatocellular necrosis. The experimental depletion of liver glutathione in the diethyl maleate-treated rats resulted in increased severity of necrosis even though the rate of bromobenzene metabolism was not significantly different from that of rats that were not depleted of glutathione experimentally. Conversely, addition of the glutathione precursor (cysteine) was protective of liver necrosis. Not only do the results demonstrate that metabolism of bromobenzene is correlated with hepatotoxicity, since CYP-induction (phenobarbital-treated rats) increased hepatotoxicity and CYP-inhibition (SKF 525A-treated rats) decreased hepatotoxicity, but they further indicate that acute hepatotoxicity is related to depletion of glutathione.

The liver appears to develop a tolerance to acute bromobenzene insult after repeated exposure. Kluwe et al. (1984) assessed bromobenzene-induced effects on liver glutathione levels, serum ALT and SDH levels, and histopathologic liver lesions in male Fischer 344/N rats following single or repeated oral dosing (1 time/day for 10 days). Nonprotein sulfhydryl group concentrations were used as a measure of glutathione levels. A single oral dose of 628 mg/kg resulted in >50% decrease in liver glutathione between 3 and 12 hours posttreatment, partial recovery by 24 hours, and marked increase above control levels at 48 hours. Differences in minimum glutathione levels between treated animals and controls became less pronounced during repeated oral treatment until, following the tenth treatment, there was no significant difference from controls. Within 24 hours posttreatment, the single 628 mg/kg dose of bromobenzene produced moderate focal centrilobular and midzonal hepatocellular necrosis, as well as an inflammatory response. Although these liver lesions were somewhat more severe 24 hours following the second treatment, only minimal necrosis was noted following the fourth treatment and was not detected following the tenth treatment. Serum ALT activity was increased following the first, second, and fourth treatments, but not after the tenth treatment.

In a similarly-designed dose-response study (0, 9.8, 78.5, or 315 mg/kg-day), a single 315 mg/kg dose resulted in glutathione depletion, liver lesions, and increased ALT and SDH (Kluwe et al., 1984). Following the tenth dose, glutathione depletion was less pronounced, ALT and SDH were no longer increased, and liver lesions were not seen. NTP (1985a,b) assessed serum ALT, AST, and SDH levels in male and female Fischer 344/N rats and B6C3F1 mice administered bromobenzene by oral gavage at doses of 0, 50, 200, or 600 mg/kg-day, 5 days/week for 90 days. Significantly increased mean serum ALT, AST, and SDH levels (approximately 30- to 100-fold) were noted after the first treatment. After the third treatment, levels of all three enzymes remained significantly elevated on day 3, but the magnitude decreased to approximately 2- to 6-fold above control levels. Serum ALT, AST, and SDH levels were no longer significantly different from controls at terminal sacrifice on day 94. Collectively, these results indicate that acute hepatotoxic levels of bromobenzene may be tolerated upon

repeated exposure and that such an adaptive effect may be due to chemically-induced increased production of liver glutathione.

As noted in the proposed metabolic scheme for bromobenzene (Section 3.3, Figure 3-1), candidates for reactive metabolites of the 3,4-oxide pathway that may elicit hepatotoxicity include the 3,4-oxide itself, the oxide derivative of 4-bromophenol, the quinone (4-bromoquinone) formed from 4-bromocatechol, and reactive oxygen species formed via redox cycling of 4-bromoquinone. The relative importance of these metabolites to bromobenzene hepatotoxicity is uncertain. There is some evidence that 4-bromophenol and its further metabolites may not be involved in hepatotoxicity since centrilobular hepatic necrosis was observed in rats that were administered bromobenzene (400 mg/kg-day) intraperitoneally, but not in other rats administered 4-bromophenol (up to 440 mg/kg-day) or 4-bromocatechol (up to 485 mg/kg-day) (Monks et al., 1984a).

4.5.2. Mechanistic Studies of Kidney Effects

Nephrotoxicity also has been associated with acute exposure to bromobenzene in mice and rats, albeit at doses much higher than those eliciting hepatotoxicity. Mice appear to be more sensitive to the nephrotoxic effects than rats. For example, extensive renal necrosis was observed in male C57 Black/6J mice following a single intraperitoneal injection of a 760 mg/kg-day dose of ^{14}C-bromobenzene, whereas a 1460 mg/kg-day dose to male Sprague-Dawley rats resulted in less severe effects (ranging from swollen and vacuolated tubular cells to dilated convoluted tubules filled with protein casts) (Reid, 1973).

The nephrotoxic effects appear to be associated with covalent binding of reactive metabolites to cellular macromolecules in cells of the proximal convoluted tubules, as evidenced by findings that (1) covalent binding of ^{14}C-compounds to kidney proteins in the convoluted tubules peaked several hours prior to the appearance of histopathologic lesions and (2) pretreatment with piperonyl butoxide (a CYP inhibitor) decreased both the rate of metabolism of bromobenzene and the severity of kidney lesions (Reid, 1973). These results, together with demonstrations that intraperitoneal administration of either 2-bromophenol or 2-bromoquinone in rats resulted in histopathologic kidney lesions similar to those induced by bromobenzene, implicate reactive metabolites formed via the 2,3-oxide pathway (see Section 3.3, Figure 3-1) as the most likely source(s) of covalent binding and associated nephrotoxicity, at least in the rat.

Lau et al. (1984b) suggested that bromobenzene nephrotoxicity in rats is caused by a metabolite that is produced in the liver and transported to the kidney. In rats, intraperitoneally-injected 2-bromophenol (a metabolite of bromobenzene) resulted in renal necrosis similar to that observed following bromobenzene administration, but at a dose about one-fifth as large as the dose of bromobenzene required to produce lesions of similar severity. Renal glutathione levels

were rapidly and significantly decreased within 90 minutes following administration of 2-bromophenol, whereas hepatic glutathione levels were not decreased in the same time period. Conversion of 2-bromophenol to covalently bound material in the kidney was 4-fold greater than that observed in the liver. Furthermore, intraperitoneal administration of another major metabolite of bromobenzene, namely 2-bromohydroquinone, caused renal lesions that were indistinguishable from those induced following bromobenzene treatment (Lau et al., 1984a). In the presence of glutathione, 2-bromohydroquinone gave rise to several hydroquinone-glutathione conjugates, including the very potent nephrotoxicant (2-bromo-bis[glutathione-S-yl]hydroquinone), which is the most likely candidate for a bromobenzene metabolite produced in the liver and transported to the kidney to ultimately exert its toxic effect (Lau and Monks, 1997b; Monks et al., 1985).

The 3,4-oxide pathway may also be involved in the nephrotoxic effects observed in mice. Histopathologic lesions of the convoluted tubules were demonstrated in male ICR mice following single parenteral administration of any of the bromophenols (2-, 3-, or 4-bromophenol) or 4-bromocatechol (Rush et al., 1984).

4.5.3. Genomic/Proteomic Responses of the Liver to Bromobenzene

Toxicogenomics involves the application of functional genomics technologies to conventional toxicology. The development of recent analytical techniques allows for the simultaneous detection of numerous biomolecules, thus facilitating complete description of the genome for a particular organism (genomics). These techniques can be applied to analysis of multiple gene transcripts (transcriptomics), proteins (proteomics), and metabolites (metabolomics) as well.

Heijne and coworkers (Stierum et al., 2005; Heijne et al., 2004, 2003) used these techniques to identify changes in gene expression in the rodent liver in response to bromobenzene. As previously discussed, bromobenzene undergoes CYP-mediated epoxidation to form the electrophilic 3,4-epoxide, which has been demonstrated to irreversibly bind to proteins such as glutathione *S*-transferase, liver fatty acid binding protein, and carbonic anhydrase (Koen et al., 2000). Heijne et al. (2003) administered acute intraperitoneal hepatotoxic doses of bromobenzene (0.5–5 mM/kg) to rats and assessed liver tissue for physiological signs of toxicity and changes in protein and gene expression 24 hours posttreatment. Vehicle controls were included in the study. Bromobenzene treatment resulted in glutathione depletion (primarily due to conjugation) within 24 hours, which coincided with the induction of more than 20 liver proteins, including γ-glutamylcysteine synthetase (a key enzyme in glutathione biosynthesis). Bromobenzene-induced oxidative stress was indicated by the strong upregulation of a number of genes, including heme oxygenase and peroxiredoxin 1. Transient

changes were also noted in the transcriptional expression of numerous other genes, including ones involved in drug metabolism, intracellular signaling, metabolism, and the acute phase response.

Heijne et al. (2004) demonstrated dose-and time-related changes in bromobenzene-induced liver gene expression profiles by administering bromobenzene to groups of rats by oral gavage at doses of 0.5, 2.0, or 4.0 mM/kg and assessing changes in the liver transcriptome at 6, 24, and 48 hours posttreatment. Dose- and time-related changes were observed in the transcriptional expression of numerous genes involved in GSH depletion, drug metabolism, intracellular signaling, metabolism (cholesterol, fatty acid, and protein metabolism), and the acute phase response. At the highest dose, the time-course of altered gene expression coincided with that of histopathological evidence of bromobenzene-induced liver lesions, with few signs of adverse effects at 6 hours and increased evidence of histopathologic liver lesions and altered transcriptional expression at 24 and 48 hours. Although histopathologic liver lesions were not observed at the two lower doses, dose-related altered transcriptional expression was noted and recovery was apparent in the mid-dose group at 48 hours posttreatment. Results of available toxicogenomics assessments provide suggestive evidence for the involvement of some genes in particular aspects of bromobenzene hepatotoxicity. However, the toxicogenomics studies available do not establish key events in the mode of action for bromobenzene-induced hepatotoxicity.

4.5.4. Similarities Between Bromobenzene and Chlorobenzene

Bromobenzene and chlorobenzene (structures shown below) are monohalogenated benzene compounds that are distinguished from one another structurally by the particular halogen, bromine in the case of bromobenzene, and chlorine in the case of chlorobenzene. The two chemicals are structurally similar, with similar Pauling electronegativites of 3.16 and 2.96 for chlorine and bromine (Loudon, 1988), respectively. In addition, neither the chlorine nor the bromine atoms are removed from the benzene ring through metabolism.

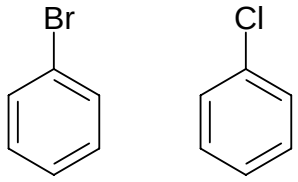

Figure 4-1. Chemical structure of bromobenzene and chlorobenzene

Independent *in vivo* and *in vitro* studies indicate that bromobenzene and chlorobenzene have similar toxicokinetic properties and share the same critical target of toxicity (liver).

Bromobenzene and chlorobenzene each exhibit the ability to enter the systemic circulation of laboratory animals following inhalation or oral exposure (see Section 3.1 for a detailed discussion of the toxicokinetics of bromobenzene and Hellman (1993) for a summary of toxicokinetic information for chlorobenzene). Results of parenteral injection studies in animals indicate that, following absorption, bromobenzene and its metabolites are widely distributed, with highest levels found in adipose tissue (Ogino, 1984b; Zampaglione et al., 1973; Reid et al., 1971). Similar distribution of chlorobenzene has been observed in rats following inhalation exposure to radiolabeled chlorobenzene (Sullivan et al., 1983). Metabolic schemes for both bromobenzene and chlorobenzene include initial CYP-catalyzed epoxidation to reactive epoxide intermediates and subsequent formation of corresponding dihydrodiol derivatives, phenols, glutathione conjugates, catechols, and quinones. Elimination is mainly accomplished via the urinary excretion of bromobenzene- and chlorobenzene-derived metabolites.

In a recent study, Chan et al. (2007) demonstrated the usefulness of isolated normal and phenobarbital induced rat hepatocytes for predicting *in vivo* toxicity caused by a series of halobenzene congeners, including bromobenzene and chlorobenzene. The underlying molecular mechanism of halobenzene hepatotoxicity was elucidated using Quantitative structure-activity relationships (QSARs) and accelerated cytotoxicity mechanism screening (ACMS) techniques in rat and human hepatocytes. The *in vivo* and *in silico* studies suggest that halobenzene interaction with cytochrome P450 for oxidation is the rate limiting step for toxicity and is similar in both species.

The subchronic oral toxicity studies of bromobenzene in Fischer 344/N rats (NTP, 1985a) and B6C3F1 mice (NTP, 1985b) and chlorobenzene in Fischer 344/N rats and B6C3F1 mice (NTP, 1985e) are the best available data from which to compare the toxicities of repeated exposure to bromobenzene and chlorobenzene. These studies identified the liver and kidney as the most sensitive targets of bromobenzene and chlorobenzene toxicity. Tables 4-12 and 4-13 summarize the liver and kidney effects observed for chlorobenzene.

The database for bromobenzene does not include reproductive or developmental toxicity studies. However, chlorobenzene was assessed for reproductive toxicity in a two-generation study of rats exposed to chlorobenzene vapor concentrations of 0, 50, 150, or 450 ppm daily, 6 hours/day for 10 or 11 weeks prior to mating and throughout mating, gestation, and lactation (Nair et al., 1987). Statistically significantly increased incidences of rats with histopathologic liver and kidney lesions were observed in F_0 and F_1 male rats at exposure levels $\geq$150 ppm. The NOAEL for hepatic effects in this study was 50 ppm. The highest exposure level (450 ppm) did not elicit any clear signs of reproductive toxicity in either generation. Furthermore,

chlorobenzene did not induce developmental effects in the fetuses of pregnant rats exposed to vapor concentrations as high as 590 ppm for 6 hours/day on gestation days 6-15 (John et al., 1984).

The oral database for chlorobenzene includes one developmental study in which Charles River albino rat dams were administered chlorobenzene at oral dose levels of 100 or 300 mg/kg-day on gestation days 6-15 (IBT, 1977). Although no developmental toxicity was elicited, it is

Table 4-12. Incidences of male and female Fischer 344/N rats with liver and kidney lesions following administration of chlorobenzene by gavage 5 days/week for 13 weeks

Endpoint	Dose (mg/kg-day)				
	0	125	250	500	750[a]
Males					
Liver					
Necrosis	0/10	0/10	2/10	3/10	7/10[b]
Degeneration	0/10	0/10	0/10	2/10	1/10
Kidney					
Nephropathy	0/10	0/10	1/10	2/10	2/10
Females					
Liver					
Necrosis	0/10	0/10	1/10	1/10	6/10[b]
Degeneration	0/10	0/10	0/10	0/10	4/10[b]
Kidney					
Nephropathy	0/10	0/10	0/10	0/10	7/10[b]

[a]Significantly decreased survival in the 750 mg/kg-day dose groups may have influenced observed incidences of animals with liver and/or kidney lesions.
[b]Statistically significantly different from control groups according to Fisher's exact test ($p<0.05$), performed by Syracuse Research Corporation.
Source: NTP (1985e)

Table 4-13. Incidences of male and female B6C3F1 mice with liver and kidney lesions following administration of chlorobenzene by gavage 5 days/week for 13 weeks

Endpoint	Dose (mg/kg-day)					
	0	60	125	250	500[a]	750[a]
Males						
Liver						
Necrosis	0/10	1/10	1/10	7/10[b]	10/10[b]	10/10[b]
Degeneration	0/10	0/10	0/10	2/10	0/10	0/10
Kidney						
Nephropathy	0/10	NE	0/10	4/10[b]	9/10[b]	8/10[b]
Females						
Liver						
Necrosis	0/10	0/10	0/10	10/10[b]	8/10[b]	1/10
Degeneration	0/10	0/10	0/10	0/10	9/10[b]	4/10[b]
Kidney						
Nephropathy	0/10	NE	0/10	4/10[b]	0/10	0/10

[a]Significantly decreased survival in the 500 and 750 mg/kg-day dose groups may have influenced observed incidences of animals with liver and/or kidney lesions.
[b]Statistically significantly different from control groups according to Fisher's exact test ($p<0.05$), performed by Syracuse Research Corporation.
NE = not examined, due to the absence of lesions at the next higher dose
Source: NTP (1985e)

uncertain whether repeated oral doses of chlorobenzene as high as those known to induce histopathologic liver lesions in rats (750 mg/kg-day) might also cause developmental effects.

Significantly increased mean relative (but not absolute) testis weight was noted in 400 and 600 mg/kg treatment groups of male rats administered bromobenzene via oral gavage 5days/week for 13 weeks (NTP, 1985a). However, gross and histopathologic examinations of these dose groups did not reveal other significant treatment-related testicular effects. No treatment-related effects were observed at any exposure level among female rats or male or female mice in the oral study (NTP, 1985a,b). There were no indications of significant exposure-related effects on reproductive organs or tissues in male or female rats or mice exposed to bromobenzene at any of the vapor concentrations used in the 13-week inhalation study of NTP (NTP, 1985c,d). Taken together, these results indicate that reproductive and developmental endpoints do not appear to be more sensitive targets of chlorobenzene or bromobenzene toxicity than the liver.

Although no chronic-duration oral or inhalation animal studies are available for bromobenzene, a 2-year toxicity and carcinogenicity study is available for chlorobenzene (NTP, 1985e). Groups of male and female F344/N rats and B6C3F1 mice (50/sex/species) were administered chlorobenzene by oral gavage at doses of 0, 60, or 120 mg/kg-day (0, 30, or 60 mg/kg-day for male mice), 5 days/week for 2 years. There was no evidence of treatment-related increased incidences of nonneoplastic liver lesions in female rats or male or female mice, including the highest dose level tested (120 mg/kg-day for female rats and mice, 60 mg/kg-day for male mice). There was equivocal evidence for treatment-related increased incidence of hepatocellular necrosis in high-dose (120 mg/kg-day) male rats. The original pathology report noted necrosis in 7/50 high-dose males (0/50 in vehicle controls). However, an independent pathological review resulted in a diagnosis of hepatocellular necrosis in one vehicle control male rat (1/50) and a single high-dose male rat (1/50). The NTP 2-year oral study of chlorobenzene identified a free-standing no-observed-adverse-effect level (NOAEL) of 120 mg/kg-day in female rats and equivocal evidence of a lowest-observed-adverse-effect level (LOAEL) of 120 mg/kg-day for hepatocellular necrosis in male rats. In male and female mice, free-standing NOAELs were 60 and 120 mg/kg-day, respectively, for nonneoplastic liver effects. In a similarly-designed subchronic (90-day) oral toxicity study in mice, a NOAEL of 125 mg/kg-day was identified in both males and females; the LOAEL was 250 for chlorobenzene-induced liver lesions (NTP, 1985e). These results suggest the development of some degree of tolerance to chlorobenzene during chronic exposure (i.e., dose-response relationships for subchronic and chronic exposure appear to be similar). It is reasonable to expect such similarities in dose-response relationships for subchronic and chronic exposure to bromobenzene as well because

mechanistic studies have demonstrated the development of some degree of tolerance upon repeated exposure to bromobenzene (Kluwe et al., 1984).

4.6. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

No data are available on health effects in humans following oral exposure to bromobenzene. No chronic-duration toxicity, reproductive toxicity, or developmental toxicity studies are available in animals following oral exposure to bromobenzene. Pertinent information on health effects in animals is restricted to results from studies of rats and mice administered bromobenzene by oral gavage at doses of 0, 50, 100, 200, 400, or 600 mg/kg-day, 5 days/week for 90 days (NTP, 1985a,b, 1986a). The liver was the most sensitive toxicity target in these NTP studies. Results of mechanistic studies involving acute oral exposures support this finding (e.g., Heijne et al., 2004; Bambal and Hanzlik, 1995; Kluwe et al., 1984). Dose-related significantly increased liver weights were observed in all treated groups of female rats and mice (50-600 mg/kg-day) and all but the 50 mg/kg-day groups of male rats and mice (liver weights were not available for the 50 mg/kg-day group of male rats). Oral doses $\geq$200 mg/kg-day resulted in significantly increased incidences of histopathologic liver lesions in male and female rats and male mice ($\geq$400 mg/kg-day in female mice).

Subchronic-duration oral exposure to bromobenzene also resulted in statistically significantly increased incidences of renal lesions such as necrosis and degeneration (without observable regeneration) in the proximal convoluted tubules in male and female rats and male mice, but only at the highest (600 mg/kg-day) dose level (NTP, 1985a).

The Pathology Working Group (NTP, 1986a) reported that lesions in the brain, stomach, thymus and bone marrow of the rats were present primarily or solely at the 600 mg/kg-day level. Liver and kidney lesions persisted through the 400 mg/kg-day dosed rats, but were essentially absent or present to a minimal degree in the rats at the 200 mg/kg-day dose level. In the NTP study in mice (NTP, 1985b), bromobenzene lesions were limited to the liver and were of less severity at 400 and 200 mg/kg-day and were essentially absent at 100 and 50 mg/kg-day.

Relatively high single oral doses ($\geq$785 mg/kg-day) have been shown to elicit hepatic, renal, and pulmonary effects in laboratory animals (Casini et al., 1986; Forkert, 1985; Kluwe et al., 1984; Patrick and Kennedy, 1964). However, pulmonary effects were not observed in the subchronic oral studies of NTP (1985a,b).

4.6.2. Inhalation

No data are available on health effects in humans following inhalation exposure to bromobenzene. No chronic-duration toxicity, reproductive toxicity, or developmental toxicity studies are available in animals following inhalation exposure to bromobenzene. Pertinent information on health effects in animals is restricted to results from studies in rats and mice exposed to bromobenzene at vapor concentrations of 0, 10, 30, 100, or 300 ppm, 6 hours/day, 5 days/week for 13 weeks (NTP, 1985c,d). The liver appeared to be the most sensitive toxicity target in these studies. Liver weights (absolute and relative-to-body weight) were significantly increased at exposure concentrations $\geq$100 ppm in both sexes of rats. The liver-to body weight ratio was significantly increased in 100-ppm male mice (the study did not include a 300-ppm male group). Statistically significantly increased liver-to-body weight ratios occurred in female mice at all bromobenzene exposure concentrations (including 10 ppm). Statistically significantly increased absolute liver weights occurred at all exposure concentrations $\geq$30 ppm.

A statistically significantly increased incidence of cytomegaly was observed only in female mice of the highest exposure level (300 ppm; male mice were not exposed at this concentration). The Pathology Working Group (NTP, 1986b) agreed with the diagnosis of cytomegaly, hepatic necrosis, and mineralization in the 300 ppm group, but considered necrosis and inflammation in the liver of female mice to be minimal or not present in the 100 ppm or lower exposure groups. There was no clear evidence of renal toxicity in mice repeatedly exposed to bromobenzene vapor concentrations up to and including the highest concentration tested (100 ppm in males and 300 ppm in females) (NTP, 1985d).

The liver was shown to be a target of bromobenzene toxicity in mice following a single 4-hour exposure to bromobenzene vapor concentrations as low as 250 ppm (Becher et al., 1989; Brondeau et al., 1983). Necrosis was also noted in the lungs of mice following a single 4-hour exposure to bromobenzene at a vapor concentration of 1000 ppm (Becher et al., 1989). However, lung lesions were not seen in rats or mice repeatedly exposed to bromobenzene vapors at concentrations up to 300 ppm (NTP, 1985c,d).

4.6.3. Mode of Action Information

No human data are available for health effects following exposure to bromobenzene by any exposure route for any duration. Animal studies demonstrate that relatively high single oral doses ($\geq$785 mg/kg) of bromobenzene elicit lesions in the liver, kidney, and lung. Parenteral injection studies support these findings. Hepatic effects have also been elicited in mice following a single 4-hour exposure to bromobenzene vapors at a concentration of 250 ppm; a higher concentration (1000 ppm) resulted in lung lesions. Subchronic-duration (90-day) oral and

inhalation studies in rats and mice have identified the liver as the most sensitive target of repeated exposure to bromobenzene.

The results of several mechanistic studies in animals demonstrate that bromobenzene hepatotoxicity is associated with metabolism of the parent compound and that cytotoxicity likely results from modifications of hepatocellular macromolecules by one or more reactive metabolites.

Available data further indicate that reactive metabolites are formed via the metabolic pathway that involves the 3,4-oxide (rather than the 2,3-oxide) derivative of bromobenzene. Supporting evidence includes the findings that:

- Induction of β-naphthoflavone- or 3-methylcholanthrene-induced CYP isozymes results in increased urinary excretion of 2-bromophenol (formed via the 2,3-oxide pathway) and decreased hepatotoxicity (Jollow et al., 1974; Lau and Zannoni, 1979; Lau et al., 1980; Zampaglione et al., 1973), whereas

- Induction of phenobarbital-induced CYP isozymes results in increased urinary excretion of 4-bromophenol (formed via the 3,4-oxide pathway) as well as increases in severity of hepatocellular necrosis and increases in the extent of covalent binding of radioactivity from ^{14}C-bromobenzene to hepatocellular macromolecules in the region of observed hepatocellular necrosis (Brodie et al., 1971).

Candidates for reactive metabolites of the 3,4-oxide pathway that may elicit hepatotoxicity include the 3,4-oxide itself, the oxide derivative of 4-bromophenol, the quinone (4-bromoquinone) formed from 4-bromocatechol, and reactive oxygen species formed via redox cycling of 4-bromoquinone. The relative importance of these metabolites to bromobenzene hepatotoxicity is uncertain. There is some evidence that 4-bromophenol and its further metabolites may not be involved in hepatotoxicity since centrilobular hepatic necrosis was observed in rats that were administered bromobenzene (400 mg/kg) intraperitoneally but not in other rats administered 4-bromophenol (up to 440 mg/kg) or 4-bromocatechol (up to 485 mg/kg) (Monks et al., 1984a).

Molecular mechanisms responsible for bromobenzene hepatotoxicity may include bromobenzene-induced alterations in liver proteins and gene expression. Heijne and coworkers used a toxicogenomics approach to study molecular mechanisms of bromobenzene hepatotoxicity (Heijne et al., 2003, 2004). Rats were administered bromobenzene intraperitoneally (0.5-5 mM/kg), and liver tissue was assessed for physiological signs of toxicity and changes in protein and gene expression for up to 48 hours posttreatment. Bromobenzene treatment resulted in glutathione depletion (primarily due to conjugation) within 24 hours, which coincided with induction of more than 20 liver proteins, including γ-glutamylcysteine synthetase (a key enzyme in glutathione biosynthesis). Transient changes were also noted in the

transcriptional expression of numerous genes involved in drug metabolism, oxidative stress, glutathione depletion, the acute phase response, metabolism, and intracellular signaling.

Nephrotoxicity has also been observed in animals following acute-duration exposure to bromobenzene, albeit at higher doses than the lowest hepatotoxic doses. Repeated-dose oral and inhalation studies in rats and mice provide evidence for kidney effects but only at the highest exposure levels tested, which also resulted in lethality. Nephrotoxicity also appears to result from modification of macromolecules in cells of the proximal convoluted tubule by one or more reactive metabolites.

4.7. EVALUATION OF CARCINOGINICITY

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is inadequate information available for an assessment of the human carcinogenic potential of bromobenzene. Cancer studies in humans and cancer bioassays in animals exposed to bromobenzene were not found. As discussed in Section 4.4.2, bromobenzene was not mutagenic in the Ames assay and did not consistently produce marked cytogenetic effects *in vitro* with mammalian cells, even in the presence of rat liver S-9 preparations. Bromobenzene increased formation of micronucleated polychromatic erythrocytes in bone marrow of mice given acute oral doses of 125 mg/kg and was bound to DNA and RNA following intraperitoneal injection. The available genotoxicity data, therefore, is inadequate to assess bromobenzene genotoxicity.

4.8. SUSCEPTIBLE POPULATIONS

4.8.1. Possible Childhood Susceptibility

Limited data were located regarding age-related susceptibility to bromobenzene. Single intraperitoneal injection of bromobenzene at concentrations that produced extensive centrilobular necrosis in the liver of adult rats failed to produce similar lesions in neonatal rats (Green et al., 1984; Mitchell et al., 1971). The lack of hepatotoxicity in the neonatal rats was presumably the result of a generally low level of hepatic microsomal enzymes observed in early neonatal stages of development (Kato et al., 1964).

4.8.2. Possible Gender Differences

Available information regarding gender-related susceptibility to bromobenzene is restricted to animal studies. In rats (NTP, 1985a), results of subchronic-duration oral exposure to bromobenzene indicate that males are somewhat more susceptible than females to hepatocellular effects such as centrilobular necrosis and cytomegaly (see Table 4-2). Male-female differences were not as apparent following subchronic-duration oral exposure in mice (see Table 4-4) (NTP, 1985b).

4.8.3. Other

No data are available regarding the effects of bromobenzene on other potentially susceptible populations. However, since the experimental depletion of glutathione in bromobenzene-treated animals has been demonstrated to potentiate bromobenzene hepatotoxicity (Jollow et al., 1974), individuals with abnormally low levels of glutathione, such as those with GSH synthetase deficiency (Meister, 1982), could potentially be at increased risk for bromobenzene hepatotoxicity. The importance of glutathione conjugation as a protective mechanism for bromobenzene hepatotoxicity may also make individuals exposed to other glutathione depleting chemicals more susceptible to bromobenzene hepatotoxicity.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE

5.1.1. Subchronic Oral RfD

5.1.1.1. Choice of Principal Study and Critical Effect - with Rationale and Justification

As discussed in Section 4.1, there are no human studies available for development of a subchronic RfD. The toxicity database for repeated oral exposure in laboratory animals that are available for selection of a subchronic RfD consists of two 90-day gavage studies: one in rats (NTP, 1985a) and one in mice (NTP, 1985b). No reproductive or developmental toxicity studies are available.

The liver appears to be the principal target organ for bromobenzene toxicity in rodents. Significantly increased incidences of hepatocellular necrosis (a clear indicator of an adverse effect) were observed at doses of 400 and 600 mg/kg-day in male and female B6C3F1mice and male Fischer 344/N rats (600 mg/kg-day in female Fischer 344/N rats) (NTP, 1985a,b). These dose levels also resulted in greater than 3-fold increases (statistically and biologically significant) in serum concentrations of SDH, an enzyme indicative of liver damage. Significantly increased incidences of cytomegaly were observed at doses $\geq$200 mg/kg-day in male and female mice and male rats ($\geq$400 mg/kg-day in female rats). Significantly increased mean liver weights were observed at doses as low as 50 mg/kg-day in female rats and mice and 100 mg/kg-day in male rats and mice.

Kidney lesions were associated with the proximal convoluted tubule and consisted of degeneration, casts, necrosis (rats only), and mineralization in male and female rats and male mice. The incidence of kidney lesions was not considered for the development of the subchronic RfD because the lowest dose associated with a statistically significant increase in the incidence of renal lesions (600 mg/kg-day in rats and mice) was higher than the lowest dose (400 mg/kg-day rats and mice) resulting in a clear treatment-related adverse liver effect (hepatocellular necrosis), indicating that the liver effects are a more sensitive indicator of bromobenzene toxicity.

Comprehensive histopathologic examinations of all major tissues and organs in the subchronic studies of rats and mice revealed no significantly increased incidences of exposure-related lesions at sites other than liver and kidney.

The increase in the incidence of liver lesions and the increase in absolute and relative liver weight in rats and mice, and the increase in serum concentrations of SDH in male and female mice, were considered in the selection of the critical effect for the development of the subchronic RfD. The increase in liver weight and enzyme levels may be considered to be on a

continuum leading to the observed liver toxicity. It is difficult to ascertain which liver lesions are most important or occur first in the development of liver toxicity. Therefore, liver lesions were combined so that an animal with any of the four observed lesions (centrilobular cytomegaly, necrosis, inflammation, or mineralization) was counted as having a liver lesion. The rationale for combining the liver lesions in this manner includes findings that: (1) all four lesions were principally observed in the centrilobular region of the liver; (2) statistically significantly increased incidences of hepatocellular necrosis or inflammation were observed and associated only with doses equal to or greater than those eliciting statistically significantly increased incidences of cytomegaly; and (3) inflammation and mineralization were considered, by the NTP study authors, to be direct results of hepatocellular necrosis (NTP, 1985a,b).

5.1.1.2. Methods of Analysis - Including Models (PBPK, BMD, etc.)

All available models in the EPA Benchmark Dose Software (BMDS version 1.3.2) were fit to the incidence data for the combined incidence of animals with one or more of the histopathologic liver lesions (centrilobular cytomegaly, necrosis, inflammation, mineralization). All models were also fit to the increases in absolute liver weight and liver-to-body weight ratios in male and female rats and mice and the increases in SDH levels in male and female mice from the subchronic oral gavage studies (NTP, 1985a,b). Modeling results are presented in Appendix B.

The modeled liver lesion data are shown in Table 5-1. Results of the best fitting models (lowest Akaike Information Criterion [AIC]) for incidences of male and female rats and mice with liver lesions are presented in Table 5-2. The female mouse liver lesion data produced the lowest $BMDL_{10}$ (24.8 mg/kg-day), indicating that female mice have a lower point of departure for bromobenzene hepatotoxicity (BMDs and BMDLs for 10, 5, and 1% extra risk are shown in Table 5-3). The conventional BMR of 10% extra risk (U.S. EPA, 2000c) was selected because the small group sizes (n=10) in the principal study preclude selecting a lower benchmark risk level.

The modeled data for absolute liver weight and liver-to-body weight ratio (relative liver weight) for rats and mice are shown in Table 5-4. Dose-related statistically significantly increased mean liver weights (absolute, relative-to-body weight) were observed in male rats at doses of 100-400 mg/kg-day and at all dose levels in female rats. Changes in the 600 mg/kg-day males were similar in magnitude to changes in the 400 mg/kg-day males, but could not be assessed for statistical significance because only one survivor remained in this group at study termination. In male mice, absolute liver weight was significantly increased at dose levels $\geq$200 mg/kg-day, while the liver-to-body weight ratio was significantly increased at dose levels $\geq$100 mg/kg-day. In female mice, both measures of liver weight were significantly increased in a

Table 5-1. Incidences of male and female Fischer 344/N rats and B6C3F1 mice with liver lesions[a] following administration of bromobenzene by gavage 5 days/week for 90 days

	Dose (mg/kg-day)					
	0	**50**	**100**	**200**	**400**	**600**
Male rats	2/10	2/10	2/10	7/10[b]	10/10[b]	10/10[b]
Female rats	2/10	2/10	4/10	5/10	10/10[b]	10/10[b]
Male mice	1/10	0/10	2/10	6/10[b]	10/10[b]	10/10[b]
Female mice	0/10	1/10	2/10	6/10[b]	9/10[b]	10/10[b]

[a]Incidences of rats with one or more of the liver lesion types (cytomegaly, necrosis, inflammation, mineralization), extracted from individual animal histopathologic results provided to Syracuse Research Corporation by NTP. Liver lesions were not seen in 2/10 male rats of the 200 mg/kg-day dose level that died early due to gavage error.
[b]Statistically different from control groups according to Fisher's exact test ($p<0.05$), performed by Syracuse Research Corporation.
Source: NTP (1985a,b)

Table 5-2. Benchmark doses (BMD_{10}s and $BMDL_{10}$s) from best fitting models predicting combined incidences of Fischer 344/N rats or B6C3F1 mice with liver lesions (see Appendix B)

Data set	**Model**	**BMD_{10}s and $BMDL_{10}$s (mg/kg-day)**		**Fit statistics**	
		BMD_{10}	**$BMDL_{10}$**	**x^2 p-value**	**AIC**
Male rats	Log-logistic	172.1	69.2	1.00	46.2
Female rats	Log-logistic	184.7	66.1	0.85	52.7
Male mice	Multi-stage	98.0	38.8	0.87	35.9
Female mice	Weibull	56.1	24.8	0.99	40.8

Table 5-3. Weibull model-estimated BMDs and BMDLs (mg/kg-day) associated with 10, 5, and 1% extra risk for liver lesions in female B6C3F1 mice administered bromobenzene by oral gavage 5 days/week for 90 days

BMDs and BMDLs (mg/kg-day)					
10% Extra risk		**5% Extra risk**		**1% Extra risk**	
BMD_{10}	$BMDL_{10}$	BMD_{05}	$BMDL_{05}$	BMD_{01}	$BMDL_{01}$
56.1	24.8	36.0	12.7	13.2	2.8

Source: NTP (1985b)

Table 5-4. Data for absolute liver weight and liver-to-body weight ratio for male and female Fischer 344/N rats and male and female B6C3F1 mice following administration of bromobenzene by gavage 5 days/week for 90 days (mean +/- standard deviation)

	Dose (mg/kg-day)					
	0	**50**	**100**	**200**	**400**	**600**
Absolute liver weight (grams)						
Rats						
Male	9.16 ± 0.66	NA	10.64* ± 0.76	11.29* ± 0.69	11.87* ± 0.80	--
Female	4.68 ± 0.35	5.23* ± 0.37	5.55* ± 0.36	6.28* ± 0.40	7.85* ± 0.49	--
Mice						
Male	1.05 ± 0.14	1.13 ± 0.15	1.12 ± 0.12	1.25* ± 0.22	1.27* ± 0.11	1.56* ± 0.16
Female	0.86 ± 0.06	0.96* ± 0.08	1.01* ± 0.08	1.08* ± 0.06	1.12* ± 0.07	1.30* ± 0.06
Liver-to-Body Weight Ratio (relative liver weight)						
Rats						
Male	26.72 ± 1.88	NA	31.08* ± 1.18	34.10* ± 0.68	40.56* ± 3.16	--
Female	24.25 ± 1.13	26.55* ± 1.23	28.69* ± 1.20	33.48* ± 1.37	43.11* ± 2.38	--
Mice						
Male	33.4 ± 2.41	33.9 ± 3.52	36.0* ± 1.91	37.3* ± 4.48	45.3* ± 1.83	51.2* ± 3.48
Female	38.1 ± 1.42	40.2* ± 2.02	42.5* ± 1.62	44.4* ± 2.12	48.0* ± 2.13	55.2* ± 2.56

*Statistically significantly different from controls ($p<0.05$) based on Student's two-tailed t-test.
Source: NTP (1985a,b)

dose-related manner in all bromobenzene treatment groups. Results for the best fitting models (lowest AIC) for absolute liver weight and liver-to-body weight ratio in male and female rats and mice are presented in Table 5-5. The lowest $BMDL_{1sd}$ value for liver weight effects was 25.8 mg/kg-day for absolute liver weight in female mice. A 0.5 standard deviation (0.5sd) change from the control mean was also considered as a potential benchmark response (BMR) for absolute liver weight in female mice (see Table 5-6).

Table 5-5. Benchmark doses (BMD_{10} and $BMDL_{10}$) from best fitting models for increased absolute liver weight and liver-to-body weight ratio in Fischer 344/N rats and B6C3F1 mice administered bromobenzene by gavage 5 days/week for 90 days

Data set	Model	BMD_{1sd} and $BMDL_{1sd}$ (mg/kg-day)		Fit-statistics	
		BMD_{1sd}	$BMDL_{1sd}$	X^2p-value	AIC
Absolute liver weight (grams)					
Male rats	Polynomial (2°)	48.82	35.4	0.47	16.10
Female rats	Linear	49.18	41.44	0.80	-42.58
Male mice	Linear	215.16	164.36	0.29	-139.46
Female mice	Polynomial (3°)	34.78	25.79	0.90	-242.57
Liver-to-body weight ratio (relative liver weight)					
Male rats	Linear	41.29	31.15	0.52	89.98
Female rats	Linear	30.90	26.27	0.96	91.83
Male mice	Linear	97.91	81.36	0.49	169.81
Female mice	Polynomial (3°)	40.61	29.32	0.79	136.58

Source: NTP (1985a,b)

Table 5-6. The third-degree polynomial model-estimated BMDs and BMDLs (mg/kg-day) associated with 1 and 0.5 standard deviation extra risk for increased absolute liver weight in female B6C3F1 mice administered bromobenzene by oral gavage 5 days/week for 90 days

BMDs and BMDLs (mg/kg-day)			
BMD_{1sd}	$BMDL_{1sd}$	$BMD_{0.5sd}$	$BMDL_{0.5sd}$
34.78	25.79	16.43	12.34

Source: NTP (1985b)

ALT, AST, and SDH serum levels in F-344/N rats generally showed increases over controls. ALT and AST serum levels in B6CF1 mice did not demonstrate a clear dose response, had a large variance and, as such, were not used for benchmark dose modeling. Statistically increased serum SDH values were observed at dose levels ≥200 mg/kg-day relative to sex matched controls in male and female mice.

The linear, polynomial, power, and Hill models were used to model the SDH serum levels for male and female mice data shown in Table 5-7. The power model for female mice data provided the best fit for SDH modeling. The results for the power model are shown in Table 5-8.

Table 5-7. Data for SDH for male and female B6C3F1 mice following administration of bromobenzene by gavage 5 days/week for 90 days (mean +/- standard deviation)

Sex	Dose mg/kg-day					
	0	**50**	**100**	**200**	**400**	**600**
Male	25 ± 2.5	27 ± 3.1	27 ± 3.2	41* ± 19.3	89* ± 28.3	101* ± 29.0
Female	13 ± 1.9	12 ± 1.6	14 ± 1.8	15* ± 1.7	23* ± 4.6	43* ± 18.8

* Statistically significantly increased from controls (p<0.05) based on students two tailed t-test.

Table 5-8. The power model estimated BMD and BMDLs associated with 10% extra risk for increased SDH serum levels in B6C3F1 female mice exposed to bromobenzene by gavage 5 days/week for 90 days

Data Set	BMD (mg/kg-day)	BMDL (mg/kg-day)	Fit-statistics	
			x^2 p-value	AIC
Female mice	196.47	145.79	1.33	192.63

In summary, female mice have a lower point of departure for hepatotoxicity of bromobenzene than male mice or male or female rats as indicated by the BMDLs in Tables 5-2 (liver lesions) and 5-5 (absolute liver weight and liver-to-body weight ratio). The increase in SDH levels in male and female mice was a less sensitive effect and was highly variable. The lowest $BMDL_{1sd}$ from the best fitting model for liver weight changes was 25.8 mg/kg-day, which was very similar to the lowest $BMDL_{10}$ from the best fitting model for combined liver lesions of 24.8 mg/kg-day. For this reason, liver toxicity in female mice, as defined by an increase in liver weight and liver lesions was selected as the critical effect for deriving the subchronic RfD. The average BMDL of 25 mg/kg-day was selected as the point of departure to derive the subchronic RfD for bromobenzene.

5.1.1.3. Subchronic RfD Derivation - Including Application of Uncertainty Factors (UFs)

Benchmark dose (BMD) analysis of liver toxicity data for female mice yielded an average BMDL of 25 mg/kg-day, which was selected as the point of departure for deriving a subchronic RfD for bromobenzene (see Section 5.1.2). The point of departure (25 mg/kg-day for mice that were administered bromobenzene by gavage 5 days/week for 90 days) was adjusted to account for daily exposure (25 mg/kg-day x 5 days/7 days = 17.8 mg/kg-day) and divided by a total UF of 1000. The UF consists of three areas of uncertainty: (1) interspecies extrapolation, (2) interindividual human variability, and (3) database deficiencies.

A 10-fold UF was used to account for laboratory animal-to-human interspecies differences (UF_A). No information is available on toxicokinetic or toxicodynamic differences or similarities for bromobenzene in animals and humans.

A 10-fold UF for intraspecies differences (UF_H) was used to account for variability in susceptibility in human populations. The default value of 10 was selected in the absence of information indicating the degree to which humans may vary in susceptibility to bromobenzene hepatotoxicity.

A 10-fold UF was used to account for database deficiencies (UF_D). Subchronic studies in rats and mice are available. Well-designed developmental toxicity and multi-generation reproductive toxicity studies are lacking. Therefore, an uncertainty factor if 10 was applied.

Bromobenzene and chlorobenzene exhibit striking similarities in structure, toxicokinetic properties, and critical target of toxicity (liver) in rats and mice (see Section 4.5.4 for a detailed discussion). Therefore, the toxicity database for chlorobenzene was assessed for its potential to address database deficiencies for bromobenzene. For example, in a 2-generation reproductive toxicity study in rats, chlorobenzene did not elicit any clear signs of reproductive toxicity in either generation at an exposure level of 450 ppm (Nair et al., 1987). In the same study, both F_0 and F_1 male rats exhibited chlorobenzene-induced hepatotoxicity from inhalation exposure at concentrations as low as 150 ppm. Chlorobenzene did not induce developmental effects in the fetuses of pregnant rats exposed to vapor concentrations as high as 590 ppm for 6 hours/day on gestation days 6-15 (John et al., 1984) or in fetuses of rat dams administered chlorobenzene at oral dose levels of 100 or 300 mg/kg-day on gestation days 6-15 (IBT, 1977). In addition to the chlorobenzene data, the subchronic oral gavage studies of bromobenzene in rats and mice did not reveal evidence of significant treatment-related effects on reproductive organs or tissues at dose levels that were clearly hepatotoxic (NTP, 1985a,b). Collectively, these results indicate that reproductive and developmental endpoints may not be particularly sensitive targets of bromobenzene or chlorobenzene toxicity. However, because database deficiencies for chlorobenzene include the lack of a developmental toxicity study in a second animal species, the

default value of 10 for deficiencies in the bromobenzene database was not reduced.

The subchronic RfD for bromobenzene was calculated as follows:

Subchronic RfD = (average BMDL x 5/7) ÷ UF
= (25 mg/kg-day x 5/7) ÷ 1000
= 17.8 mg/kg-day ÷ 1000
= 0.02 mg/kg-day (rounded to one significant figure)

5.1.2. Chronic Oral RfD

5.1.2.1. Choice of Principal Study and Critical Effect - with Rationale and Justification

As discussed in Section 4.1, there are no human studies available for development of a chronic RfD. The toxicity database for repeated oral exposure in laboratory animals that are available for selection of a chronic RfD consists of two 90-day gavage studies: one in rats (NTP, 1985a) and one in mice (NTP, 1985b). No chronic-duration, reproductive toxicity, or developmental toxicity studies are available.

The choices of principal study and critical effect for development of a chronic RfD for bromobenzene are the same as those described for the development of a subchronic RfD (see Section 5.1.1.1). The increase in the incidence of liver lesions and the increase in absolute and relative liver weight in rats and mice, and the increase in serum concentrations of SDH in male and female mice were considered in the selection of the critical effect for the development of the chronic RfD. Liver toxicity in female mice, as defined by an increase in liver weight and liver lesions was selected as the critical effect for deriving the chronic RfD.

5.1.2.2. Methods of Analysis - Including Models (PBPK, BMD, etc.)

The methods of analysis used to derive the subchronic RfD for bromobenzene apply to the derivation of the chronic RfD as well (see Section 5.1.1.2).

5.1.2.3. Chronic RfD Derivation - Including Application of Uncertainty Factors (UFs)

The lowest $BMDL_{1sd}$ from the best fitting model for liver weight changes was 25.8 mg/kg-day, which was very similar to the lowest $BMDL_{10}$ from the best fitting model for combined liver lesions of 24.8 mg/kg-day. An average dose of 25 mg/kg-day was selected as the point of departure for deriving a chronic RfD for bromobenzene (see Section 5.1.2). The point of departure (25 mg/kg-day for female mice administered bromobenzene by gavage 5 days/week for 90 days) was adjusted to account for daily exposure (25 mg/kg-day x 5 days/7 days = 17.8 mg/kg-day) and divided by a total UF of 3000. The UF consists of four areas of uncertainty: (1)

interspecies extrapolation, (2) interindividual human variability, (3) subchronic to chronic duration extrapolation, and (4) database deficiencies.

A 10-fold UF was used to account for laboratory animal-to-human interspecies differences (UF_A). No information is available on toxicokinetic or toxicodynamic differences or similarities for bromobenzene in animals and humans.

A 10-fold UF for intraspecies differences (UF_H) was used to account for variability in susceptibility in human populations. The default value of 10 was selected in the absence of information indicating the degree to which humans may vary in susceptibility to bromobenzene hepatotoxicity.

A 3-fold UF was used to account for extrapolating from a subchronic study to chronic exposure scenarios (UFs). Subchronic oral studies in both male and female rats and mice identify the liver as a critical target of bromobenzene toxicity. As discussed in Section 4.5, the liver appears to develop a tolerance to bromobenzene insult during repeated exposure. For example, a single 315 mg/kg oral dose of bromobenzene administered to male rats resulted in marked glutathione depletion, increased serum ALT and SDH concentrations, and observed histopathologic liver lesions (Kluwe et al., 1984). Following 10 days of dosing at 315 mg/kg-day, glutathione depletion was less pronounced, serum ALT and SDH concentrations were no longer increased, and histopathologic liver lesions were no longer detected. NTP (1985a,b) also found increased serum levels of ALT, AST, and SDH were not significantly different from the controls after 90 days of bromobenzene exposure.

Although chronic oral or inhalation animal studies are not available for bromobenzene, a chronic oral toxicity study is available for chlorobenzene. As discussed in detail in Section 4.5.4, bromobenzene and chlorobenzene exhibit striking similarities in structure, toxicokinetic properties, and critical target of toxicity (liver) in rats and mice. Mice appear to be more sensitive than rats to nonneoplastic hepatotoxicity induced by either bromobenzene or chlorobenzene. The NTP 2-year oral study of chlorobenzene concluded that, nonneoplastic lesions clearly attributable to chlorobenzene were not observed, and identified free-standing NOAELs of 60 and 120 mg/kg-day in male and female mice, respectively (NTP, 1985e). In a similarly-designed subchronic (90-day) oral toxicity study in mice, a NOAEL of 125 and a LOAEL of 250 mg/kg-day were identified in both males and females for chlorobenzene-induced liver lesions (NTP, 1985e). These results suggest that the dose-response relationships for liver effects from subchronic and chronic exposure are similar. It is reasonable to expect such similarities in dose-response relationships for subchronic and chronic exposure to bromobenzene as well, due to the similarity between the two chemicals with respect to chemical reactivity and structure, including similar Pauling electronegativities of chlorine (3.16) and bromine (2.96) (Loudon, 1988). In addition, a study by Chan et al. 2007 suggests that halobenzene congeners

interact with cytochrome P450 for oxidation as the primary metabolic activating pathway for toxicity. Mechanistic studies, demonstrating possible hepatic tolerance to repeated bromobenzene exposure (NTP, 1985a,b; Kluwe et al., 1984), further support the similarity between the two compounds. The available data for chronic exposure to chlorobenzene lend support to the database for bromobenzene. Therefore, a UF of 3 was selected to account for extrapolation from subchronic to chronic exposure to bromobenzene.

A 10-fold UF was used to account for database deficiencies (UF_D). As discussed previously (Section 5.1.1.3), the oral database for bromobenzene lacks well-designed developmental toxicity and multi-generation reproductive toxicity studies. Therefore, the default value of 10 for database deficiencies was not reduced.

The chronic RfD for bromobenzene was calculated as follows:

Chronic RfD = (average BMDL x 5/7) ÷ UF
= (25 mg/kg-day x 5/7) ÷ 3000
= 17.8 mg/kg-day ÷ 3000
= 0.006 mg/kg-day (rounded to one significant figure)

5.1.3. Previous Oral Assessment

An RfD was not previously available on IRIS.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Subchronic Inhalation RfC

5.2.1.1. Choice of Principal Study and Critical Effect - with Rationale and Justification

As discussed in Section 4.6.2, there are no available reports of health effects in humans following inhalation exposure to bromobenzene. The toxicity database for repeated inhalation exposure in laboratory animals consists of two 13-week studies, one in rats (NTP, 1985c) and one in mice (NTP, 1985d). No chronic-duration toxicity, reproductive toxicity, or developmental toxicity studies are available.

An adverse effect level was not identified in the 13-week inhalation study in male and female Fischer 344/N rats repeatedly exposed to bromobenzene vapor concentrations as high as 300 ppm (NTP, 1985c). Significantly increased mean liver weights in 100- and 300-ppm male and female rats may be indicators of an adaptive liver effect of questionable toxicological significance in the absence of more overt toxicity, e.g., liver lesions or necrosis. It should be noted also that this finding is in general agreement with the available oral studies in rats (NTP, 1985a) indicating that, unlike mice, this species does not exhibit overt liver toxicity following bromobenzene exposure. Cortical tubular regeneration in the kidney of male rats appeared to be

slightly more pronounced in severity in 300-ppm male rats, compared to controls. However, a statistically significant effect on incidence or severity of this kidney lesion could not be discerned. Therefore, this study is not selected for deriving the subchronic RfC.

The liver was the most sensitive toxicity target in female B6C3F1 mice exposed to bromobenzene vapors for 6 hours/day, 5 days/week for 13 weeks. Treatment-related significantly increased liver weights were seen in male mice at exposure concentrations $\geq$100 ppm and in all exposure groups of female mice (including the 50 ppm level). A significantly increased incidence of cytomegaly was observed in 300-ppm female mice (10/10 versus 0/10 controls). Necrosis was noted in 5/10 of the 300-ppm female mice, but the incidence of this lesion was not significantly greater than the incidence in controls (2/10). In the 90-day oral studies of rats and mice discussed earlier (NTP, 1985a,b), significantly increased incidences of cytomegaly were observed at doses equal to or slightly lower than those eliciting significantly increased incidences of necrosis. Therefore, it is reasonable to expect that somewhat higher exposure levels in the 90-day inhalation studies (NTP, 1985c,d) would have also resulted in hepatocellular necrosis in the female mice. The 300-ppm exposure level may represent an effect level in female mice that is near the threshold for bromobenzene hepatotoxicity. Therefore, the treatment-related increased occurrence of cytomegaly and increased liver weight may provide early indication of liver toxicity that could occur at higher levels of exposure. For these reasons, the subchronic inhalation study in mice (NTP, 1985d) was selected as the principal study and the increased occurrence of cytomegaly and increased absolute and relative liver weight in female mice was selected as potential critical effects for deriving the subchronic RfC.

Other effects in rats and mice were considered for the critical effect but were discounted. In rats, renal histopathology was associated with bromobenzene only at the highest exposure level tested (300 ppm) (NTP, 1985c). Although this lesion was observed in all male rats of the highest exposure group, it was also noted (albeit in slightly lesser severity) in all control males. The increased severity of the renal lesion (cortical tubular regeneration without observable degeneration or necrosis) at the highest exposure level (300 ppm) may represent a treatment-related renal effect in the male rats. However, the Pathology Working Group considered this effect to be mild in all rats in the high-exposure group (NTP, 1986b). Exposure of female rats at levels up to and including 300 ppm did not result in adverse renal effects. Evidence of renal effects was not detected in male or female mice at exposure concentrations up to and including the highest level tested (300 ppm for females; 100 ppm for males). Comprehensive histopathologic examinations of all major tissues and organs in the subchronic inhalation studies of rats and mice revealed no clear evidence of exposure-related lesions at sites other than the kidney (rats) and liver (mice).

5.2.1.2. Methods of Analysis - Including Models (PBPK, BMD, etc.)

Available models in U.S. EPA BMDS version 1.3.2 were fit to the liver lesion (cytomegaly) data for female B6C3F1 mice and to absolute liver weight and liver-to-body weight data for male and female B6C3F1 mice from the 90-day inhalation studies (NTP, 1985c,d). Modeling results are presented in Appendix C.

Table 5-9 presents incidence data for microscopically detected cytomegaly and necrosis in the centrilobular region of the liver in female mice exposed to bromobenzene vapors for 6 hours/day, 5 days/week for 13 weeks (NTP, 1985d). Cytomegaly was the lesion used for BMD analysis because the Pathology Working Group (NTP, 1986b) agreed with the diagnosis of cytomegaly, hepatic necrosis, and mineralization in the 300-ppm group, but considered necrosis and inflammation in the liver of the female mice to be minimal or not present in the 100-ppm or lower exposure groups. Based on statements of the original study pathologist, quality assurance pathologist, and the Pathology Working Group, hepatic necrosis and associated effects observed in the 300-ppm female mice were apparently distinguishable from the necrosis, inflammation, and mineralization observed in some of the control, 10-, 30-, and 100-ppm female mice. In a summary statement, the Pathology Working Group (NTP, 1986b) considered the 100-ppm exposure level to represent a NOAEL for liver effects in the female mice. Regardless, the statistically significant increase in liver weight at lower doses may be indicative of liver toxicity in this study. Given the available data sets, it is difficult to determine the region of the dose-response curve where precursor effects for liver toxicity might occur.

Table 5-9. Incidences of female B6C3F1 mice with cytomegaly in the centrilobular region of the liver following inhalation exposure to bromobenzene vapors for 6 hours/day, 5 days/week for 13 weeks

Lesion	Exposure concentration (ppm)				
	0	10	30	100	300
Cytomegaly	0/10	0/10	0/10	2/10	10/10*
Necrosis	2/10	1/10	0/10	2/10	5/10
Inflammation	4/10	3/10	2/10	2/10	2/10
Mineralization	0/10	0/10	0/10	0/10	2/10

*Statistically significantly different from control incidences according to Fisher's exact test ($p<0.05$), performed by Syracuse Research Corporation
Source: NTP (1985d)

Consideration was given to using a NOAEL/LOAEL approach for the cytomegaly data set since there is little change in effect until a dose of 100 ppm. However, it was decided that the use of the entire dataset in a BMD modeling approach would be a more sound method since the

curve was sigmoidal in shape. It was expected that a number of sigmoidal models would fit such data adequately and equivalently (e.g., gamma, probit, logistic, higher degree multistage). As a consequence, considerable uncertainty about the 'best' model among sigmoidal models is expected.

Sigmoidal models and two non-sigmoidal models (quantal quadratic and quantal linear) in the U.S. EPA BMDS (version 1.3.2.) were fit to the data in Table 5-9. Modeling results, presented in Table 5-10, show that: (1) all sigmoidal models provided excellent fit to the data (as expected due to the nature of the data); (2) the non-sigmoidal models provided poorer fits to the data; and (3) all sigmoidal models provided similar estimates of BMC_{10}s (ranging from about 77 ppm to 97 ppm, a 1.3-fold range) and $BMCL_{10}$s (ranging from about 40 ppm to 60 ppm, a 1.5-fold range). The conventional BMR of 10% extra risk (U.S. EPA, 2000c) was selected because the small group sizes (n=10) in the principal study preclude selecting a lower benchmark risk level. Following U.S. EPA (2000c) guidance for selecting models for point of departure computation, the model with the best fit and the lowest AIC is selected to calculate the BMCL which in this case corresponds to the log-logistic and gamma models (Table 5-10). The $BMCL_{10}$s from these best-fitting models (from the log-logistic and gamma models) were averaged (55 ppm) to arrive at the point of departure for deriving the RfC, as per U.S. EPA (2000c) guidance. Table 5-11 shows BMCs and BMCLs associated with 10, 5, and 1% extra risk levels.

The data for absolute liver weight and liver-to-body weight ratios (relative liver weight) for male and female mice are shown in Table 5-12. Although a significantly increased liver-to-body weight ratio was observed in 100-ppm male mice, there was no evidence of bromobenzene-induced histopathologic liver lesions. Therefore, the male mouse liver weight data were not modeled.

All continuous variable models in the U.S. EPA BMDS (version 1.3.2.) were fit to the absolute and relative liver weight data for female mice. As shown in Table 5-13, all models provided adequate fits to the data for absolute liver weight and liver-to-body weight ratio in female B6C3F1 mice as assessed by a chi-square goodness-of-fit test. Second-degree polynomial models provided the best fits for both variables as determined by the AIC (Table 5-13). One standard deviation change from the control mean corresponds to an excess risk of approximately 10% for the proportion of individuals above the 98^{th} percentile (or below the 2^{nd} percentile) of the control distribution for normally distributed effects (see Appendix C). Predicted BMC_{1sd} values were 52.38 ppm for absolute liver weight and 52.42 ppm for relative liver weight; associated 95% lower confidence limits ($BMCL_{1sd}$s) were 33.51 ppm for absolute liver weight and 33.90 ppm for relative liver weight (see Table 5-13). A 0.5 standard deviation

Table 5-10. BMC modeling results for the incidence of liver cytomegaly in female B6C3F1 mice exposed to bromobenzene vapors 6 hours/day, 5 days/week for 13 weeks

Model	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)	x^2 *p*-value	AIC
Log-logistic[a]	95.59	**58.73**	1.00	12.01
Gamma[b]	89.24	**51.42**	1.00	12.01
Multi-stage[c]	77.09	40.33	0.999	12.17
Weibull[b]	92.34	47.08	1.00	14.01
Log-probit[a]	92.95	57.45	1.00	14.01
Logistic	96.75	59.75	1.00	14.01
Probit	93.71	54.94	1.00	14.01
Quantal quadratic	55.15	40.15	0.87	14.05
Quantal linear	21.38	13.18	0.16	22.78

[a]Slope restricted to >1
[b]Restrict power > = 1
[c]Restrict betas > = 0; degree of polynomial = 3 (maximum degree restricted to #dose groups minus 2)
Source: NTP (1985d)

Table 5-11. BMCs and BMCLs predicted from the log-logistic and gamma models for 10, 5, and 1% extra risk for hepatocellular cytomegaly in female B6C3F1 mice exposed to bromobenzene vapors for 6 hours/day, 5 days/week for 13 weeks

10% Extra risk		5% Extra risk		1% Extra risk	
BMC_{10}	$BMCL_{10}$	BMC_{05}	$BMCL_{05}$	BMC_{01}	$BMCL_{01}$
Log-logistic model					
95.59	58.73	91.71	46.09	83.67	26.47
Gamma model					
89.24	51.42	80.98	38.52	66.93	20.53

Source: NTP (1985d)

Table 5-12. Data for absolute liver weight and liver-to-body weight ratio for male and female B6C3F1 mice following inhalation exposure to bromobenzene vapors for 6 hours/day, 5 days/week for 13 weeks (mean +/- standard deviation)

Exposure concentration (ppm)					
	0	**10**	**30**	**100**	**300**
Absolute liver weight (grams)					
Male	1.84 ± 0.21	1.73 ± 0.14	1.73 ± 0.18	1.87 ± 0.21	--
Female	1.43 ± 0.15	1.52 ± 0.09	1.54[a] ± 0.07	1.68[a] ± 0.10	2.37[b] ± 0.21
Liver-to-body weight ratio (relative liver weight)					
Male	50.71 ± 3.66	51.86 ± 3.57	51.57 ± 2.78	54.28[a] ± 2.42	--
Female	52.00 ± 3.22	55.25[a] ± 3.49	54.66[a] ± 1.80	59.37[b] ± 3.43	79.73[b] ± 5.27

[a]Statistically significantly different from controls ($p<0.05$) based on Student's two-tailed t-test
[b]Outside 3 standard deviations from the control mean
Source: NTP (1985d)

Table 5-13. Model output for increased absolute liver weight and liver-to-body weight ratio in female B6C3F1 mice following inhalation exposure to bromobenzene for 6 hours/day, 5 days/week for 13 weeks

Model[a]	BMC_{1sd} (ppm)	$BMCL_{1sd}$ (ppm)	x^2 p-value	AIC
Absolute liver weight[b]				
Linear	35.24	28.39	0.1838	-150.18
Polynomial (2^o)	52.38	33.51	0.3922	-151.16
Polynomial (3^o)	32.67	14.45	0.2891	-149.91
Power	56.82	32.56	0.2901	-150.55
Liver-to-body weight ratio[b]				
Linear	41.03	34.52	0.08619	183.82
Polynomial (2^o)	52.42	33.90	0.09284	182.19
Polynomial (3^o)	45.52	18.56	0.09301	184.05
Power	57.55	34.12	0.07211	182.77

[a]Statistical tests indicated that variances were not constant across exposure groups. Model results are for non-homogeneous variance, with the exception of the linear and 3-degree polynomial models for liver-to-body weight ratio.
[b]Modeled as a continuous variable using one standard deviation as the BMR.
Source: NTP (1985d)

(0.5sd) change from the control mean was also considered as a potential BMR for absolute liver weight and liver-to-body weight ratio (see Table 5-14).

Table 5-14. The second-degree polynomial model-estimated BMCs and BMCLs associated with 1 and 0.5 standard deviation extra risk for increased absolute liver weight and liver-to-body weight ratio in female B6C3F1 mice exposed to bromobenzene vapors for 6 hours/day, 5 days/week for 90 days

Endpoint	BMCs and BMCLs (ppm)			
	BMC_{1sd}	$BMCL_{1sd}$	$BMC_{0.5sd}$	$BMCL_{0.5sd}$
Absolute liver weight	52.38	33.51	27.65	16.83
Liver-to-body weight ratio	52.42	33.90	27.76	17.08

Source: NTP (1985d)

The $BMDL_{10}$ for absolute and relative liver weight changes in female mice was 34 ppm. The $BMDL_{10}$ for the incidence of cytomegaly was 55 ppm derived from an average of the $BMDL_{10}$s from the two best-fitting models. There is some uncertainty associated with the choice of the critical effect and the point of departure. Although cytomegaly in the absence of necrosis or other indicators of degenerative effects may represent an adaptive hepatic effect rather than an adverse effect, necrosis and mineralization observed in livers of some of the 300-ppm female mice was considered by the Pathology Working Group (NTP, 1986b) to be an exposure-related effect. Therefore, the 300-ppm exposure level may represent an effect level in female mice that is near the threshold of significantly detectable bromobenzene hepatotoxicity. For this reason, the average $BMCL_{10}$ of 55 ppm (from the log-logistic and gamma models) for cytomegaly in female mice was selected as the point of departure to derive the subchronic RfC for bromobenzene. There is less uncertainty in choosing this endpoint over the increase in liver weight due to the lack of directly observable statistically significant toxicity at higher doses.

5.2.1.3. Subchronic RfC Derivation - Including Application of Uncertainty Factors (UFs)

Following U.S. EPA (1994b) methodology, the human equivalent concentration (HEC) for an extra respiratory effect produced by a category 3 gas, such as bromobenzene (not highly water soluble or reactive in the respiratory tract, the liver as the critical extrarespiratory target), is calculated by multiplying the duration-adjusted BMCL or NOAEL by the ratio of the blood:gas partition coefficients in animals and humans $[(H_{b/g})_A / H_{b/g})_H]$. Because bromobenzene blood:gas partition coefficients are not available for humans or mice, a default value of 1 is used for this ratio. The $BMCL_{10}$ of 55 ppm for hepatocellular cytomegaly in female mice was converted to 353.2 mg/m^3 (55 ppm x MW[157] / 24.45 = 353.2 mg/m^3), which was then converted to a

continuous exposure basis (353.2 mg/m^3 x 6/24 hr x 5/7 days = 63 mg/m^3) and multiplied by a default blood:gas partition coefficient ratio of 1 to obtain the $BMCL_{10HEC}$ of 63 mg/m^3. The $BMCL_{10HEC}$ of 63 mg/m^3 was divided by a total UF of 300. The UF consists of three areas of uncertainty: (1) interspecies extrapolation, (2) interindividual human variability, and (3) database deficiencies.

A factor of 3 was selected to account for uncertainties in extrapolating from mice to humans (UF_A). Although no human data are available, it appears reasonable to assume that hepatic effects observed in female mice would be relevant to humans. The default value of 10 was reduced to 3 because dosimetric adjustment methodology (U.S. EPA, 1994b) for a category gas 3, with a default value of 1 for the ratio of the blood:gas partition coefficients in animals and humans [$(H_{b/g})_A$ / $H_{b/g})_H$]), was applied to derive the $BMCL_{10HEC}$ point of departure for the subchronic RfC.

A default 10-fold UF was selected to account for interindividual toxicokinetic and toxicodynamic variability in humans (UF_H). Although hepatotoxicity was observed only in female mice, a 300-ppm (1926 mg/m^3) group of male mice was not included in the study. Due to the lack of conclusive information concerning gender-specific differences in bromobenzene hepatotoxicity following inhalation exposure, as well as the lack of data concerning the extent of variation in sensitivity to bromobenzene within the human population, the default value of 10 was not reduced.

A 10-fold UF was used to account for database deficiencies (UF_D). Subchronic studies in rats and mice are available. Developmental toxicity and multi-generation reproductive toxicity studies are lacking. Therefore, the default value of 10 was not reduced.

The subchronic RfC for bromobenzene was calculated as follows:

$$\text{Subchronic RfC} = BMCL_{10HEC} \div UF$$
$$= 63 \text{ mg/m}^3 \div 300$$
$$= 0.2 \text{ mg/m}^3 \text{ (rounded to one significant figure)}$$

5.2.2. Chronic Inhalation RfC

5.2.2.1. Choice of Principal Study and Critical Effect - with Rationale and Justification

As discussed in Section 4.6.2, there are no available reports of health effects in humans following inhalation exposure to bromobenzene. The toxicity database for repeated inhalation exposure in laboratory animals consists of two 13-week studies, one in rats (NTP, 1985c) and one in mice (NTP, 1985d). No chronic-duration toxicity, reproductive toxicity, or developmental toxicity studies are available.

The choices of principal study and critical effect for development of the chronic RfC for bromobenzene are the same as those described for the development of a subchronic RfC (see Section 5.2.1.1). The increase in incidence of cytomegaly and the increase in absolute and relative liver weight in female mice (NTP, 1985d) were considered in the selection of the critical effect for development of the subchronic RfC for bromobenzene.

5.2.2.2. Methods of Analysis - Including Models (PBPK, BMD, etc.)

The methods of analysis used to derive the subchronic RfC for bromobenzene apply to the derivation of the chronic RfC as well (see Section 5.2.1.2).

5.2.2.3. Chronic RfC Derivation - Including Application of Uncertainty Factors (UFs)

As described in detail in Section 5.2.1.3, the average $BMCL_{10}$ of 55 ppm for cytomegaly in female mice was selected as the point of departure to derive the subchronic RfC for bromobenzene. The same point of departure was used to derive the chronic RfC. The $BMCL_{10}$ of 55 ppm for hepatocellular cytomegaly in female mice was converted to a $BMCL_{10HEC}$ of 63 mg/m^3 (see Section 5.2.1.3 for details regarding conversion to the HEC). The $BMCL_{10HEC}$ of 63 mg/m^3 was divided by a total UF of 1000. The UF consists of four areas of uncertainty: (1) interspecies extrapolation, (2) interindividual human variability, (3) extrapolation from subchronic- to chronic-duration exposure, and (4) database deficiencies.

A factor of 3 was selected to account for uncertainties in extrapolating from mice to humans (UF_A). Although no human data are available, it appears reasonable to assume that hepatic effects observed in female mice would be relevant to humans. The default value of 10 was reduced to 3 because dosimetric adjustment methodology (U.S. EPA, 1994b) for a category gas 3, with a default value of 1 for the ratio of the blood:gas partition coefficients in animals and humans [$(H_{b/g})_A / (H_{b/g})_H$]), was applied to derive the $BMCL_{10HEC}$ point of departure for the chronic RfC.

A 10-fold UF was selected to account for interindividual toxicokinetic and toxicodynamic variability in humans (UF_H). Although hepatotoxicity was observed only in female mice, a 300-ppm (1926 mg/m^3) group of male mice was not included in the study. Due to the lack of conclusive information concerning gender-specific differences in bromobenzene hepatotoxicity following inhalation exposure, as well as the lack of data concerning the extent of variation in sensitivity to bromobenzene within the human population, the default value of 10 was not reduced.

A 3-fold UF was used to account for extrapolating from a subchronic study to chronic exposure scenarios (UFs). Subchronic oral studies in both male and female rats and mice identify the liver as a critical target of bromobenzene toxicity. A subchronic inhalation study in

mice provides supporting evidence for the hepatotoxicity of bromobenzene. There are no chronic exposure studies for bromobenzene, but results of chronic exposure to chlorobenzene indicate the subchronic and chronic dose-responses are similar (see Section 5.1.2.3). It is reasonable to expect the subchronic and chronic dose-responses from exposure to bromobenzene to be similar as well. Therefore, a UF of 3 was selected to account for extrapolation from subchronic to chronic exposure to bromobenzene.

A 10-fold UF was used to account for database deficiencies (UF_D). Subchronic studies in rats and mice are available. Developmental toxicity and multi-generation reproductive toxicity studies are lacking. Therefore, the default value of 10 was not reduced.

The chronic RfC for bromobenzene was calculated as follows:

$$\text{Chronic RfC} = BMCL_{10HEC} \div UF$$
$$= 63 \text{ mg/m}^3 \div 1000$$
$$= 0.06 \text{ mg/m}^3 \text{ (rounded to one significant figure)}$$

5.3. CANCER ASSESSMENT

No studies of cancer risks of humans or cancer bioassays in animals exposed to bromobenzene were located. Bromobenzene was not mutagenic in the Ames assay and did not consistently produce marked cytogenetic effects *in vitro* with mammalian cells, even in the presence of rat liver S-9 preparations. Bromobenzene induced micronuclei in bone marrow of mice given acute oral doses of 125 mg/kg and was bound to DNA and RNA following intraperitoneal injection. Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess the carcinogenic potential" of brombenzene.

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

6.1. HUMAN HAZARD POTENTIAL

No human data are available for health effects following exposure to bromobenzene by any exposure route for any duration. Animal studies demonstrate that relatively high single oral doses (≥785 mg/kg-day) of bromobenzene elicit hepatic, renal, and pulmonary effects (Becher et al., 1989; Casini et al., 1986; Forkert, 1985; Kluwe et al., 1984; Rush et al., 1984; Roth, 1981; Reid et al., 1973; Patrick and Kennedy, 1964). Hepatic effects have been elicited in mice following a single 4-hour exposure to bromobenzene vapors at a concentration of 250 ppm; a higher concentration (1000 ppm) resulted in lung lesions (Becher et al., 1989). Subchronic-duration (90-day) oral and inhalation studies in rats and mice identify the liver as the most sensitive target of bromobenzene toxicity (NTP, 1985a,b,c,d). The threshold for renal effects appears to be somewhat higher than that for hepatic effects. Bromobenzene has not been assessed for reproductive or developmental toxicity or for carcinogenicity in animals. It is reasonable to assume that bromobenzene-induced human health effects would be similar to those demonstrated in laboratory animals.

Results of additional well-designed studies of bromobenzene toxicity in animals would be helpful in assessing the hazards associated with exposure to bromobenzene. The chronic oral and inhalation toxicity of bromobenzene should be assessed in two animal species at exposure concentrations that include a clear advese effect level. In addition, as discussed in Section 4.6.1, a well-designed developmental toxicity study and a multi-generation reproductive toxicity study should be performed using the oral and/or inhalation exposure route.

Following EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess the carcinogenic potential" of bromobenzene due to the lack of data on the possible carcinogenicity of bromobenzene in humans or animals. Bromobenzene was not mutagenic in bacterial assays and did not consistently produce marked cytogenetic effects *in vitro* with mammalian cells, even in the presence of rat liver metabolizing preparations. Bromobenzene increased formation of micronucleated polychromatic erythrocytes in bone marrow of mice given acute oral doses of 125 mg/kg and was bound to DNA and RNA following intraperitoneal injection. The available genotoxicity data, therefore, provide only limited evidence of bromobenzene genotoxicity.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

The liver was selected as the critical target of bromobenzene toxicity because it is the most sensitive indicator of bromobenzene toxicity. BMD analysis of the incidence data for combined liver lesions (centrilobular inflammation, cytomegaly, mineralization or necrosis), absolute liver weight, liver-to-body weight ratio, and SDH levels in rats and mice (NTP, 1985a,b) indicated that female mice have a lower point of departure than male mice or male or female rats. Liver toxicity defined as the combined incidence of hepatic lesions and liver weight changes in female mice was selected as the critical effect for deriving the chronic and subchronic RfD.

The average of the lower 95% confidence limit for a BMD of 10% extra risk for liver weight changes ($BMDL_{10}$ = 25.8 mg/kg-day) and combined liver lesions (24.8 mg/kg-day) was used as the point of departure. The average BMDL of 25 mg/kg-day was adjusted to account for daily exposure (25 mg/kg-day x 5 days/7 days = 17.8 mg/kg-day). The subchronic RfD was derived by dividing the average $BMDL_{ADJ}$ of 17.8 mg/kg-day by a composite UF of 1000 to account for three areas of uncertainty (10 for interspecies extrapolation, 10 for interindividual human variability, and 10 for database deficiencies). The resulting RfD is 17.8 mg/kg-day ÷ 1000 = 0.02 mg/kg-day. The derivation of the chronic RfD included an additional UF of 3 to account for extrapolation from a subchronic study to chronic exposure scenarios for a composite UF of 3000. The resulting chronic RfD is 17.8 mg/kg-day ÷ 3000 = 0.006 mg/kg-day.

6.2.2. Noncancer/Inhalation

The NTP 90-day inhalation studies in rats and mice provided adequate exposure-response data for bromobenzene (NTP, 1985a,b). The liver was selected as the critical target of bromobenzene toxicity because the liver was the only target that provided clear evidence of bromobenzene toxicity. Significantly increased incidences of cytomegaly were observed in female mice of the highest exposure level (300 ppm). Incidences of histopathologic liver lesions in bromobenzene-exposed groups of male and female rats and male mice were not significantly different from controls up to and including the highest exposure level tested (300 ppm in male and female rats, 100 ppm in male mice). Significantly increased liver weights were noted in 100- and 300-ppm male and female rats, 100-ppm male mice, and all bromobenzene-exposed groups (50-300 ppm) of female mice. The incidence of hepatocellular cytomegaly in female mice was selected as the critical effect for deriving the chronic and subchronic RfC.

The average $BMCL_{10}$ of 55 ppm (from the log-logistic and gamma models) for cytomegaly in female mice was selected as the point of departure. The $BMCL_{10}$ was converted to 353.2 mg/m^3 (55 ppm x MW[157] / 24.45 = 353.2 mg/m^3), which was then converted to a

continuous exposure basis (353.2 mg/m^3 x 6/24 hours x 5/7 days = 63 mg/m^3) and multiplied by a default blood:gas partition coefficient ratio of 1 to obtain the $BMCL_{10HEC}$ of 63 mg/m^3. The subchronic RfC was derived by dividing the $BMCL_{10HEC}$ of 63 mg/m^3 by a composite UF of 300 to account for three areas of uncertainty (3 for interspecies extrapolation using dosimetric conversion, 10 for interindividual human variability, and 10 for database deficiencies). The resulting subchronic RfC is 63 mg/m^3 ÷ 300 = 0.2 mg/m^3. The derivation of the chronic RfC included an additional UF of 3 to account for extrapolation from a subchronic study to chronic exposure scenarios. The resulting chronic RfC is 63 mg/m^3 ÷ 1000 = 0.06 mg/m^3.

6.2.3. Cancer/Oral

The lack of cancer studies in humans and cancer bioassays in animals precludes a cancer dose-response assessment for bromobenzene.

6.2.4. Cancer/Inhalation

The lack of cancer studies in humans and cancer bioassays in animals precludes a cancer dose-response assessment for bromobenzene.

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

[to be provided]

APPENDIX B. BENCHMARK DOSE CALCULATIONS FOR THE RfD

All available models in the EPA BMDS (version 1.3.2) were fit to incidence data for histopathologic liver lesions in male and female Fischer 344/N rats and male and female B6C3F1 mice from the 90-day oral gavage studies (NTP, 1985a,b). The data that were modeled are shown in Table 5-1.

All models provided adequate fits to the data for histopathologic liver lesions (centrilobular inflammation, cytomegaly, mineralization, or necrosis; combined) in the NTP (1985a,b) studies, as assessed by a chi-square goodness-of-fit test (see Tables B-1, B-2, B-3, and B-4 below and respective plots of observed and predicted values from the various models [Figures B-1, B-2, B-3, and B-4]).

Table B-1. BMD modeling results for the incidence of combined liver effects in male Fischer 344/N rats exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10}s and $BMDL_{10}$s (mg/kg-day)		x^2 p-value	AIC
	BMD_{10}	$BMDL_{10}$		
Log-logistic[a]	172.07	69.23	1.00	46.24
gamma[b]	134.60	54.59	1.00	46.25
Multi-stage[c]	127.91	27.49	1.00	46.27
Quantal quadratic	65.62	49.47	0.88	47.67
Log-probit[a]	160.78	67.44	1.00	48.24
Weibull[b]	156.79	47.09	1.00	48.24
Probit	45.50	31.74	0.73	48.37
Logistic	49.24	33.29	0.73	48.45
Quantal linear	20.13	13.61	0.20	53.93

[a]Slope restricted to >1
[b]Restrict power >=1
[c]Restrict betas >=0; Degree of polynomial = 5

Table B-2. BMD modeling results for the incidence of combined liver effects in female Fischer 344/N rats exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10}s and $BMDL_{10}$s (mg/kg-day)		x^2 p-value	AIC
	BMD_{10}	$BMDL_{10}$		
Log-logistic[a]	184.67	66.05	0.85	52.66
gamma[b]	161.04	37.75	0.85	52.69
Quantal quadratic	73.60	54.85	0.86	53.01
Multi-stage[c]	56.75	21.35	0.92	53.83
Probit	46.29	32.82	0.81	53.31
Logistic	49.08	33.73	0.77	53.68
Weibull[b]	126.69	35.45	0.79	54.40
Log-probit[a]	181.98	59.88	0.71	54.66
Quantal linear	21.40	14.41	0.34	57.45

[a]Slope restricted to >1
[b]Restrict power >=1
[c]Restrict betas >=0; Degree of polynomial = 5

Table B-3. BMD modeling results for the incidence of combined liver effects in male B6C3F1 mice exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10}s and $BMDL_{10}$s (mg/kg-day)		x^2 p-value	AIC
	BMD_{10}	$BMDL_{10}$		
Multi-stage[a]	97.99	38.82	0.87	35.86
Logistic	77.20	50.47	0.65	36.89
Probit	69.43	46.08	0.60	37.07
Quantal quadratic	68.85	53.53	0.72	37.16
Weibull[b]	98.67	53.72	0.74	37.86
Gamma[b]	99.40	57.87	0.71	37.97
Log-probit[c]	100.10	63.56	0.66	38.25
Log-logistic[c]	107.28	64.0	0.62	38.61
Quantal linear	22.64	15.65	0.09	46.35

[a]Restrict power >=1
[b]Restrict betas >=0; Degree of polynomial = 5
[c]Slope restricted to >1

Table B-4. BMD modeling results for the incidence of combined liver effects in female B6C3F1 mice exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10}s and $BMDL_{10}$s (mg/kg-day)		x^2 p-value	AIC
	BMD_{10}	$BMDL_{10}$		
Weibull[a]	56.08	24.81	0.99	40.84
Gamma[a]	59.27	24.92	0.98	40.98
Quantal quadratic	74.86	59.49	0.87	41.65
Log-probit[b]	63.34	35.33	0.91	41.68
Log-logistic[b]	65.47	34.62	0.92	41.70
Quantal linear	23.08	16.27	0.73	42.22
Probit	74.52	50.54	0.84	42.30
Logistic	78.28	52.22	0.83	42.38
Multi-stage[c]	50.55	20.62	0.95	42.83

[a]Restrict power >=1
[b]Slope restricted to >1
[c]Restrict betas >=0; Degree of polynomial = 5

The log-logistic model provided the best fit to the male rat data (see Table B-1), and was thus selected to estimate a BMD for the male rats from the NTP (1985a) data. The BMD_{10} associated with a 10% extra risk for histopathologic liver lesions in male rats was 172.1 mg/kg-day and its lower 95% confidence limit ($BMDL_{10}$) was 69.2 mg/kg-day (see Figure B-1 for a plot of observed and predicted values). The form and parameters of the log-logistic model for male rat liver effects (NTP, 1985a) are:

$$P(d) = B_0+(1-B_0)/[1+\exp(-\text{intercept}-\text{slope}*\log(d))] \quad \text{(Eq. B-1)}$$

d = exposure dose
B_0 = 0.199998 (se = 0.0730298)
intercept = -94.8589 (se = 0.786551)
slope = 18; no standard error because this parameter hit a bound

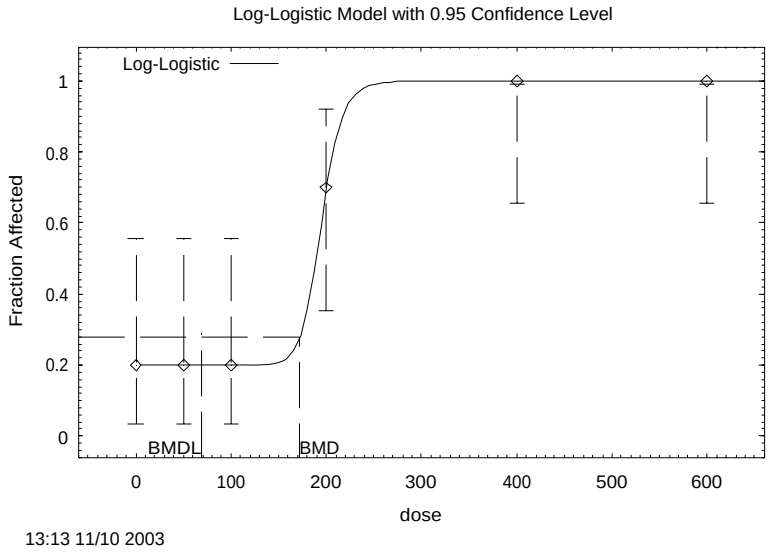

Figure B-1. Observed and predicted incidences of male Fischer 344/N rats exhibiting bromobenzene-induced combined liver lesions following gavage treatment 5 days/week for 90 days. BMD=ED_{10}; BMDL=LED_{10}

The log-logistic model provided the best fit to the female rat data (see Table B-2 and Figure B-2) and was thus selected to estimate a BMD for the female rats from the NTP (1985a) data. The BMD_{10} associated with a 10% extra risk for histopathologic liver lesions in female rats was 184.7 mg/kg-day and its lower 95% confidence limit ($BMDL_{10}$) was 66.1 mg/kg-day (see Figure B-2 for a plot of observed and predicted values). The form and parameters of the log-logistic model for female rat liver effects are as follows:

$$P(d) = B_0+(1-B_0)/[1+\exp(-\text{intercept}-\text{slope}*\log(d))] \quad \text{(Eq. B-2)}$$

d = exposure dose

B_0 = 0.266665 (se = 0.0807368)

intercept = -96.1318 (se = 1.05229)

slope = 18; no standard error because this parameter hit a bound

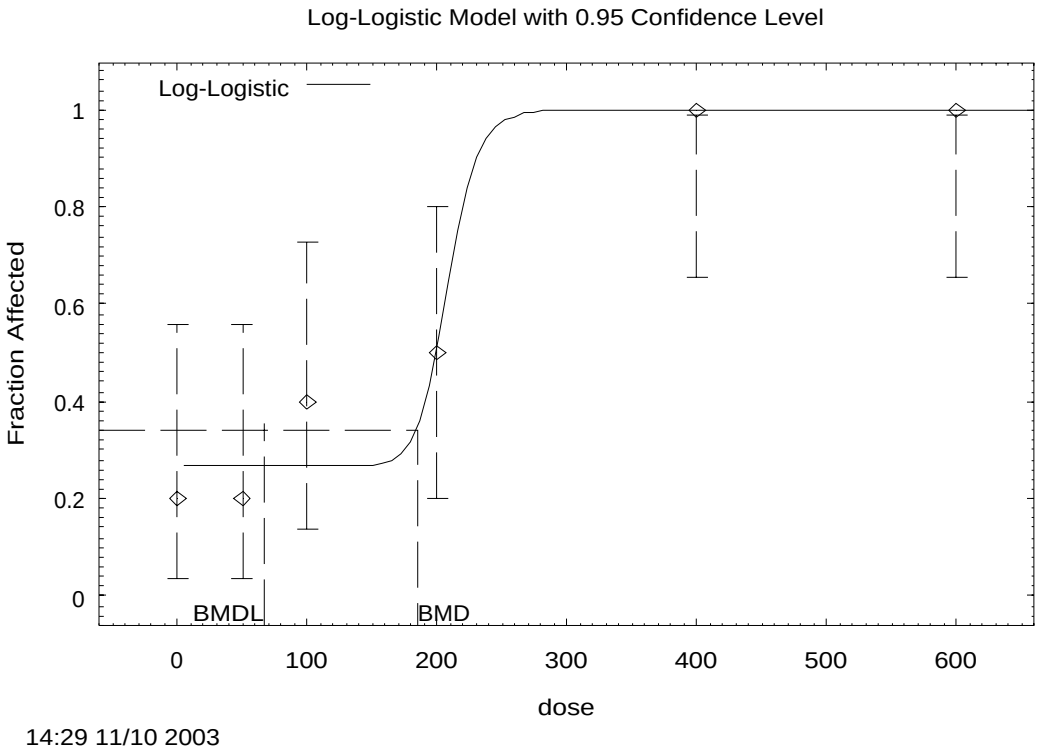

Figure B-2. Observed and predicted incidences of female Fischer 344/N rats exhibiting bromobenzene-induced combined liver lesions following gavage treatment 5 days/week for 90 days. BMD=ED_{10}; BMDL=LED_{10}

The multi-stage model provided the best fit to the male mouse liver lesion data (see Table 5-1), and was thus selected to estimate a BMD for the male mice from the NTP (1985a) data. The BMD_{10} associated with a 10% extra risk for histopathologic liver lesions in male mice was 97.99 mg/kg-day and its lower 95% confidence limit ($BMDL_{10}$) was 38.82 mg/kg-day (see Figure B-3 for a plot of observed and predicted values). The form of the multi-stage model for male mouse liver effects are as follows:

$$P(d) = background + (1-background)*[1-EXP(-\beta1*dose-\beta2*d^2-\beta3d^3-\beta4d^4)] \quad \text{(Eq. B-3)}$$

background = 0
β1 = 1.94919e+017
β2 = 1.63151e+013
β3 = 0
β4 = 0.

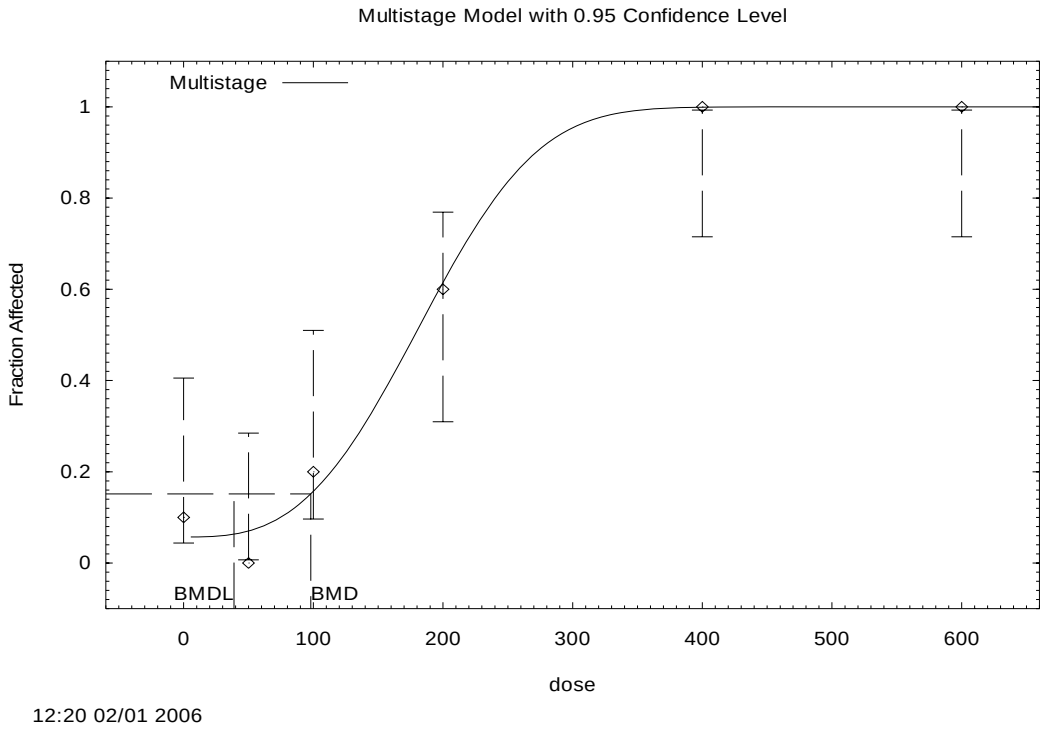

Figure B-3. Observed and predicted incidences of male B6C3F1 mice exhibiting bromobenzene-induced combined liver lesions following following gavage treatment 5 days/week for 90 days. BMD=ED_{10}; BMDL=LED_{10}

The Weibull model provided the best fit to the female mouse liver lesion data (see Table B-4), and was thus selected to estimate a BMD for liver lesions in female mice from the NTP (1985b) data. The BMD_{10} associated with a 10% extra risk for histopathologic liver lesions in female mice was 56.1 mg/kg-day and its lower 95% confidence limit ($BMDL_{10}$) was 24.8 mg/kg-day (see Figure B-4 for a plot of observed and predicted values). Estimated BMDs and BMDLs associated with 5% and 1% extra risk are presented in Table 5-3 (see Figures B-5 and B-6 for a plot of observed and predicted values associated with 5% and 1% extra risk, respectively). The form and parameters of the Weibull model for female mouse liver effects are as follows:

$$P(d) = B_0+(1-B_0)*[1-\exp(-slope*d^{power})] \quad \text{(Eq. B-4)}$$

d = exposure dose

B_0 = 0

slope = 0.00152103 (se = 0.000322079)

power = 1.62425 (se = 0.383589)

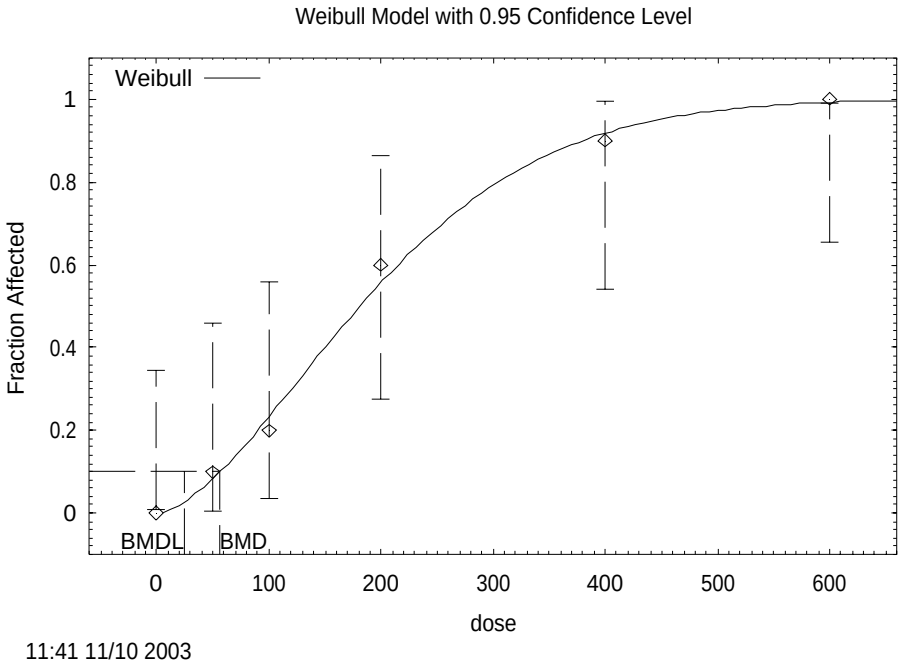

Figure B-4. Observed and predicted incidences of female B6C3F1 mice exhibiting bromobenzene-induced 10% extra risk for liver lesions following gavage treatment 5 days/week for 90 days. BMD=ED_{10}; BMDL=LED_{10}

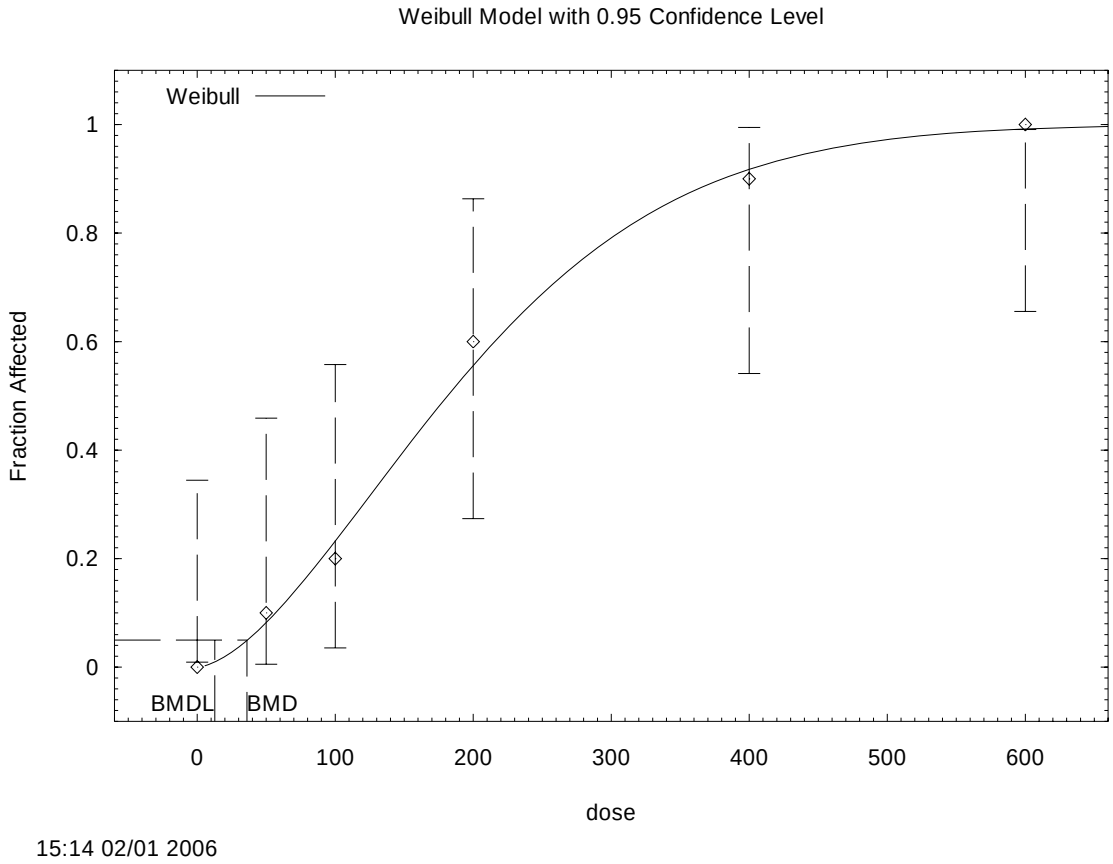

Figure B-5. Observed and predicted incidences of female B6C3F1 mice exhibiting bromobenzene-induced 5% extra risk for liver lesions following gavage treatment 5 days/week for 90 days

The form and parameters for the Weibull model for female mouse liver effects are as follows:

P[response] = background = (1-background)*[1-EXP(-slope*dose^power)] (Eq. B-5)

background = 0

slope = 0.000152103 (se = 0.000322079)

power = 1.62425 (se = 0.383589)

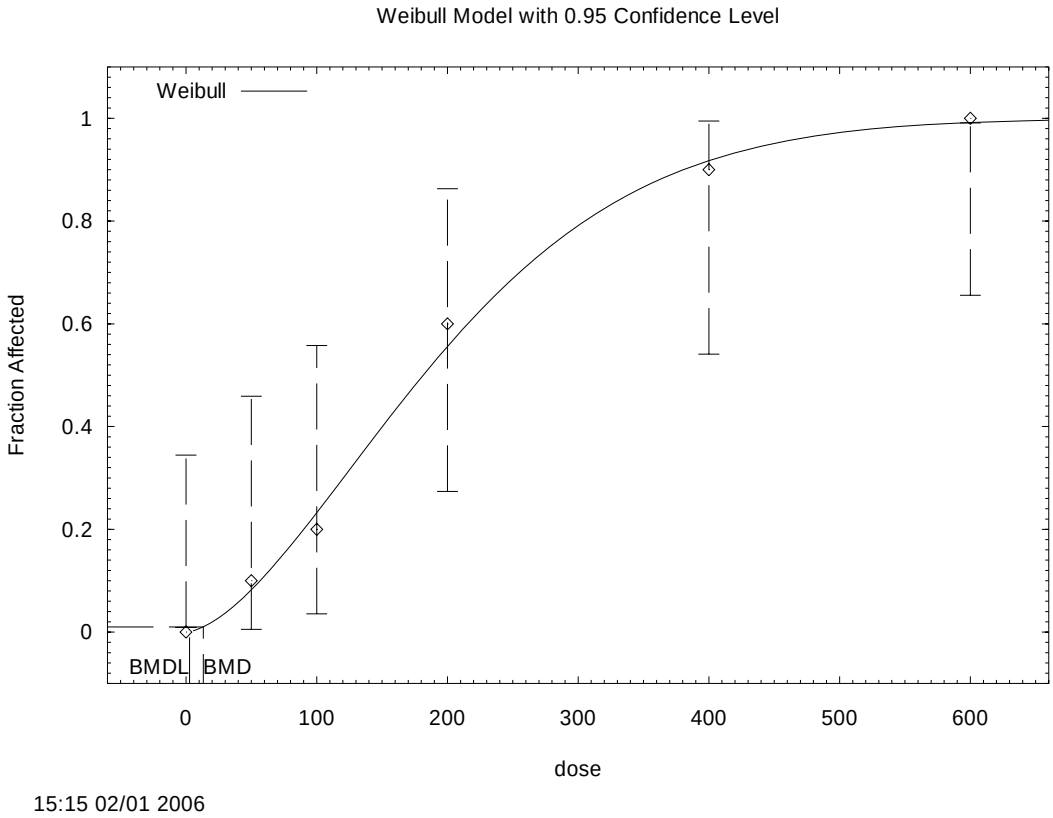

Figure B-6. Observed and predicted incidences of female B6C3F1 mice exhibiting bromobenzene-induced 1% extra risk for liver lesions following gavage treatment 5 days/week for 90 days

The form and parameters for the Weibull model for female mouse liver effects are as follows:

P[response] = background + (1-background)*[1-EXP(-slope*dose^power)] (Eq. B-6)

background = 0

slope = 0.000152103 (se = 0.000322079)

power = 1.62425 (se = 0.383589)

All available models in the EPA BMDS (version 1.3.2) were fit to absolute liver weight and liver-to-body weight data in male and female Fischer 344/N rats and male and female B6C3F1 mice from the 90-day oral gavage studies (NTP, 1985a,b). The data that were modeled are shown in Table 5-4.

Results from the best fitting models for absolute liver weight and liver-to-body weight ratio in male and female rats and mice are presented in Table 5-5. The $BMDL_{1sd}$ of 25.8 mg/kg-day for increased absolute liver weight in female mice represents the lowest $BMDL_{1sd}$ among the male and female rat and mouse data (see Figure B-7 for a plot of observed and predicted values). The $BMD_{0.5sd}$ and $BMDL_{0.5sd}$ are presented in Table 5-6 (see Figure B-8 for a plot of observed and predicted values).

The 3-degree polynomial model form of the response function for the female mice absolute liver weight ratio data is:

$$Y[dose] = beta\_0 + beta\_1*dose + beta\_2*dose\^2 + ... \quad \text{(Eq. B-7)}$$

A constant variance was assumed.

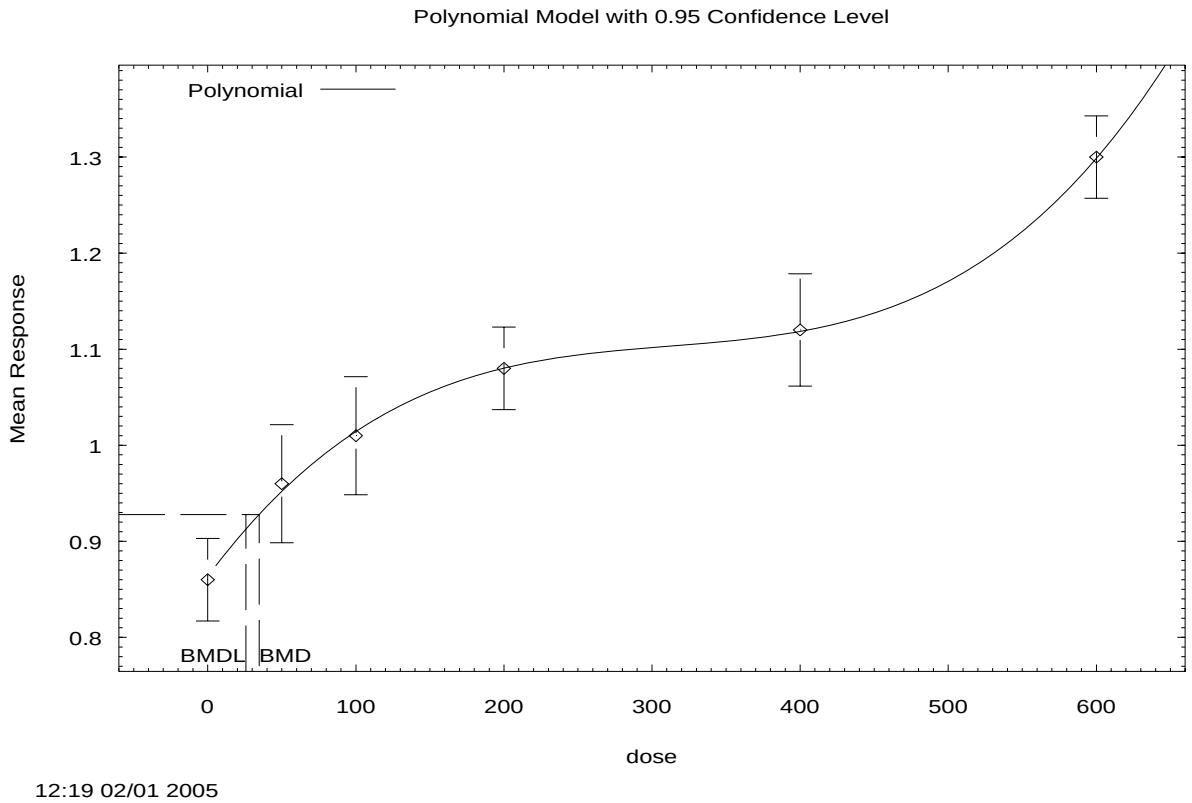

Figure B-7. Observed and predicted 1 standard deviation extra risk for absolute liver weight changes in female B6C3F1 mice administered bromobenzene by gavage 5 days/week for 90 days. BMD=ED_{10}; BMDL=LED_{10}

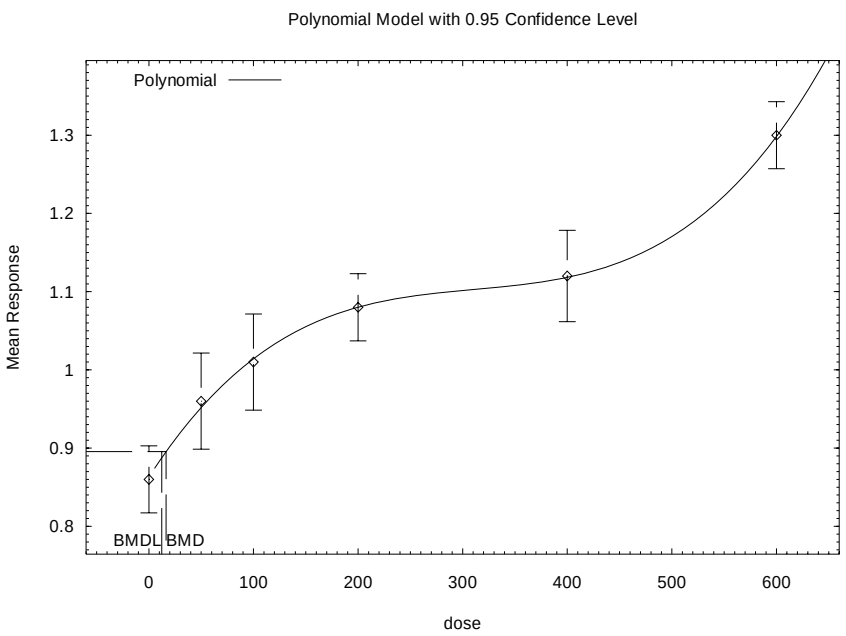

Figure B-8. Observed and predicted 0.5 standard deviation extra risk for absolute liver weight changes in female B6C3F1 mice administered bromobenzene by gavage 5 days/week for 90 days

The form of the response function is:

$$Y[dose] = beta\_0 + beta\_1*dose + beta\_2*dose\text{^}2 + ... \quad \text{(Eq. B-8)}$$

Third degree parameter estimates for 0.5 standard deviation for absolute liver weight data in the female mice are presented in Table B-5.

Table B-5. Third degree polynomial estimates for 0.5 standard deviation for absolute liver weight data

Variable	Estimate	Standard Error
beta_0	0.863152	0.0184339
beta_1	0.002071	0.000348955
beta_2	-6.25619e-006	1.51029e-006
beta_3	6.69735e-009	1.68304e-009

The third-degree polynomial model parameter estimates for 1 standard deviation for absolute liver weight data in female mice are presented in Table B-6.

Table B-6. Third-degree polynomial model parameter estimates for the female mice absolute liver weight data

Variable	Estimate	Standard error
beta 0	0.863152	0.0184339
beta 1	0.002071	0.000348955
beta 2	-6.25619e-006	1.51029e-006
beta 3	6.69735e-009	1.68304e-009
alpha	0.00419238	0.000792286

Serum levels for sorbital dehydrogenase (SDH) for male and female mice were modeled using the linear, polynomial, power and hill models. The power model results for female mice provided the best fit and the results of that model follow.

POWER MODEL FOR SDH FEMALE MICE

The form of the response function is:

$$Y[dose] = control + slope * dose^{power} \quad \text{(Eq. B-9)}$$

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

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 68.6796
rho = 0
control = 12
slope = 0.00131174
power = 1.53281

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-0.99	-0.07	-0.3	0.33
rho	-0.99	1	0.037	0.31	-0.34
control	-0.07	0.037	1	-0.54	0.53
slope	-0.3	0.31	-0.54	1	-1
power	0.33	-0.34	0.53	-1	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
alpha	0.000143716	0.000212936	-0.000273631	0.000561064
rho	0.85033	0.525075	2.8212	4.87945
control	12.9423	0.351905	12.2526	13.6321
slope	1.91405e-006	4.65239e-006	-7.20447e-006	1.10326e-005
power	2.5891	0.392941	1.81895	3.35925

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	13	12.9	1.9	1.66	0.11
50	9	12	13	1.6	1.67	-1.78
100	9	14	13.2	1.8	1.73	1.33
200	10	15	14.7	1.7	2.11	0.481
400	8	23	23.4	4.6	5.18	-0.212
600	10	43	42.8	18.8	16.6	0.0405

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-143.251459	7	300.502917
A2	-87.617373	12	199.234745
A3	-88.708442	8	193.416884
fitted	-91.313743	5	192.627486
R	-174.876017	2	353.752034

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	174.517	10	<0.0001
Test 2	111.268	5	<0.0001
Test 3	2.18214	4	0.7023
Test 4	5.2106	3	0.157

The p-value for Test 1 is less than 0.05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data.

The p-value for Test 2 is less than 0.1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than 0.1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is greater than 0.1. The model chosen seems to adequately describe the data.

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 196.474

BMDL = 145.789

The lowest $BMDL_{1sd}$ from the best fitting model for liver weight changes was 25.8 mg/kg-day, which was very similar to the lowest $BMDL_{10}$ from the best fitting model for combined liver lesions of 24.8 mg/kg-day. For this reason, liver toxicity in female mice, as defined by an increase in liver weight and liver lesions was selected as the critical effect for deriving the subchronic RfD. The average $BMDL_{10}$ of 25 mg/kg-day was selected as the point of departure to derive the chronic and subchronic RfD for bromobenzene. Full modeling results for 10% extra risk for combined liver lesions in the Weibull model in female B6C3F1 mice are presented after Figure B-9.

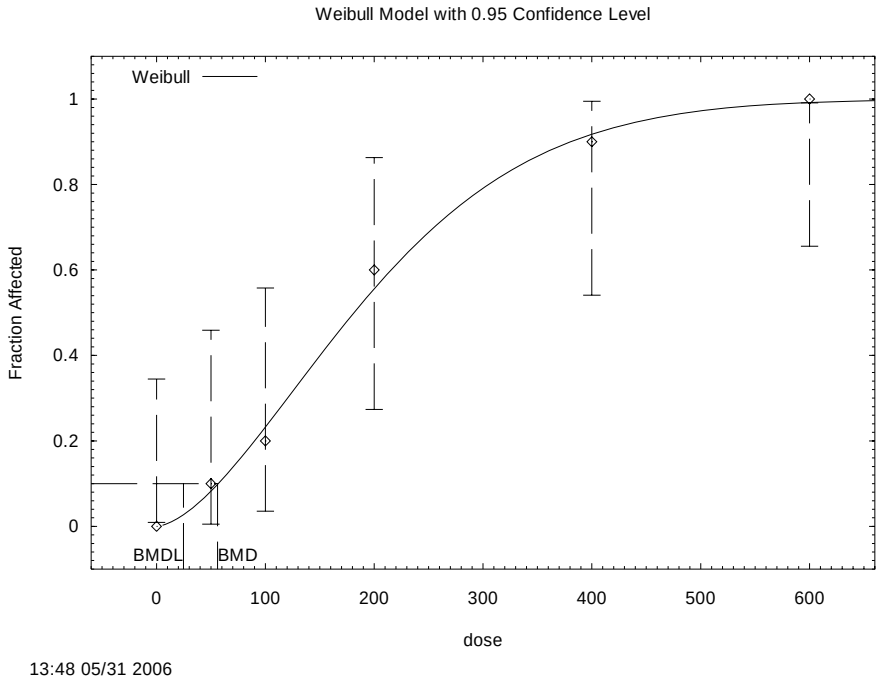

Figure B-9. Full modeling results for 10% extra risk for combined liver lesions in the Weibull model in female B6C3F1 mice treated by oral gavage that were used to estimate the RfD

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-slope*dose^power)] (Eq. B-10)

background = 0

slope = 0.000152103 (se = 0.000322079)

power = 1.62425 (se = 0.383589)

APPENDIX C. BENCHMARK DOSE CALCULATIONS FOR THE RfC

Liver Lesion Data

Incidence data for centrilobular cytomegaly in the liver of female B6C3F1 mice were considered as a potential basis of the RfC, based on the results from the 13-week NTP inhalation study indicating that female mice have a lower point of departure for bromobenzene hepatotoxicity than male mice or male or female rats. The data considered for BMD modeling are shown in Table 5-7. Based on the lack of data points from which to readily characterize exposure-response relationships between no-effect and effect levels (i.e., 100 and 300 ppm), it is expected that a number of sigmoidal models will fit such data adequately and equivalently (e.g., gamma, probit, logistic, higher degree multistage). As a consequence, considerable uncertainty about the 'best' model among sigmoidal models is expected.

Sigmoidal models and two non-sigmoidal models (quantal quadratic and quantal linear) in the U.S. EPA BMDS (version 1.3.2.) were fit to the data in Table 5-7. Modeling results are presented in Table C-1 showing that (1) all sigmoidal models provided excellent fit to the data (as expected due to the nature of the data) (2) the non-sigmoidal models provided poorer fits to the data, and (3) all sigmoidal models provided similar estimates of BMC_{10} values (ranging from about 77 to 97 ppm, a 1.3-fold range) and $BMCL_{10}$ values (ranging from about 40 to 60 ppm, a 1.5-fold range). Following U.S. EPA (2000c) guidance for selecting models for point of departure computation, the model with the best fit and the lowest AIC is selected to calculate the BMCL. The log-logistic and gamma models both have the best fit and the lowest AIC value (Table C-1). The $BMCL_{10}$s from these best-fitting models (log-logistic and gamma models) were averaged (55 ppm) to arrive at the point of departure for deriving the RfC, as per U.S. EPA (2000c) guidance. Estimated BMCs and BMCLs associated with 5 and 1% extra risk are presented in Table 5-9. Figures C-1, C-2 and C-3 are plots of the log-logistic models for 10%, 5% and 1% extra risk, respectively. Figures C-4, C-5 and C-6 are plots of the gamma models for 10%, 5% and 1% extra risk, respectively. Figures C-1 and C-4 are plots of observed and predicted values for 10% extra risk from the log-logistic and gamma models, respectively, which were used for the RfC determination. Full modeling details for the 10% log-logistic and gamma models appear at the end of Appendix C.

Table C-1. BMC modeling results for the incidence of liver cytomegaly in female B6C3F1 mice exposed to bromobenzene vapors 6 hours/day, 5 days/week for 13 weeks

Model	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)	x^2 p-value	AIC
Log-logistic[a]	95.59	58.73	1.00	12.01
Gamma[b]	89.24	51.42	1.00	12.01
Multi-stage[c]	77.09	40.33	0.999	12.17
Weibull[b]	92.34	47.08	1.00	14.01
Log-probit[a]	92.95	57.45	1.00	14.01
Logistic	96.75	59.75	1.00	14.01
Probit	93.71	54.94	1.00	14.01
Quantal quadratic	55.15	40.15	0.87	14.05
Quantal linear	21.38	13.18	0.16	22.78

[a]Slope restricted to >1
[b]Restrict power > = 1
[c]Restrict betas > = 0; degree of polynomial = 3 (maximum degree restricted to #dose groups minus 2)

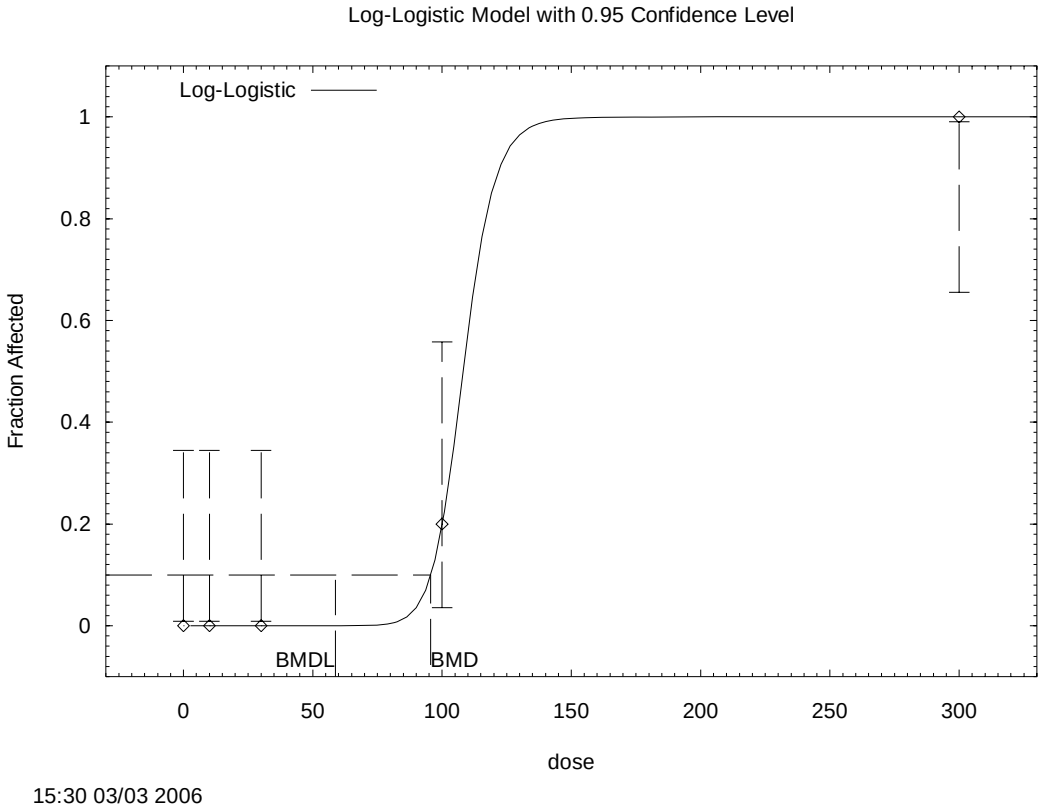

Figure C-1. Observed and predicted incidences of female B6C3F1 mice exhibiting 10% extra risk of bromobenzene-induced hepatocellular cytomegaly following inhalation exposure for 6 hours/day, 5 days/week for 13 weeks. Log-logistic model predictions. dose=concentration in ppm.

The form and parameters of the log-logistic model for the incidence of female mouse cytomegaly are as follows:

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

background = 0

intercept = -84.2793 (se = 0.790565)

slope = 18

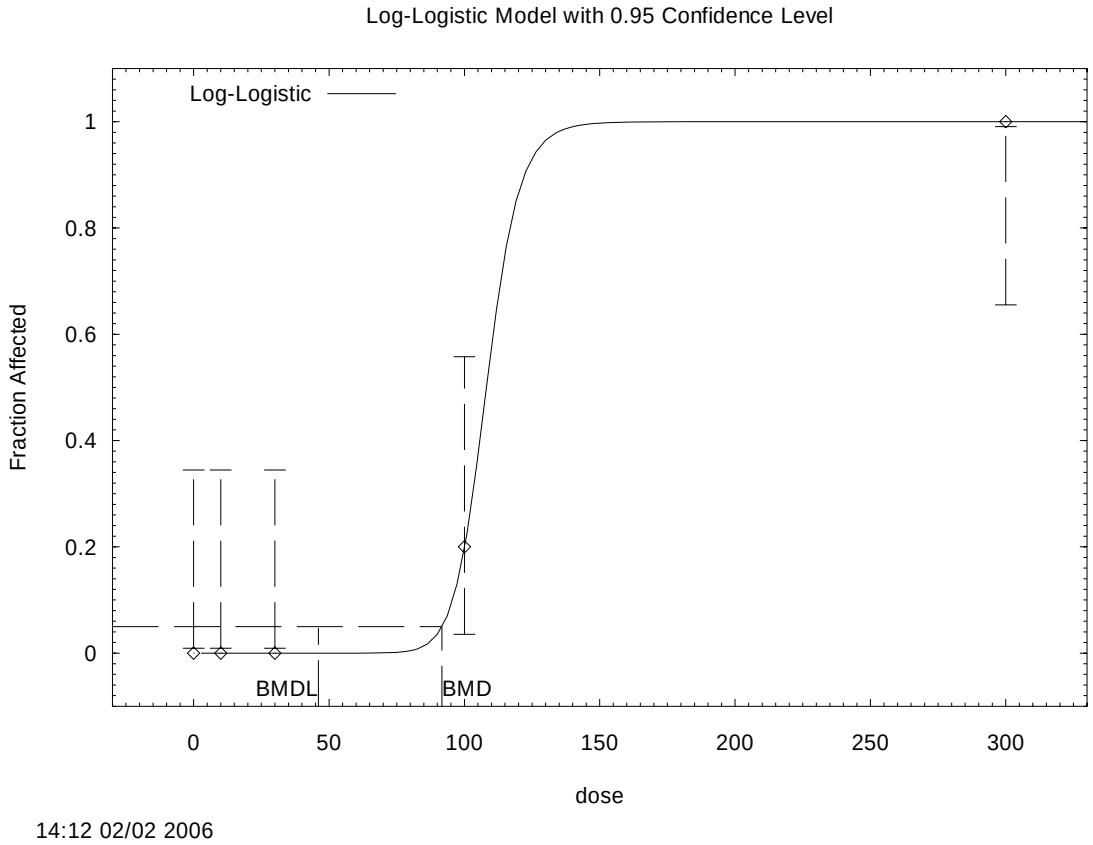

Figure C-2. Observed and predicted incidences of B6C3F1 mice exhibiting 5% extra risk of bromobenzene-induced hepatocellular cytomegaly following inhalation exposure for 6 hours/day, 5 days/week for 13 weeks. Log-logistic model predictions. dose=concentration in ppm.

The form and parameters of the log-logistic model for incidence of female mice cytomegaly are as follows:

P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))] (Eq. C-2)

background = 0

intercept = -84.2793 (se = 0.790565)

slope = 18

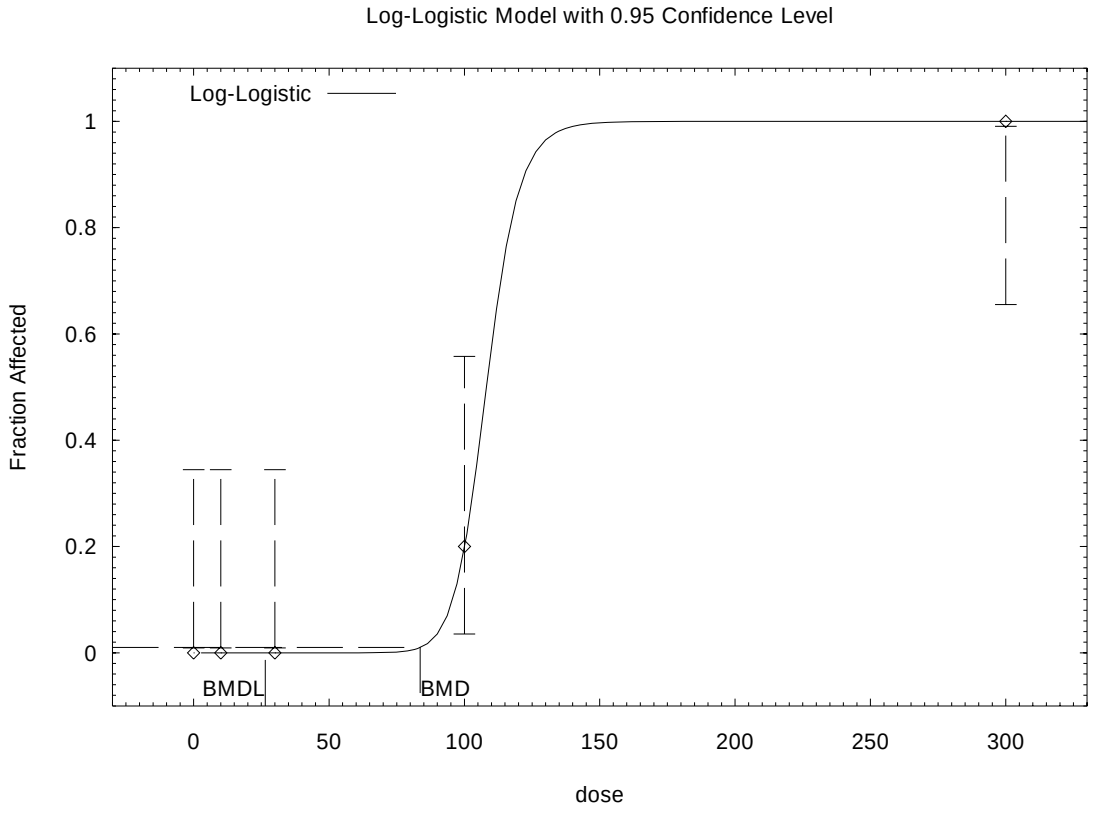

Figure C-3. Observed and predicted incidences of female B6C3F1 mice exhibiting 1% extra risk for bromobenzene-induced hepatocellular cytomegaly following inhalation exposure for 6 hours/day, 5 days/week for 13 weeks. Log-logistic model predictions. dose=concentration in ppm.

The form and parameter estimates of the log-logistic model for incidence of female mice cytomegaly are as follows:

P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))] (Eq. C-3)

background = 0

intercept = -84.2793 (se = 0.790565)

slope = 18

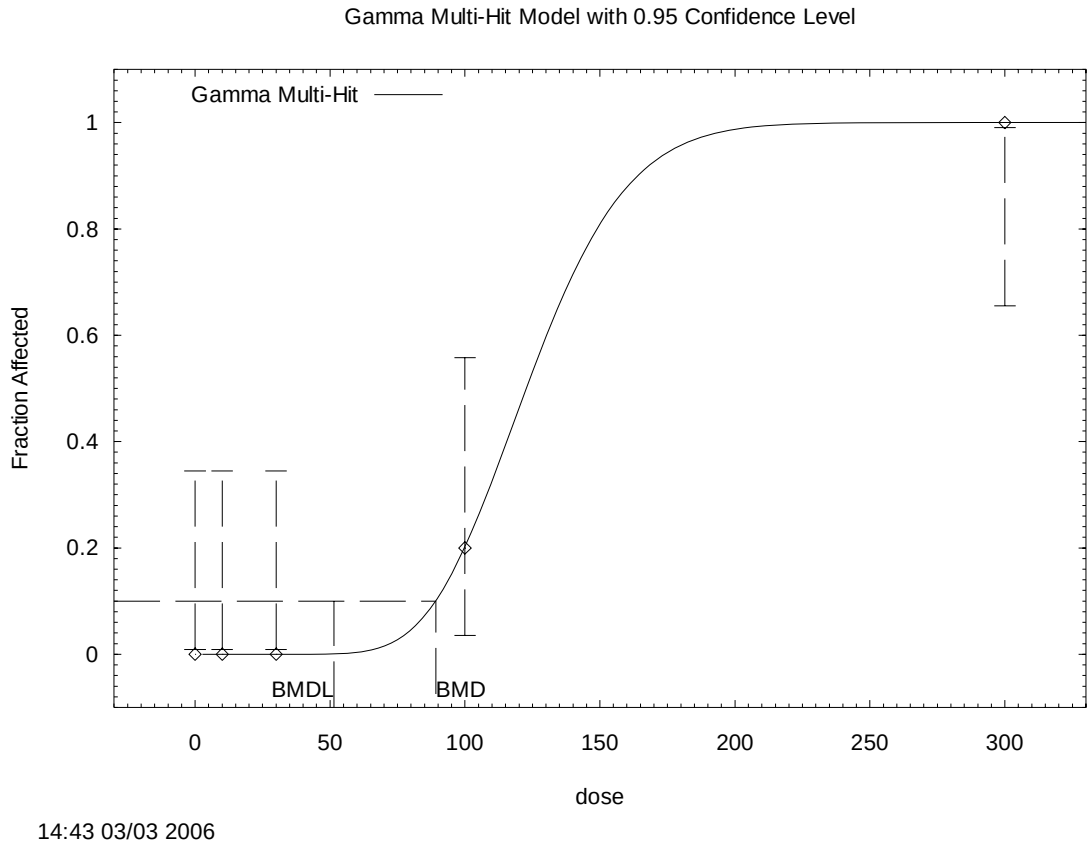

Figure C-4. Observed and predicted incidences of female B6C3F1 mice exhibiting 10% extra risk of bromobenzene-induced hepatocellular cytomegaly following inhalation exposure for 6 hours/day, 5 days/week for 13 weeks. Gamma model predictions. dose=concentration in ppm.

The form and parameters of the gamma model for the incidence of female mouse cytomegaly are as follows:

P[response]= background+(1-background)*CumGamma[slope*dose,power] (Eq. C-4)

where CumGamma(.) is the cumulative Gamma distribution function]

background = 0

slope = 0.143677 (se = 0.0164918)

power = 18

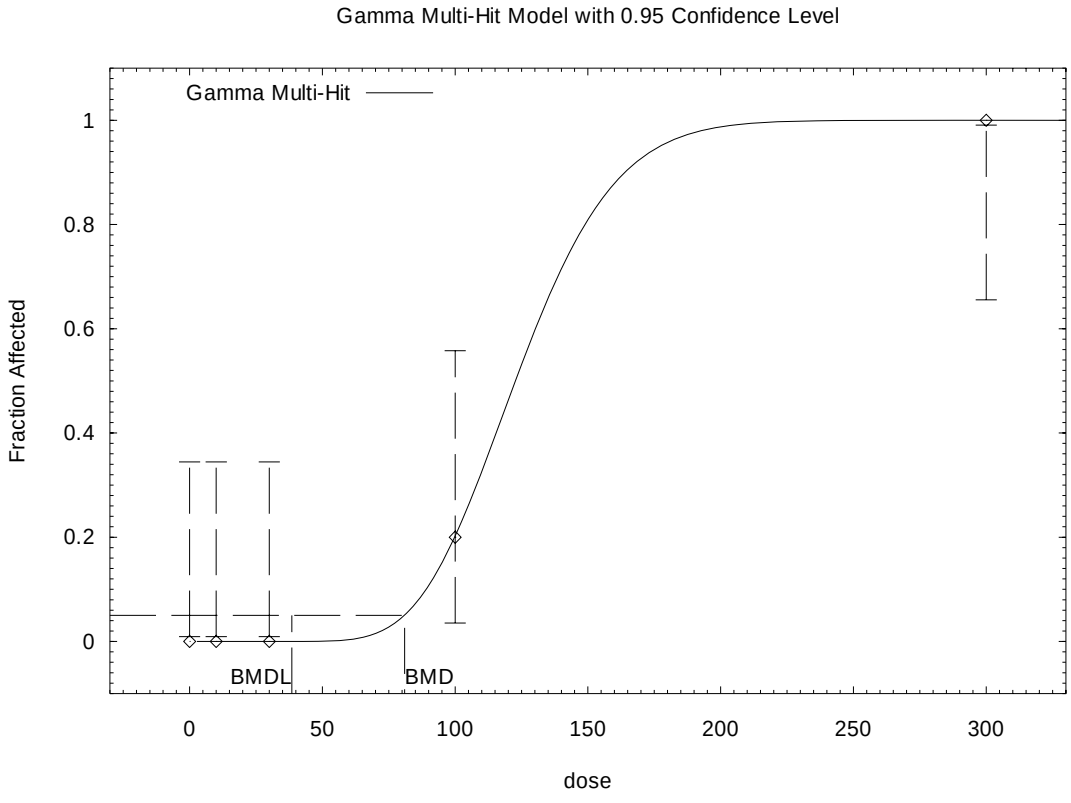

Figure C-5. Observed and predicted incidences of female B6C3F1 mice exhibiting 5% extra risk of bromobenzene-induced hepatocellular cytomegaly following inhalation exposure for 6 hours/day, 5 days/week for 13 weeks. Gamma model predictions. dose=concentration in ppm.

The form and parameters of the gamma model for 5% extra risk for female mouse cytomegaly are as follows:

P[response]= background+(1-background)*CumGamma[slope*dose,power] (Eq. C-5)

where CumGamma(.) is the cumulative Gamma distribution function

background = 0

slope = 0.0143677 (se = 0.0164918)

power = 18

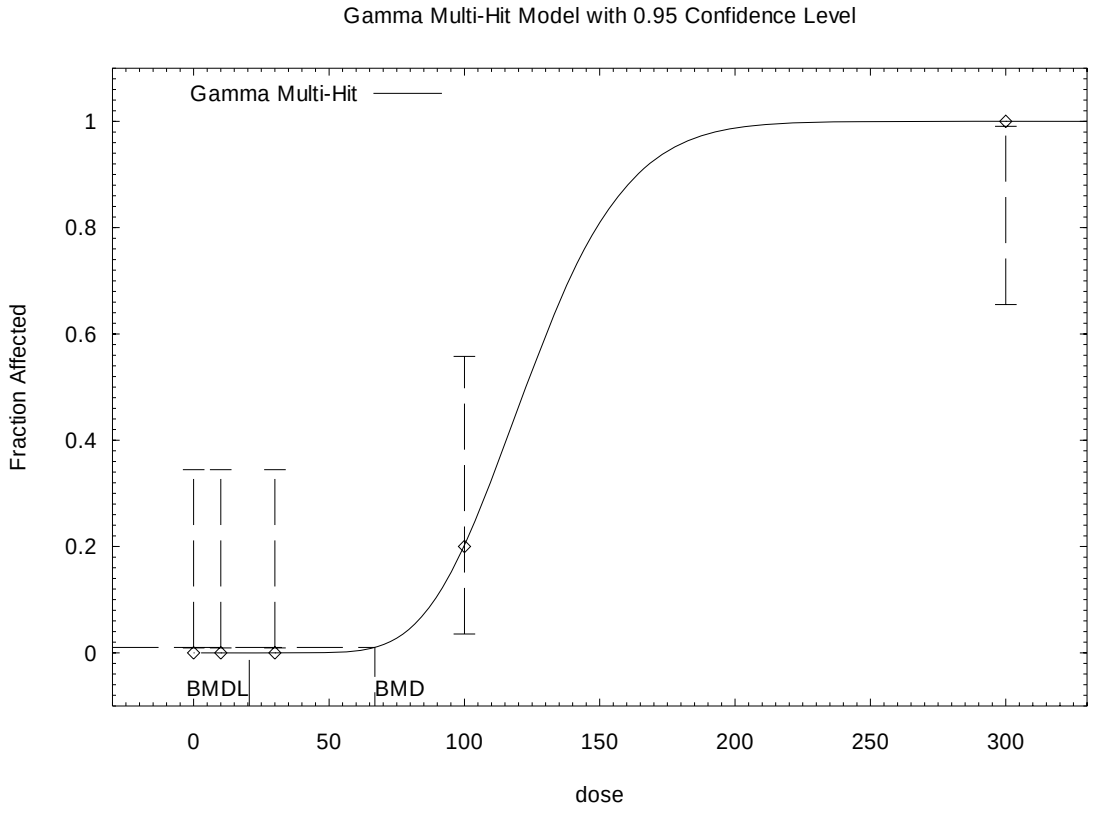

Figure C-6. Observed and predicted incidences of female B6C3F1 mice exhibiting 1% extra risk of bromobenzene-induced cytomegaly following inhalation exposure for 6 hours/day, 5 days/week for 13 weeks. Gamma model predictions. dose=concentration in ppm.

The form and parameters of the gamma model for 1% extra risk for female mice cytomegaly are as follows:

P[response]= background+(1-background)*CumGamma[slope*dose,power] (Eq. C-6)

where CumGamma(.) is the cumulative Gamma distribution function

background = 0

slope = 0.143677 (se = 0.0164918)

power = 18

Liver Weight Data

Absolute liver weight and liver-to-body weight ratio (relative liver weight) in female mice were also considered as potential bases of the RfC. All available models in the EPA BMDS (version 1.3.2) were fit to absolute liver weight data and liver-to-body weight ratio for female B6C3F1 mice from the 13-week inhalation study (NTP, 1985d). The data that were modeled are shown in Table 5-12. The model outputs are displayed in Table C-2. Second-degree polynomial models provided the best fit for the absolute and relative liver weight data for female mice, as determined by the AIC, and yielded $BMCL_{1sd}$s of 33.51 ppm and 33.90 ppm, respectively (Table C-2). See Figures C-7 and C-8 for a plot of observed and predicted values 1sd and 0.5sd, respectively, for absolute liver weight. See Tables C-3 and C-4 for model outputs for the second-degree polynomial models for 1sd and 0.5sd extra risk for absolute liver weight. See Figures C-9 and C-10 for a plot of the observed and predicted values for the second-degree polynomial model for 1sd and 0.5sd extra risk for relative liver weight. Model outputs are displayed in Tables C-5 and C-6. The $BMC_{0.5sd}$ and $BMCL_{0.5sd}$ are presented in Table 5-14.

Table C-2. Model output for increased absolute liver weight and liver-to-body weight ratio in female B6C3F1 mice following inhalation exposure to bromobenzene for 6 hours/day, 5 days/week for 13 weeks

Model[a]	BMC (ppm)	$BMCL_{1sd}$ (ppm)	x^2 p-value	AIC
Absolute liver weight[b]				
Linear	35.24	28.39	0.1838	-150.18
Polynomial (2^o)	**52.38**	**33.51**	**0.3922**	**-151.16**
Polynomial (3^o)	32.67	14.45	0.2891	-149.91
Power	56.82	32.56	0.2901	-150.55
Liver-to-body weight ratio[b]				
Linear	41.03	34.52	0.08619	183.82
Polynomial (2^o)	**52.42**	**33.90**	**0.09284**	**182.19**
Polynomial (3^o)	45.52	18.56	0.09301	184.05
Power	57.55	34.12	0.07211	182.77

[a]Statistical tests indicated that variances were not constant across exposure groups. Model results are for non-homogeneous variance, with the exception of the linear and third-degree polynomial models for liver-to-body weight ratio.
[b]Modeled as a continuous variable using one standard deviation as the BMR.

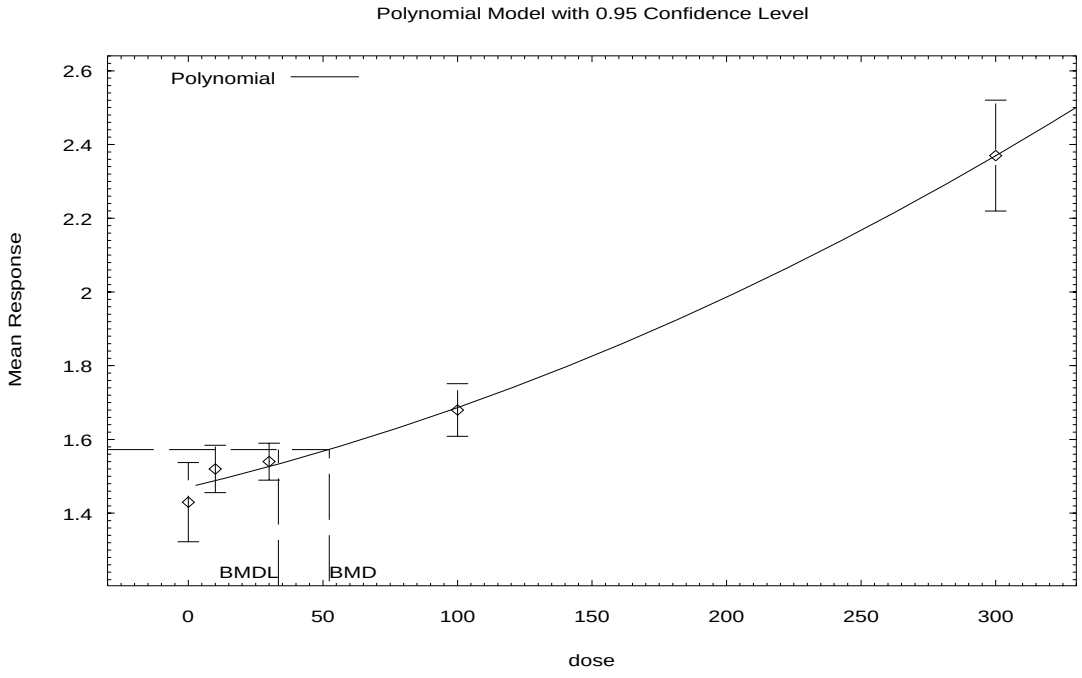

Figure C-7. The second-degree polynomial model prediction for changes 1 standard deviation extra risk in absolute liver weight in female B6C3F1 mice exposed to bromobenzene vapors for 6 hours/day, 5 days/week for 13 weeks

The second-degree polynomial model form of the response function for the female mouse absolute liver weight data is:

$$Y[dose] = beta\_0 + beta\_1*dose + beta\_2*dose\text{^}2 + ... \quad \text{(Eq. C-1)}$$

The variance was modeled as:

$$Var(i) = alpha*mean(i)\text{^}rho \quad \text{(Eq. C-2)}$$

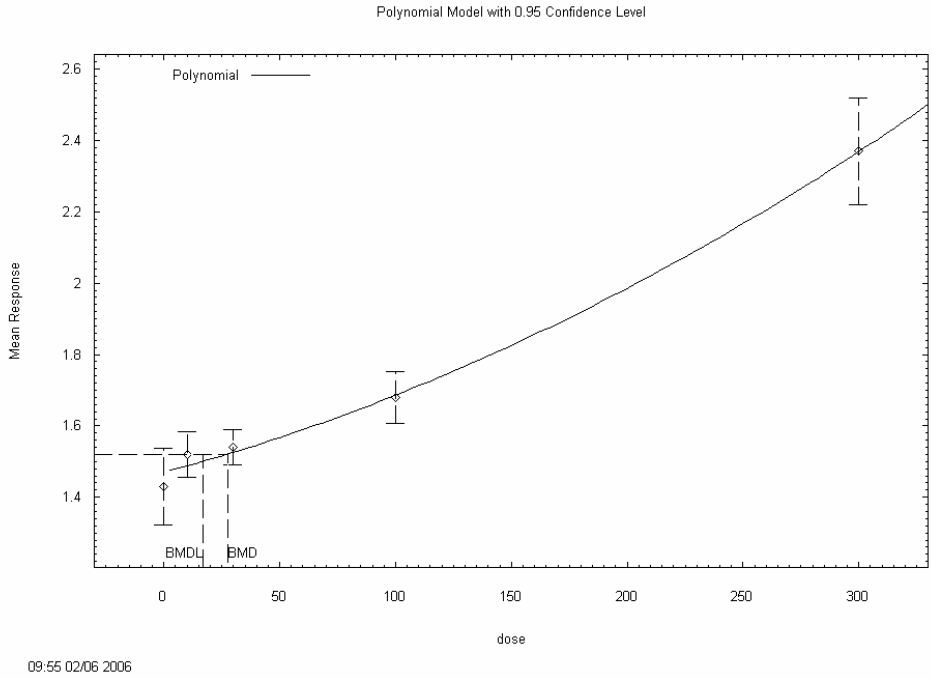

Figure C-8. The second-degree polynomial model prediction for changes 0.5 standard deviation extra risk in absolute liver weight in female B6C3F1 mice exposed to bromobenzene vapors for 6 hours/day, 5 days/week for 13 weeks

The second-degree polynomial model parameter estimates for the absolute liver weight data in the female mice are presented in Table C-2.

The form of the response function is:

$$Y[dose] = beta\_0 + beta\_1*dose + beta\_2*dose\^2 + ... \quad \text{(Eq. C-7)}$$

Table C-3. Second-degree polynomial model parameter estimates for 1 standard deviation extra risk in absolute liver weight for the female B6C3F1 mice with variance as a power function of dose

Variable	Estimate	Standard error
beta 0	1.47	0.02
beta 1	0.002	0.0007
beta 2	0.000004	0.000002
alpha	0.004	0.002
rho	2.45	1.03

Table C-4. Second-degree polynomial model parameter estimates for 0.5 standard deviation extra risk in absolute liver weight for female B6C3F1 mice with variance as a power function of dose

Variable	Estimate	Standard error
beta 0	1.46979	0.0233122
beta 1	0.00174434	0.000695665
beta 2	4.19465e-006	2.34312e-006
alpha	0.00412809	0.00234934
rho	2.44506	1.02732

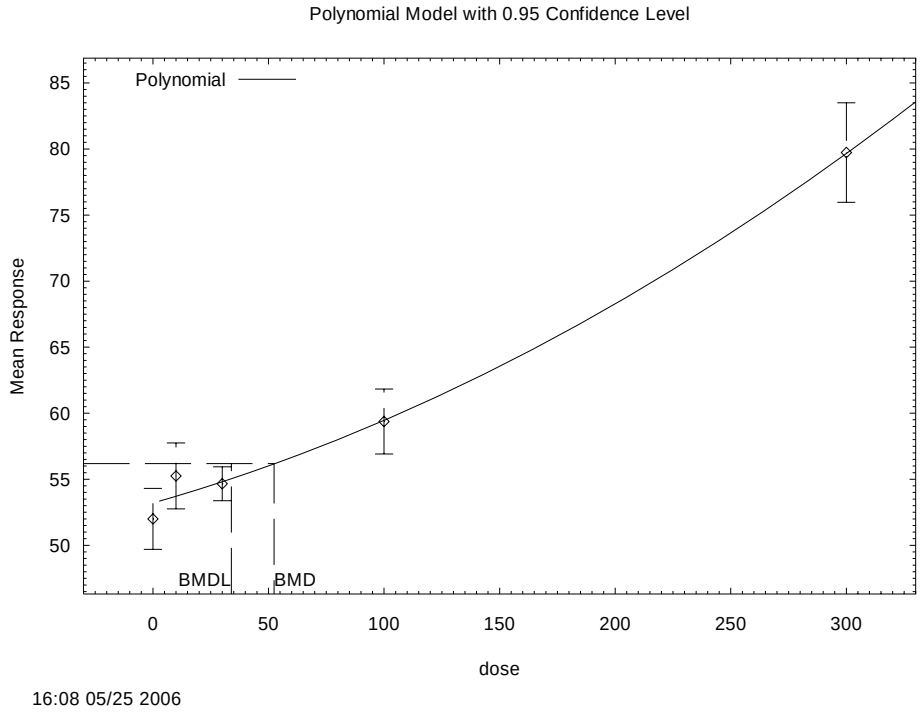

Figure C-9. The second-degree polynomial prediction for 1 standard deviation extra risk in relative liver weight in female B6C3F1 mice exposed to bromobenzene vapors for 6 hours/day, 5 days/week for 13 weeks

The second degree polynomial model form of the response function for the female mice relative liver weight is:

$$Y[dose] = beta\_0 + beta\_1*dose + beta\_2*dose\text{^}2 + \quad \text{(Eq. C-8)}$$

The variance was modeled as:

$$Var(i) = alpha*mean(i)\text{^}rho \quad \text{(Eq. C-9)}$$

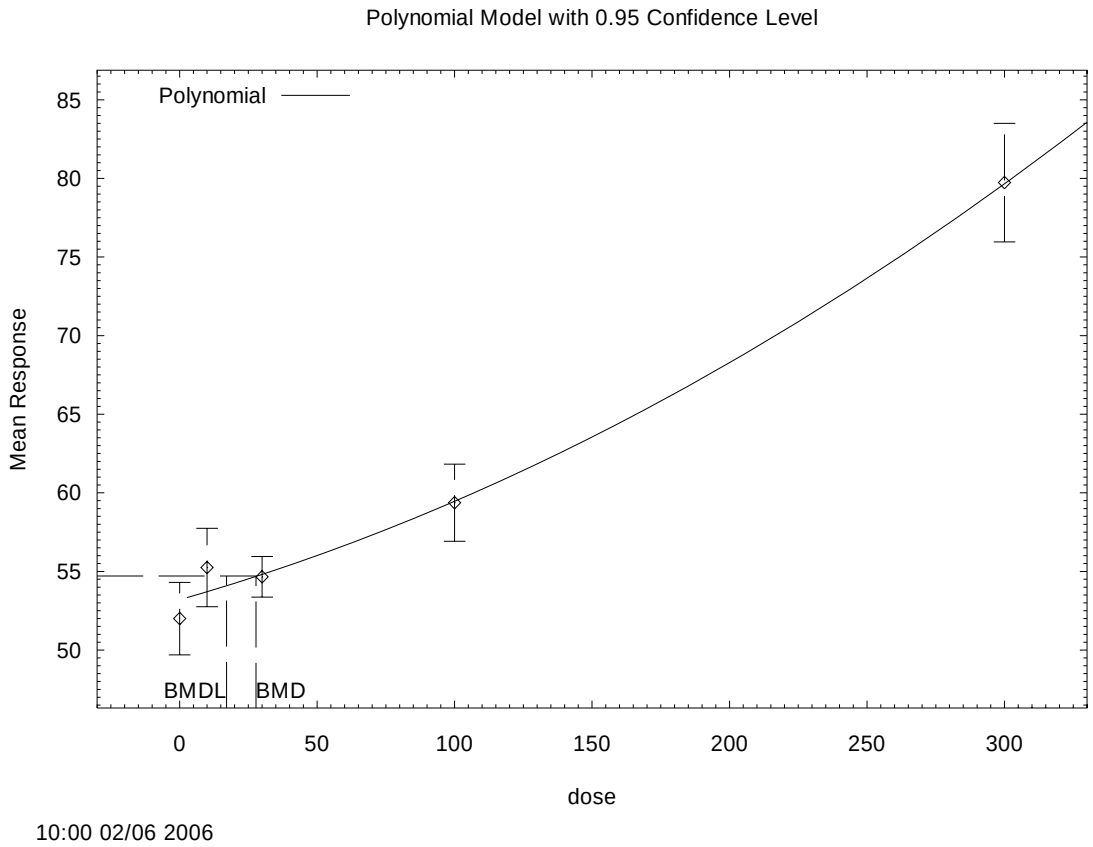

Figure C-10. The second-degree polynomial prediction for 0.5 standard deviation changes in relative liver weight in female B6C3F1 mice exposed to bromobenzene vapors for 6 hours/day, 5 days/week for 13 weeks

The second degree polynomial model form of the response function is:

$$Y[dose] = beta\_0 + beta\_1*dose + beta\_2*dose\text{^}2 + ... \quad \text{(Eq. C-10)}$$

Table C-5. Second-degree polynomial model parameter estimates for 1 standard deviation extra risk in relative liver weight for female B6C3F1 mice with variance as a power function of dose

Variable	Estimate	Standard error
beta 0	53.2265	0.668405
beta 1	0.0498228	0.0195324
beta 2	0.000128264	6.5069e-005
alpha	0.000538819	0.00280452
rho	2.44035	1.27305

Table C-6. Second-degree polynomial model parameter estimates for 0.5 standard deviation extra risk in relative liver weight for female B6C3F1 mice with variance as a function of dose

Variable	Estimate	Standard error
beta 0	53.2265	0.668405
beta 1	0.0498228	0.0195324
beta 2	0.000128264	6.5069e-005
alpha	0.000538819	0.00280452
rho	2.44035	1.27305

The average $BMCL_{10}$ from the log-logistic and gamma models for cytomegaly in female mice was selected as the point of departure to derive the subchronic and chronic RfC for bromobenzene. Full modeling results for the log-logistic model appear after Figure C-11 and full modeling results for the gamma model appear after Figure C-12.

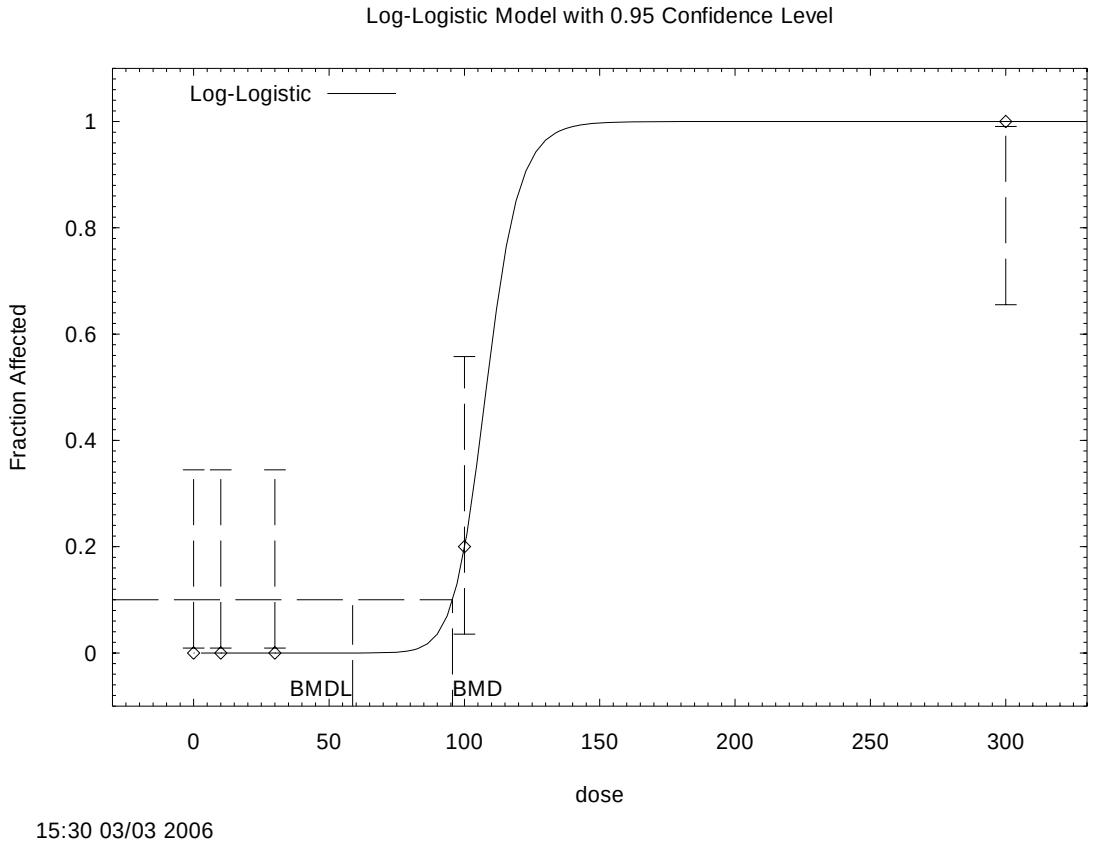

Figure C-11. Full modeling results for 10% extra risk for cytomegaly in the log-logistic model in female B6C3F1 mice treated by inhalation that were used to estimate the RfC

The form of the probability function is:

P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))] (Eq. C-11)

Dependent variable = response
Independent variable = dose
Slope parameter is restricted as slope >= 1

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = -8.09038
slope = 1.74428

Asymptotic Correlation Matrix of Parameter Estimates

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

	intercept
intercept	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0	NA
intercept	-84.2793	0.790565
slope	18	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	-5.00402			
Fitted model	-5.00402	2.08911e-007	4	1
Reduced model	-27.554	45.0999	4	<.0001

AIC: 12.008

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
0.0000	0.0000	0.000	0	10	0
10.0000	0.0000	0.000	0	10	-1.581e-009
30.0000	0.0000	0.000	0	10	-3.112e-005
100.0000	0.2000	2.000	2	10	-2.199e-005
300.0000	1.0000	10.000	10	10	0.0003213

Chi-square = 0.00 DF = 4 P-value = 1.0000

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 95.5947

BMDL = 58.7312

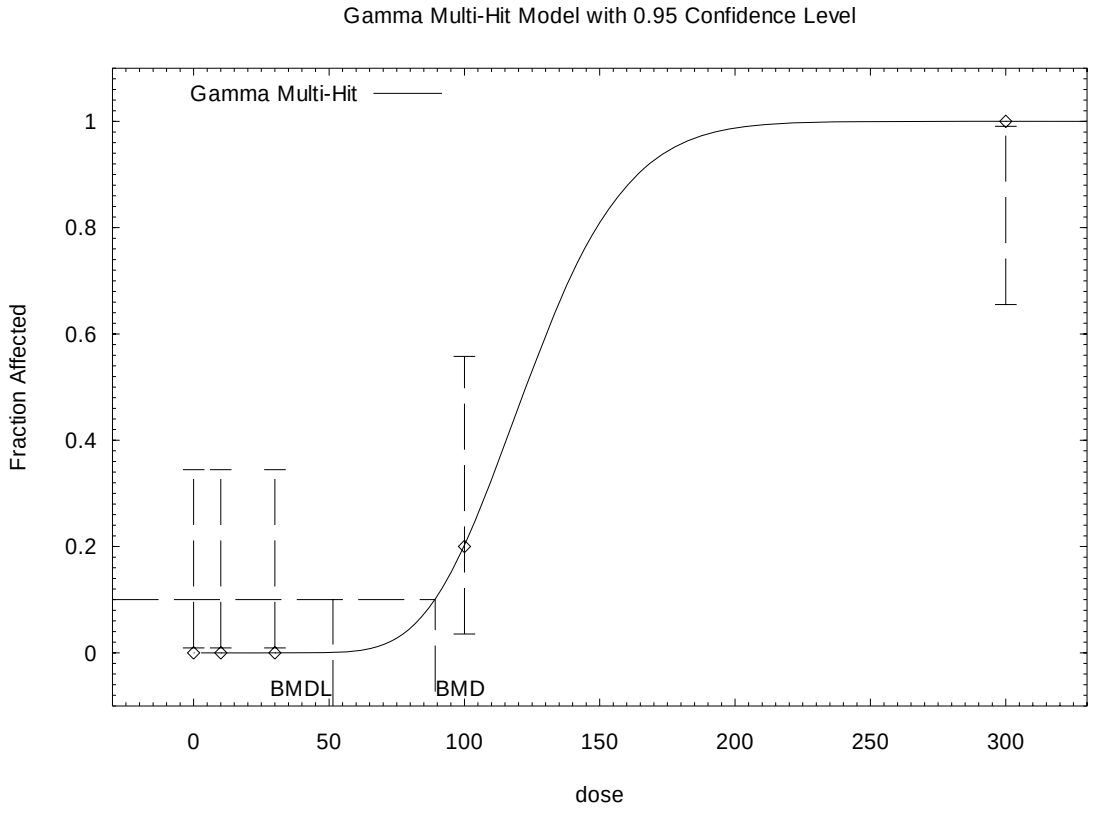

Figure C-12. Full modeling results for 10% extra risk for cytomegaly in the gamma model in female B6C3F1 mice treated by inhalation that were used to estimate the RfC

The form of the probability function is:

P[response]= background+(1-background)*CumGamma[slope*dose,power] (Eq. C-12)

where CumGamma(.) is the cumulative Gamma distribution function

Dependent variable = response
Independent variable = dose
Power parameter is restricted as power >=1

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
Default Initial (and Specified) Parameter Values
background = 0.0454545

slope = 0.00531194
power = 1.3

Asymptotic Correlation Matrix of Parameter Estimates

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

	Slope
Slope	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA
Slope	0.143677	0.0164918
Power	18	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	-5.00402			
Fitted model	-5.00408	0.000120288	4	1
Reduced model	-27.554	45.0999	4	<.0001

AIC: 12.0082

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
0.0000	0.0000	0.000	0	10	0
10.0000	0.0000	0.000	0	10	-5.228e-007
30.0000	0.0000	0.000	0	10	-0.00267
100.0000	0.2000	2.000	2	10	-0.000151
300.0000	1.0000	10.000	10	10	0.007281

Chi-square = 0.00 DF = 4 P-value = 1.0000

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 89.2392

BMDL = 51.4215