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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)

September 2009

U.S. Environmental Protection Agency
Washington, DC

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LIST OF ABBREVIATIONS AND ACRONYMS

AIC	Akaike's Information Criteria
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMC	benchmark concentration
BMC_{10}	benchmark concentration associated with a 10% response level
BMCL	95% lower confidence limit on the benchmark concentration
$BMCL_{10}$	95% lower confidence limit on the benchmark concentration associated with a 10% response level
BMD	benchmark dose
BMDL	95% lower confidence limit on the benchmark dose
$BMDL_{10}$	95% lower confidence limit on the benchmark dose associated with a 10% response level
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
CASRN	Chemical Abstract Service Registry Number
DEN	diethylnitrosamine
FEL	frank-effect level
GC-MS	gas chromatography-mass spectrometry
GST-P^+	glutathione S-transferase placental form-positive
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
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
POD	point of departure
RfC	inhalation reference concentration
RfD	oral reference dose
SDH	sorbitol dehydrogenase
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency

FOREWORD

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

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

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

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This document has been reviewed by EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A.

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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 other exposure durations, and a carcinogenicity assessment.

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

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

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 (NRC, 1983). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk*

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

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through December, 2008.

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 shown in Figure 2-1 and Table 2-1.

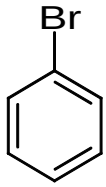


Figure 2-1. Chemical Structure of bromobenzene.

Table 2-1. Selected chemical and physical properties 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. Soluble 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	K_{oc} = 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 (4.5×10^3 kg) in 1986, 1990, 1994, 1998, and 2002 (U.S. EPA, 2002). 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 as 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 2.47×10^{-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 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 is not 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, based on 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, Massachusetts that is over 3,500 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 >10,000 and <10,000 persons. Bromobenzene was detected above 0.5 μg/L (quantitation limit) in 3 out of 280 random sample sites serving <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 >10,000 persons at 1.7 μg/L. In 2 of 321 nonrandom sample sites serving <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 >10,000 persons. Bromobenzene was detected 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). 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 2,664 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, Arkansas collected from 1976 to 1978, bromobenzene concentrations ranged from 0.83 to 2,100 ppt, with a mean concentration of 210 ppt. In 28 air samples from Magnolia, Arkansas 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, Arizona or in one air sample from Edison, New Jersey.

Heikes et al. (1995) detected bromobenzene in 2 of 234 table foods above the limit of quantitation (1.83 ppb) using U.S. 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 are frequently encountered in table-ready foods as contaminant residues and that foods high in fat had more elevated levels (>1,000 ppb).

3. TOXICOKINETICS

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., 1985, 1984; 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 headspace GC) in the blood of laboratory animals immediately following a single 4-hour inhalation exposure to bromobenzene vapors (Aarstad et al., 1990). At 1,000 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 throughout the body, 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 postinjection, the highest levels of bromobenzene were found in fat (5,600 μg/g tissue), followed by the 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 as follows: 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, the 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 the 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 the spleen and adipose tissues. Concentrations of bromobenzene were reported to show a pattern (data not reported) of peaking within 10 hours after dosing, followed by rapidly decreasing concentrations (Ogino, 1984b).

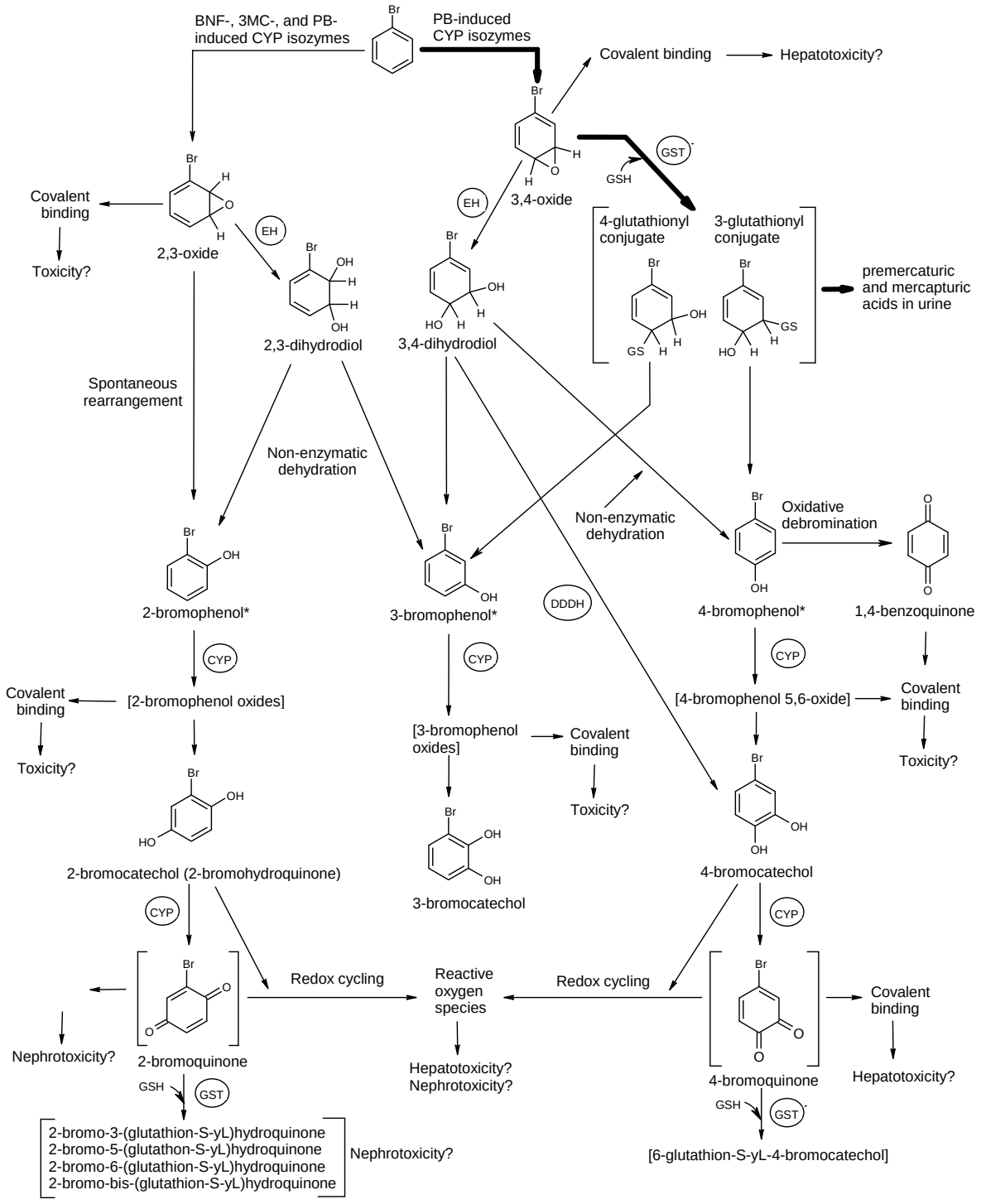
In order to monitor tissue distribution immediately following exposure, male Sprague-Dawley rats were administered [^{14}C]-bromobenzene intravenously at a dose of 10 μmol/kg and plasma levels of radioactivity were monitored (Zampaglione et al., 1973). Plasma levels dropped triphasically during 70 minutes 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 primarily by phenobarbital-induced cytochrome isozymes (e.g., CYP 450 1A2, 2A6, 2B6, and 3A4), or the 2,3-oxide derivative catalyzed primarily by 3-methylcholanthrene and β-naphthoflavone-induced CYP isozymes (e.g., CYP 450 1A1, 1A2, and 1B1). However, both inducible CYP classes are partially

involved in the formation of the 2,3 and 3,4-oxide metabolites (Girault et al., 2005; Krusekopf et al., 2003; Lau and Zannoni, 1981a, 1979; Zampaglione et al., 1973; Reid et al., 1971).



Sources: Adapted from Lertratanangkoon et al. (1993); Lau and Monks (1988).

Figure 3-1. Proposed metabolic scheme for bromobenzene in mammals.

The predominant metabolic pathway in the rat liver leads to enzymatic (glutathione-S-transferase) conjugation of the 3,4-oxide derivative with glutathione. This is 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 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. In addition to glutathione conjugation, early studies suggested that bromobenzene metabolites are also glucuronidated and sulfated (Jollow et al., 1974; Zampaglione et al., 1973). However, urinary metabolite profiles from F344 rats repeatedly exposed to nonhepatotoxic (7.9 and 79 mg/kg-day) and toxic doses (790 mg/kg-day) of bromobenzene for 1 week suggested that glucuronidation and sulfation play a minor role in metabolism at lower, potentially environmentally relevant exposure doses (Chadwick et al., 1987). Specifically, compared to vehicle control treated animals, no significant increase in urinary biomarkers of phase II metabolism such as total glucuronides, sulfates, or mercapturic acids occurred in rats treated intraperitoneally once a day with 7.9 or 79 mg/kg-day for 7 days. Conversely, at a presumably hepatotoxic dose of bromobenzene (790 mg/kg-day), several urinary biomarkers indicative of phase I or II metabolism were significantly increased compared to controls (Chadwick et al., 1987).

Both the 3,4- and 2,3-oxide derivatives may be converted to the corresponding dihydrodiols by epoxide hydrolase. 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; Lertratanangkoon and Horning, 1987). 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 non-enzymatic dehydration of the 2,3-dihydrodiol (Lertratanangkoon et al., 1993, 1987; see also Figure 3-1). Other pathways to the formation of 3-bromophenol may include non-enzymatic dehydration of the 3,4-dihydrodiol or rearrangement of the 4-S-glutathione conjugate of the 3,4-oxide derivative (Lertratanangkoon et al., 1993, 1987). Non-enzymatic dehydration 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., 1993, 1987).

The bromophenol metabolites may be subsequently oxidized by CYP enzymes 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

predominates in the rat in vivo (Miller et al., 1990). The 4-bromophenol may undergo oxidative debromination to form 1,4-benzoquinone (Zheng and Hanzlik, 1992; Slaughter and Hanzlik, 1991). Redox cycling of 2- and 4-bromocatechol and conjugation by glutathione S-transferase produce 2-bromo-3-(glutathione-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 were 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 4.5.2).

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, including those involved in metabolism. 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, cellular response to reduced glutathione levels, the acute phase response, and intracellular signaling, following intraperitoneal administration of bromobenzene to rats. Other studies (Waters et al., 2006; Minami et al., 2005; Stierum et al., 2005) have utilized toxicogenomics to characterize the relationship between bromobenzene hepatotoxicity and hepatic gene expression profiles (see Section 4.5.3).

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 the 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 1,570 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). Biliary excretion of bromobenzene metabolites was also 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 in normal non-cannulated rats in which 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), 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 (1,178 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 radio-labeled bromobenzene (Zampaglione et al., 1973). In this study, a second-phase half-life was not calculated.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

No physiologically based toxicokinetic 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 are not available.

4.2. SUBCHRONIC 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[1] have not been published by NTP; however, reports including the review comments and conclusions of NTP's Pathology Working Group (NTP, 1986a) were obtained from NTP.

Groups of 10 male and 10 female F344/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 bromobenzene 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.

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[1]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.

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 and periodic acid-Schiff 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 in the 600 mg/kg-day dose group (9/10 males and 7/10 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 in male rats exposed to lower doses of bromobenzene 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 ≥100 mg/kg-day in male rats and at all dose levels in female rats. Changes in the 600 mg/kg-day 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. Statistically significant increases in serum enzymes indicative of hepatotoxicity (ALT, AST, and SDH) were found in male rats at 400 mg/kg-day. 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 highest 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 (in both males and females) and BUN (in males only) were also observed at doses ≥400 mg/kg-day. The effects of bromobenzene exposure on the hematopoietic system were not significantly different from the controls. 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.

Table 4-1. Effects of bromobenzene on terminal body and liver weights and serum liver enzymes of male and female F344/N rats exposed by 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 three standard deviations from the control mean.
[d]Change relative to controls.

Source: NTP (1985a).

As shown in Table 4-2, histopathologic examinations revealed treatment-related, significantly increased incidences of rats exhibiting cytomegaly (at doses ≥200 mg/kg-day in males and ≥400 mg/kg-day in females), inflammation (at doses ≥200 mg/kg-day in males), and necrosis (at doses ≥400 mg/kg-day in males and females). Cytomegaly was characterized by study pathologists as an enlargement of both the cell and the nucleus of individual hepatocytes and was more common in the central parts of the hepatic lobule. Liver necrosis was primarily coagulative in nature and considered by the study authors to be 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 by the study authors to be a result of hepatocellular necrosis. Other histological findings in the liver, including cytoplasmic alterations, infiltration, and pigmentation, were generally of low incidence and did not exhibit consistent dose-response characteristics.

Table 4-2. Incidences of male and female F344/N rats with liver and kidney lesions following administration of bromobenzene by gavage 5 days/week for 90 days in the basic study

	Dose (mg/kg-day)											
	0		50		100		200		400		600[a]	
Endpoint	Incidence[b]	Severity[c]	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Male rats												
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
Kidney, tubule												
Necrosis	0/10		0/10		0/10		0/10		0/10		6/10[d]	2.2
Degeneration	2/10	1.0	1/10	1.0	2/10	2.0	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	

Table 4-2. Incidences of male and female F344/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)											
	0		50		100		200		400		600[a]	
	Incidence[b]	Severity[c]	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Female rats												
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
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]Most male and female rats in the 600 mg/kg-day dose group died during the study, which may have affected incidences of selected lesions.
[b]Incidence = number of animals in which lesion was found/number of animals in which organ was examined.
[c]Average severity score: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.
[d]Statistically significantly different from control groups according to Fisher's exact test ($p < 0.05$), performed by Syracuse Research Corporation.

Source: NTP (1985a).

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 direct results of hepatocellular necrosis (NTP, 1985a). However, the observed incidence of hepatic inflammation in control and low dose (≤100 mg/kg-day) bromobenzene-treated male and female rats in the absence of hepatocellular necrosis suggests that this lesion may be related to experimental factors other than bromobenzene exposure. In addition, hepatic mineralization was observed only at the highest bromobenzene dose (600 mg/kg-day) in male and female rats, the same dose at which mortality occurred. Therefore, liver lesions, such as mineralization, observed at a high dose, frank-effect level (FEL) are of limited usefulness for qualitative evaluation of bromobenzene-induced toxicity. Likewise, observations of inflammation in the liver of control-treated rats confound the

inclusion of this lesion into a pathological phenotype related specifically to bromobenzene exposure.

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 the absence of indications of tubular regeneration) in 600 mg/kg-day males and females. 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 unknown. NTP (1985a) 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. Incidences of other renal tubular effects (necrosis, degeneration, and casts) were statistically significantly increased only in high-dose male and female rats. Furthermore, other histopathologic effects (hyperkeratosis, ulceration, and hemorrhage in the stomach; brain mineralization and necrosis; thymus atrophy; bone marrow atrophy) were observed only in the high-dose groups of male and female rats. It is possible that the effects in the stomach were associated with bolus gavage dosing. Atrophy or necrosis of the thymus was observed in six male and six female rats in the 600 mg/kg dose group. These effects, considered the result of stress, were noted only in rats that died or that were euthanized while moribund. 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.

EPA considers the male rat no-observed-adverse-effect level (NOAEL) to be 50 mg/kg-day and the lowest-observed-adverse-effect level (LOAEL) to be 100 mg/kg-day, based on statistically significant increases in absolute and relative liver weight. The female rat LOAEL is 50 mg/kg-day (lowest dose tested), based on statistically significant increases in absolute and relative liver weight. The NOAEL could not be established.

In the mouse study (NTP, 1985b), groups of 10 male and 10 female $B6C3F_1$ 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 rat study (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, and 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.

NTP (1985b) attributed 5/10 and 2/10 deaths of male mice in the 600 mg/kg-day dose group of the basic and supplementary studies, respectively, to treatment with bromobenzene. Additional deaths noted in 1/10 males and 2/10 females in the 400 mg/kg-day dose group of the basic study and other occasional deaths in control and treated mice were attributed to 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. Absolute liver weight increased with dose, ranging from approximately 12% in the 50 mg/kg-day group to >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 twofold 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 significantly different from controls in any exposed mouse group, although ALT was increased 1.14- and 1.97-fold in female mice at the 400 and 600 mg/kg-day dose levels, respectively, compared with control values. Results of urinalysis and serum chemistry did not provide evidence of bromobenzene-induced effects on the renal system. Effects of bromobenzene exposure on the hematopoietic system were not significantly different from control animals.

Table 4-3. Effects of bromobenzene on terminal body and liver weights and levels of selected serum liver enzymes of male and female B6C3F$_1$ mice exposed by 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 ± 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).

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 ≥400 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 study pathologists as an enlargement of both the cell and the nucleus of individual hepatocytes. Liver necrosis was primarily coagulative in nature and was considered by the study authors 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 associated with hepatocellular necrosis. However, the observed incidence of hepatic inflammation in control and low dose (≤100 mg/kg-day) bromobenzene-treated male or female mice in the absence of hepatocellular necrosis suggests that this lesion may be related to experimental factors other than bromobenzene exposure.

Treatment-related, statistically significantly increased incidences of renal lesions (casts, tubular degeneration without evidence of regeneration) were observed only in high-dose (600 mg/kg-day) males. Sporadic lesions in other organs were not considered meaningful by the

Table 4-4. Incidences of male and female B6C3F$_1$ mice with liver and kidney lesions following administration of bromobenzene by gavage 5 days/week for 90 days in the basic study

	Dose (mg/kg-day)											
	0		50		100		200		400		600[a]	
Endpoint	Incidence[b]	Severity[c]	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Male mice												
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
Kidney, tubule												
Degeneration	0/10	–	NE[e]	–	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	

Table 4-4. Incidences of male and female B6C3F$_1$ 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)											
	0		50		100		200		400		600[a]	
	Incidence[b]	Severity[c]	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Female mice												
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
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]Cytomegaly and mineralization were not diagnosed in five high-dose male mice that died on treatment day 1.
[b]Incidence = number of animals in which lesion was found/number of animals in which organ was examined.
[c]Average severity score: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.
[d]Statistically significantly different from control groups according to Fisher's exact test ($p < 0.05$), performed by Syracuse Research Corporation.
[e]NE = not examined.

Source: NTP (1985b).

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.

EPA considers the male mouse NOAEL to be 50 mg/kg-day and the LOAEL to be 100 mg/kg-day, based on significant increases in relative liver weight. The female mouse LOAEL is 50 mg/kg-day (lowest dose tested), based on significant increases in absolute and relative liver weight. The NOAEL could not be established.

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 5,130 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 on health effects in animals following chronic oral exposure to bromobenzene are available.

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[2] have not been published by NTP, but reports including the review comments and conclusions of NTP's Pathology Working Group (NTP, 1986b) were obtained from NTP.

Groups of 10 male and 10 female F344/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 1,926 mg/m^3) 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 the 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, 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. Absolute liver and kidney weights were significantly increased at concentrations ≥100 ppm in both sexes. Relative-to-body weight, liver

[2]The unpublished NTP studies are available by calling U.S. EPA's IRIS Hotline at (202)566-1676, by fax at (202)566-1749 or by email at iris@epa.gov.

and kidney weights were significantly increased in males at concentrations ≥100 ppm, and in females kidney weights were significantly increased at ≥30ppm. In males, absolute and relative liver weights increased 13 and 21% at 100 ppm and 20 and 28% at 300 ppm, respectively. In females, absolute and relative liver weights increased 12 and 12% at 100 ppm and 23 and 22% at 300 ppm, respectively. Body and organ weight data are reported in Table 4-5. MCH and MCV were statistically significantly decreased in males at concentrations ≥10 ppm and in females at 300 ppm, but the changes were small and are not considered biologically significant.

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 × 1,000 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	1.05 ± 0.05	1.07[b] ± 0.11	1.11[c] ± 0.09
Ratio right kidney/body weight × 1,000 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] ± 0.15 +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 × 1,000 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 × 1,000 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 three standard deviations from the control mean.

Source: NTP (1985c).

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 (see Table 4-6). Histopathologic examination of the kidneys revealed renal cortical tubular regeneration, characterized by basophilic (regenerative) tubules scattered throughout the renal cortex, in 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 evidence of degeneration or necrosis. NTP (1985c) noted that the severity of nephropathy in the 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 stated that the effect was mild and not life threatening.

Table 4-6. Incidences of male and female F344/N rats with liver and kidney lesions following repeated exposure to bromobenzene vapors for 13 weeks

Endpoint	Exposure concentration (ppm)									
	0		10		30		100		300	
	Incidence[a]	Severity[b]	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Male rats										
Liver Necrosis Inflammation	1/10 0/10	1.0	NE[c]	–	NE	–	NE	–	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
Female rats										
Liver Necrosis Inflammation	1/10 2/10	1 1	NE	–	NE	–	NE	–	0/10 3/10	1
Kidney, tubule regeneration	0/10	–	NE	–	NE	–	NE	–	0/10	–

[a]Incidence = number of animals in which lesion was found/number of animals in which organ was examined.
[b]Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.
[c]NE = Not examined.

Source: NTP (1985c).

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. EPA considers the rat NOAEL to be 10 ppm and the LOAEL to be 30 ppm, based on significantly increased relative kidney weight in female rats.

In the mouse study, groups of 10 male and 10 female $B6C3F_1$ mice were exposed to 0, 10, 30, 100, or 300 ppm (females only) (0, 64.2, 192.6, 642, or 1,926 mg/m^3, respectively) 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 the 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, and 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 significantly 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, although the 7% increase in relative liver weight was statistically significant. 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 biologically significant. In the original study report, histopathologic evidence of hepatic effects was presented. Cytomegaly was observed in the liver of 4/10 and 2/10 male mice and 2/10 and 10/10 female mice of the 30 and 100 ppm exposure groups, respectively. For males, the increased incidence (compared with controls) of necrosis and inflammation was noted in 2/10 and 4/10 mice, respectively, exposed to 100 ppm

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[a]
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 (%)[b]	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 × 1,000 Difference (%)[b]	50.71 ± 3.66	51.86 ± 3.57 +2.2	51.57 ± 2.78 +1.7	54.28[c] ± 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 × 1,000 Difference (%)[b]	8.13 ± 0.66	8.84 ± 0.86 +8.7	8.88[c] ± 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[d] ± 1.7
Liver weight (g) Difference (%)[b]	1.43 ± 0.15	1.52 ± 0.09 +6.3	1.54[c] ± 0.07 +7.7	1.68[d] ± 0.10 +17.5	2.37[d] ± 0.21 +65.7
Ratio liver/body weight × 1,000 Difference (%)[b]	52.0 ± 3.22	55.25[c] ± 3.49 +6.3	54.66[c] ± 1.80 +5.1	59.37[d] ± 3.43 +14.2	79.73[d] ± 5.27 +53.3
Right kidney weight	0.19 ± 0.01	0.20[d] ± 0.01	0.20 ± 0.02	0.20[d] ± 0.01	0.23[d] ± 0.02
Ratio kidney/body weight × 1,000 Difference (%)[b]	6.80 ± 0.28	7.38[d] ± 0.25 +8.5	7.04 ± 0.51 +3.5	7.14 ± 0.32 +5.0	7.64 ± 0.45 +12.4

[a]Male mice were not treated at this inhalation concentration; rationale for exclusion was not provided in the study report.
[b]Change relative to controls.
[c]Statistically significantly different from controls ($p < 0.05$) based on Student's two-tailed t-test.
[d]Outside three standard deviations from the control mean.

Source: NTP (1985d).

bromobenzene. For females, the incidences of necrosis and mineralization were increased over controls in 5/10 and 2/10 mice, respectively, exposed to 300 ppm. Additionally, females of the 300 ppm exposure group exhibited enlarged, diffusely mottled livers. Liver histopathological data are presented in Table 4-8.

Table 4-8. Incidences of male and female B6C3F$_1$ mice with liver and kidney lesions following repeated exposure to bromobenzene vapors for 13 weeks

Endpoint	Exposure concentration (ppm)									
	0		10		30		100		300	
	Incidence[a]	Severity[b]	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Male mice										
Liver										
Cytomegaly[c]	0/10		0/10		4/10[d]	2.0	2/10	1.5		
Necrosis	0/10		0/10	–	0/10		2/10	1.0	NG[e]	–
Inflammation	1/10	3.0	0/10		0/10		4/10	1.8		
Kidney, Tubule degeneration[e]	0/10	–	0/10	–	2/10	1.5	3/10	2.0	NG	–
Female mice										
Liver										
Cytomegaly	0/10		0/10		0/10		2/10	1.0	10/10[d]	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[f]	0/10		0/10		0/10		0/10		2/10	2.0
Kidney[g]	–	–	–	–	–	–	–	–	–	–

[a]Incidence = number of animals in which lesion was found/number of animals in which organ was examined.
[b]Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.
[c]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.
[d]Statistically significantly different from control groups according to Fisher's exact test ($p < 0.05$), performed by Syracuse Research Corporation.
[e]Kidney tubular degeneration could not be distinguished from artifacts of fixation or staining.
[f]Mineralization was not reported in male mice.
[g]No histopathologic renal lesions were identified in any group of female mice.
[e]NG = no group (the study did not include a 300 ppm exposure group of male mice).

Source: NTP (1985d).

The Pathology Working Group agreed with the diagnoses of cytomegaly, hepatic necrosis, and mineralization in the female mice exposed to 300 ppm bromobenzene, but did not consider the observed liver effects to be adverse in female mice at lower exposure levels (NTP, 1986b). Furthermore, the Pathology Working Group considered the cytomegaly in the 100 ppm

male mice to be more appropriately described as centrilobular hepatocellular hypertrophy or enlargement. Additionally, they considered the cytomegaly to be less severe than that 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 male mice exposed to 30 and 100 ppm bromobenzene, respectively, 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 an artifact of the 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.

EPA considers the male mouse NOAEL to be 30 ppm and the LOAEL to be 100 ppm, based on significantly increased relative liver weight. The female mouse LOAEL is 10 ppm (lowest exposure), based on significantly increased relative liver weight. The NOAEL could not be established.

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 on health effects in animals following chronic inhalation exposure to bromobenzene are available.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Reproductive Toxicity Studies

No reproductive toxicity studies are available for bromobenzene.

4.3.2. Developmental Toxicity Studies

No developmental toxicity studies are available for bromobenzene.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC 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; Kluwe et al., 1984; Rush 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 2,355 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 2,355 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 1,000 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 assays 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).

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)	1,000 mg/kg-day	1,000	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	1,000 ppm	1,000	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 (2 × 62.5–2 × 250 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	Prodi et al., 1986; Colacci et al., 1985
RNA binding in rat and mouse (in vivo)	6.35 μmol/kg (intraperitoneal)	6.35	Positive, rat and mouse liver, kidney, and lung	Prodi et al., 1986; Colacci et al., 1985

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

Bromobenzene treatment increased the 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 kidneys exhibited a much greater degree of binding to macromolecules than rat kidneys. 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). Incubation with P-450-containing liver microsomal isolates catalyzed the in vitro binding of [^{14}C]-bromobenzene to rat and liver DNA; liver microsomes of mice were 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 is 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 intermediate, 2,3-dihydrodiol, also undergoes non-enzymatic dehydration to form both 2-bromophenol and 3-bromophenol in the detoxification bromocatechol pathway (Lertratanangkoon et al., 1987). Furthermore, non-enzymatic dehydration of the 3,4-dihydrodiol is considered to be a 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., 1993, 1987). The bromophenols are subsequently oxidized by CYP enzymes 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 predominantly negative; however, clastogenic and mutagenic results in mammalian cell cultures and whole-animal studies were positive. Bromobenzene was not mutagenic in the Ames assay and did not

consistently produce marked cytogenic effects in vitro with mammalian cells. Bromobenzene increased the 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 >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 with 0.5 mmol/kg of DENA by gavage, 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 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 ⅔ 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 to reactive metabolites. The reactive metabolites are primarily formed 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). These reactive metabolites could potentially lead to decreased hepatocellular viability, including cell death, via modifications of intracellular macromolecules or organelles involved in cellular Ca^{2+}

homeostasis (Casini et al., 1987; Tsokos-Kuhn et al., 1985), mitochondrial respiration and bioenergetics (Maellaro et al., 1990; Thor and Orrenius, 1980), and cytosolic and mitochondrial glutathione levels (Wong et al., 2000; Casini et al., 1982; Jollow et al., 1974).

To demonstrate that hepatotoxic effects are elicited by metabolites of bromobenzene and not bromobenzene itself, one group of rats was administered single intraperitoneal doses (1,500 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 signs of the liver lesion, although concentrations of parent compound in plasma and liver of the CYP-inhibited rats were 5–6 times higher than those in the group not treated with the CYP-inhibitor.

Table 4-10. The effect of CYP[a] 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)[b]	Liver (μg/g)[b]
Bromobenzene (1,500 mg/kg-day)	Extensive	2.8 ± 0.3	26 ± 3
Bromobenzene (1,500 mg/kg-day) + SKF 525[c]A	No specific lesions	14.4 ± 0.5	149 ± 8

[a]CYP = cytochrome P-450 isozymes.
[b]Mean ± standard error from 5–7 rats/group.
[c]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, 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). 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 are 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, 1999, 1998, 1997; Aniya et al., 1988). While electrophilic metabolites of bromobenzene have the ability to interact with tissue macromolecules, a cause and effect relationship 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 effects are likely a result of 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 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). However, the 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 (1,570 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 the 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.

Table 4-11. The influence of various treatments on the metabolism of bromobenzene 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 ($t_{1/2}$ in minutes)[b]	Rate of glutathione depletion ($t_{1/2}$ in minutes)
Bromobenzene (1,570 mg/kg)	Minimal	10.0 ± 0.8	66 ± 8
Bromobenzene (236 mg/kg) + phenobarbital	Massive	5.5 ± 0.5[c]	20 ± 3[c]
Bromobenzene (1,570 mg/kg) + SKF 525A	None	15.5 ± 1.8[c]	230 ± 15[c]
Bromobenzene (1,570 mg/kg) + diethyl maleate	Extensive	10.2 ± 0.7	17 ± 3[c]
Bromobenzene (1,570 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]-BB.
[c]Significantly different from the values of rats treated with BB only; $p < 0.05$.

Source: Jollow et al. (1974).

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 hepatocellular necrosis. The experimental reduction 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 with reduced glutathione. 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. Many experimental studies of GSH homeostasis typically express reduction in terms of total cellular GSH level, however, mitochondrial stores may represent as much as 10% of total hepatic GSH (Meredith and Reed, 1982). Indeed, consistent with a number of model hepatotoxicants, bromobenzene reduces cytosolic GSH levels below a critical threshold (typically hypothesized to be approximately ≤20% of control or pretreatment levels), leading to a reduction in mitochondrial glutathione stores (Wong et al., 2000). Bromobenzene-induced reductions in hepatic mitochondrial GSH stores may be causally related to altered mitochondrial function. This is consistent with observed changes in hepatocyte oxygen uptake and ATP production following bromobenzene exposure (Thor and Orrenius, 1980).

Although a mode of action for bromobenzene-induced liver injury has not been established, several cellular activities critical to hepatocyte viability have been shown to be

altered following bromobenzene exposure. These include significantly decreased hepatocyte oxygen uptake and ATP levels (Thor and Orrenius, 1980), altered Ca^{2+} homeostasis (Casini et al., 1987; Tsokos-Kuhn et al., 1985), and reduced cytosolic and mitochondrial GSH levels (Wong et al., 2000; Jollow et al., 1974). Any one of these alterations in liver physiology could induce or contribute to an altered state of hepatocyte structure or function (including cell death); consistent with the histopathological observations of cytomegaly and necrosis in the liver of rats and mice exposed orally or by inhalation to bromobenzene (NTP, 1985a, b, c, d).

The liver develops 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 adult (12–16 weeks of age) male F344/N rats following single or repeated oral dosing (1 time/day for up to 10 days). Four animals, each from the control and bromobenzene-treated groups, were sacrificed 3, 6, 9, 12, or 24 hours after the final treatment. Liver and kidney tissues were harvested immediately and processed to obtain nonprotein sulfhydryl group concentrations, which 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 10th treatment. Serum ALT activity was increased following the 1st, 2nd, and 4th treatments, but not after the 10th treatment. In a similarly designed dose-response study, F344/N rats were divided into a vehicle control and three different bromobenzene treatment groups (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 10th 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 F344/N rats and $B6C3F_1$ mice administered bromobenzene by 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 two- to sixfold 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 effect may be due to chemically- induced increased synthesis of liver glutathione and/or increased half-life of the reduced glutathione pool.

4.5.2. Mechanistic Studies of Kidney Effects

Nephrotoxicity has been associated with acute exposure to bromobenzene in mice and rats, albeit at higher doses than the lowest hepatotoxic doses. Repeat-dose oral and inhalation studies in rats and mice also provide evidence for kidney effects, but only at the highest exposure levels tested, which resulted in lethality.

The nephrotoxic effects are 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 fourfold 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).

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). 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 gene expression changes 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 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 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 toxicogenomic and proteomic studies provide suggestive evidence for the involvement of various

genes and protein targets in particular aspects of bromobenzene hepatotoxicity (Kiyosawa et al., 2007; Koen et al., 2007; Tanaka et al., 2007). However, these studies do not facilitate identification of key events in the mode of action for bromobenzene-induced hepatotoxicity.

4.5.4. Similarities Between Bromobenzene and Chlorobenzene

Bromobenzene and chlorobenzene (Figure 4-1) 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 electronegativities of 3.16 and 2.96 for chlorine and bromine (Loudon, 1988), respectively.

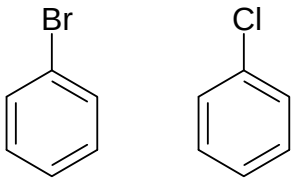

Figure 4-1. Chemical structure of bromobenzene and chlorobenzene.

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 radio-labeled 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 P-450 for oxidation is the rate limiting step for toxicity and is similar in both species.

The subchronic oral toxicity studies of bromobenzene in F344/N rats (NTP, 1985a) and $B6C3F_1$ mice (NTP, 1985b) and chlorobenzene in F344/N rats and $B6C3F_1$ 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.

Table 4-12. Incidences of male and female F344/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]
Male rats					
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
Female rats					
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 B6C3F$_1$ 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]
Male mice						
Liver Necrosis Degeneration	0/10 0/10	1/10 0/10	1/10 0/10	7/10[b] 2/10	10/10[b] 0/10	10/10[b] 0/10
Kidney Nephropathy	0/10	NE[c]	0/10	4/10[b]	9/10[b]	8/10[b]
Female mice						
Liver Necrosis Degeneration	0/10 0/10	0/10 0/10	0/10 0/10	10/10[b] 0/10	8/10[b] 9/10[b]	1/10 4/10[b]
Kidney Nephropathy	0/10	NE[c]	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.
[c]NE = not examined, due to the absence of lesions at the next higher dose.

Source: NTP (1985e).

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 F0 and F1 male rats at exposure levels ≥150 ppm. The NOAEL for hepatic effects in this study was 50 ppm. The highest exposure level (450 ppm) did not elicit any 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 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 the 400 and 600 mg/kg-day treatment groups of male rats administered bromobenzene via gavage 5 days/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 reproductive and developmental endpoints do not appear to be more sensitive to 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 B6C3F$_1$ mice (50/sex/species) were administered chlorobenzene by 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 male rats (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 freestanding NOAEL of 120 mg/kg-day in female rats and a LOAEL of 120 mg/kg-day for hepatocellular necrosis in male rats. In male and female mice, freestanding NOAELs were 60 and 120 mg/kg-day, respectively. LOAELs were not established based on the lack of 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 mg/kg-day 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 are 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 gavage at doses of 0, 50, 100, 200, 400, or 600 mg/kg-day 5 days/week for

90 days (NTP, 1986a, 1985a, b). 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 ≥200 mg/kg-day resulted in significantly increased incidences of histopathologic liver lesions indicative of injury (e.g., cytomegaly or necrosis) in male rats and male mice and female mice (≥400 mg/kg-day in female rats).

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 at which significant mortality occurred (NTP, 1985a). Mice are 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 1,460 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 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 in 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 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 is the most sensitive toxicity target in these studies. Liver weights (absolute and relative-to-body weight) were significantly increased at exposure concentrations ≥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 ≥30 ppm in female mice; no significant increase in absolute liver weight was observed in male mice.

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.

The liver was shown to be a target of bromobenzene toxicity in rats following a single 4-hour exposure to bromobenzene vapor concentrations as low as 107 ppm (Brondeau et al., 1983). Extrahepatic effects have also been noted in acute inhalation studies. Necrosis was noted in the lungs of mice following a single 4-hour exposure to bromobenzene at a vapor concentration of 1,000 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). There was no 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, 1986a, 1985d).

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 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 (1,000 ppm) resulted in lung lesions (Becher et al., 1989). 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 (NTP, 1985a, b, c, d).

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 (Lau et al., 1980; Lau and Zannoni, 1979; Jollow et al., 1974; 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 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 involved in bromobenzene hepatotoxicity may include significantly decreased hepatocyte oxygen uptake and ATP levels (Thor and Orrenius, 1980), altered Ca^{2+} homeostasis (Casini et al., 1987; Tsokos-Kuhn et al., 1985), and reduced cytosolic and mitochondrial GSH levels (Wong et al., 2000; Jollow et al., 1974). Although the significance of these bromobenzene-induced cellular alterations is still unknown, the Pathology Working Group (NTP, 1986a) identified and described liver cytomegaly as an "enlargement of both the cell and the nucleus." Importantly, necrotic cell death is commonly referred to as *oncosis* or *oncotic necrosis*; oncotic meaning "pertaining to, caused by or marked by swelling" (for review, Van Cruchten and Van Den Broeck, 2002). As such, the histopathological identification of liver cytomegaly at lower doses of bromobenzene (e.g., 200 mg/kg-day in male mice) may be an early indication of hepatocyte injury, to include potentially some state of oncotic cell death.

Bromobenzene exposure has also been shown to alter liver protein synthesis and gene expression. Heijne and coworkers used a toxicogenomics approach to study molecular mechanisms of bromobenzene hepatotoxicity (Heijne et al., 2004, 2003). 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 reduced glutathione (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, cellular response to reduced glutathione levels, the acute phase response, 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. Repeat-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 results from modification of macromolecules in cells of the proximal convoluted tubule by one or more reactive metabolites.

4.7. EVALUATION OF CARCINOGENICITY

Under U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess the human carcinogenic potential" of bromobenzene. Cancer studies in humans and cancer bioassays in animals exposed to bromobenzene are not available. 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. 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. However, 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).

4.8. SUSCEPTIBLE POPULATIONS

4.8.1. Possible Childhood Susceptibility

Limited data are available 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 studies 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.

NTP (1985a, b) conducted comprehensive histopathologic examinations of all major tissues and organs in the subchronic studies of rats and mice and observed no significantly increased incidences of exposure-related lesions at sites other than the liver and kidney. 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 lesions (600 mg/kg-day in rats and mice) was higher than the lowest dose (200 mg/kg-day in rats and mice) resulting in histopathological liver effects. Thus, the liver effects are considered a more sensitive indicator of bromobenzene toxicity (see Figure 5-1).

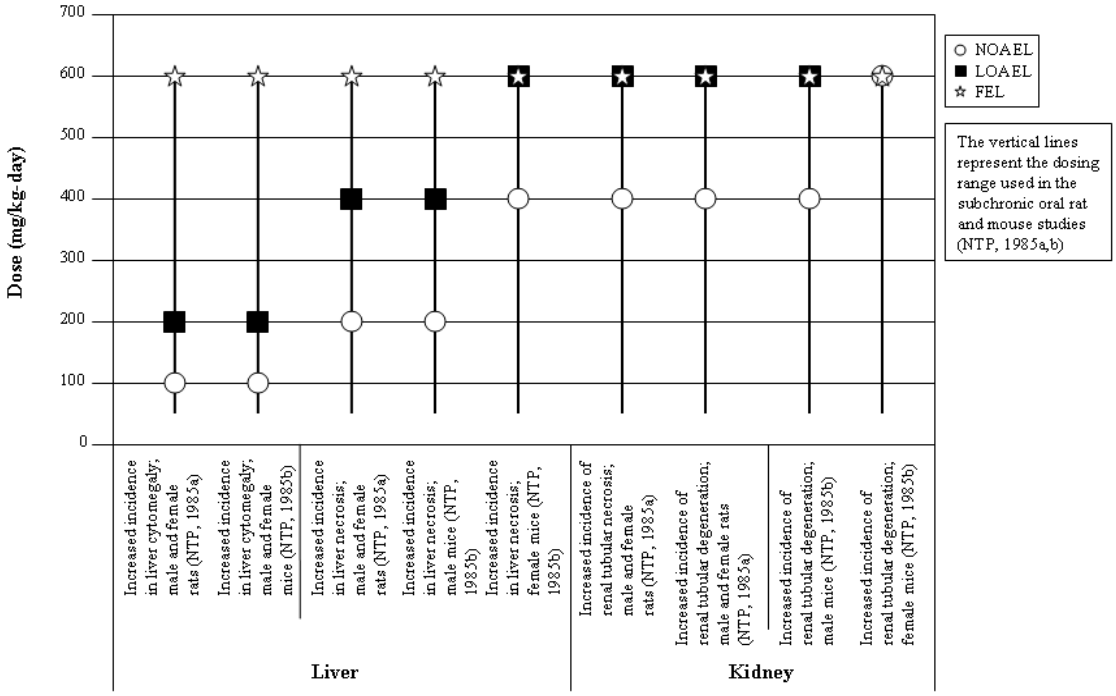

Figure 5-1. Oral exposure-response array of selected subchronic toxicity effects.

The liver is the principal target organ for bromobenzene toxicity in rodents following oral exposure. Liver observations included increased liver weights and serum enzymes and increased incidence of inflammation, cytomegaly, necrosis, and mineralization in male and female rats and mice (NTP, 1985a, b). Significantly increased mean liver weights were observed at bromobenzene doses as low as 50 mg/kg-day in female F344/N rats and $B6C3F_1$ mice and 100 mg/kg-day in the male rats and mice. Dose levels of 400 and 600 mg/kg-day resulted in ≥twofold increases (statistically and/or biologically significant) in serum concentrations of AST, ALT, and SDH in male and female rats and increased SDH in male and female mice. Statistically significant increased incidences of liver inflammation were observed at doses ≥200 mg/kg-day in male rats (also increased in females but incidence did not reach statistical significance compared to controls). Significantly increased liver inflammation was observed in female mice only at the 600 mg/kg-day dose level. Significantly increased incidences of hepatocellular necrosis were observed at doses of 400 and 600 mg/kg-day in male and female rats and male mice (600 mg/kg-day in female mice) (NTP, 1985a, b). Hepatic mineralization was slightly increased in male and female rats and female mice in the 600 mg/kg-day dose group, and significantly increased in male mice at doses ≥400 mg/kg-day. Significantly increased incidences of hepatocellular cytomegaly were observed at doses ≥200 mg/kg-day in male rats (≥400 mg/kg-day in female rats) and male and female mice.

The mode of action for bromobenzene-induced liver toxicity is not known. However, the significantly increased absolute and relative liver weights, observed in conjunction with the increased levels of systemically circulating liver enzymes and increased incidence of cytomegaly, necrosis, inflammation, and mineralization in the livers of both rats and mice are all considered manifestations of bromobenzene exposure. There is some evidence to suggest that bromobenzene-induced cytomegaly, inflammation, necrosis, and mineralization are part of a pathological continuum. Specifically, all four histopathological lesions were primarily observed in the central region of the hepatic lobules of treated rats. Furthermore, mechanistic data suggests several potential bromobenzene-induced cellular alterations that could individually, or in concert, commit hepatocytes to mixed cellular phenotypes consistent with the histopathological observations in rats and mice of the NTP studies (1985a, b). In the NTP report, inflammation and mineralization were considered to be causally associated with or a direct result of, respectively, hepatocellular necrosis (NTP, 1985a). However, the observed incidence of hepatic inflammation in the control and lower dose (≤200 mg/kg-day) groups of bromobenzene-treated male and female rats and mice, in the absence of evidence of hepatocellular necrosis, suggests that this lesion may not be associated directly with necrosis, at least not at lower doses. The hepatic mineralization was observed only at the highest bromobenzene dose (600 mg/kg-day) in male and female rats and male mice (400 mg/kg-day in female mice), the same dose at which mortality occurred. Liver lesions, such as mineralization and necrosis, and mortality, all observed at high doses, are considered frank effects.

The occurrence of cytomegaly at low doses (e.g., ≥200 mg/kg-day in rats and mice) may be an early indication of hepatocyte injury. Increased incidences of cytomegaly were observed in male and female rats and mice at doses of 200, 400, and 600 mg/kg-day. None of the controls exhibited this effect. Cytomegaly may also represent an adverse endpoint independent of its possible association with necrosis. In addition to the histological observations in the NTP studies, several reports dating back to the 1970s illustrate a number of potential biochemical and cellular events that support identification of bromobenzene-induced liver cytomegaly as an endpoint of concern. For example, a postulated mode of action of bromobenzene-induced hepatotoxicity involves metabolism of bromobenzene to reactive metabolites. The results of mechanistic studies indicate that hepatotoxicity is primarily elicited via the metabolic pathway involving the 3,4-oxide derivative of bromobenzene. The toxic effects are likely a result of 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). Modifications of hepatocellular macromolecules involved in cellular Ca^{2+} homeostasis (Casini et al., 1987; Tsokos-Kuhn et al., 1986), mitochondrial respiration and bioenergetics (Maellaro et al., 1990; Thor and Orrenius, 1980), and cytosolic and mitochondrial glutathione levels (Wong et al., 2000; Casini et al., 1982; Jollow et al., 1974) may lead to decreased hepatocellular viability (including cytomegaly and/or liver cell death), although a

causal relationship between hepatic bromobenzene metabolism and specific alterations in biochemical state or organellar status, other than intracellular GSH levels, has not been established. Therefore, liver cytomegaly is chosen as the critical effect for the derivation of the oral subchronic RfD for bromobenzene.

However, some of the external peer review panel members indicated that necrosis in the liver should be selected as the critical effect (see Appendix A: Summary of External Peer Review and Public Comments and Disposition). Three of the five external peer review panelists suggested this endpoint based on the opinion that necrosis represents the only clear indicator of bromobenzene-induced hepatotoxicity. One panelist further indicated that hepatocellular cytomegaly, liver weight changes, and inflammation are general manifestations of exposure to xenobiotics rather than bromobenzene-specific toxicity and that only necrosis could be directly linked to bromobenzene exposure according to the available mode of action information. For this reason, the results of dose-response modeling of the liver necrosis data are presented in Section 5.1.1.2 for purposes of comparison.

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

Using liver cytomegaly as the critical effect for the derivation of the subchronic RfD, potential points of departure (PODs) were estimated from cytomegaly incidence data in rats and mice using a benchmark dose (BMD) modeling approach . All available dichotomous models in the U.S. EPA's Benchmark Dose Software (BMDS, version 1.4.1) were fit to the data for animals with increased incidence of cytomegaly (Table 5-1). A benchmark response (BMR) of 10% extra risk was selected in the absence of biological information that would warrant a different choice. A 10% increase in incidence relative to controls is considered representative of a minimal biologically significant change. Detailed modeling results are presented in Appendix B.

Table 5-1. Incidences of male and female F344/N rats and B6C3F$_1$ mice with liver cytomegaly[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	0/10	0/10	0/10	4/10[b]	10/10[b]	9/10[b]
Female rats	0/10	0/10	0/10	3/10	10/10[b]	10/10[b]
Male mice	0/10	0/10	1/10	6/10[b]	4/10[b]	4/10[b]
Female mice	0/10	0/10	1/10	5/10[b]	9/10[b]	10/10[b]

[a]Incidences of rats or mice with cytomegaly, 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).

The 95% lower confidence limit on the BMD_{10} [$BMDL_{10}$]) associated with a BMR of 10% extra risk for incidences of male and female rats and mice with cytomegaly are presented for the best fitting models in Table 5-2. Male mice were the most sensitive as the estimated $BMDL_{10}$ (33.8 mg/kg-day) for cytomegaly resulted in the lowest POD.

Table 5-2. Estimated PODs from the best-fitting models predicting incidences of liver cytomegaly in F344/N rats or B6C3F$_1$ mice

Data set	Best-fit model	Estimated POD ($BMDL_{10}$ mg/kg-day)
Male rats	Log-logistic	87.5
Female rats	Log-logistic	130.2
Male mice	Log-logistic	33.8
Female mice	Multistage (2°)	47.2

Based on several external peer review panel members' recommendation of liver necrosis as the critical effect, all available dichotomous models in the BMDS (version 1.4.1) were fit to the incidence data for necrosis in male and female rats and mice (Table 5-3) for purposes of compariosn. A BMR of 10% extra risk was selected in the absence of biological information that would warrant a different choice. Detailed modeling results are presented in Appendix B. $BMDL_{10}$s estimated by the best fitting models for incidences of necrosis in male and female rats and mice are presented in Table 5-4. Male rats were the most sensitive with an estimated $BMDL_{10}$ for necrosis of 93.4 mg/kg-day, a value that is three-fold higher than the POD determined for cytomegaly.

Table 5-3. Incidences of male and female F344/N rats and B6C3F$_1$ mice with liver necrosis[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	0/10	0/10	0/10	3/10	9/10[b]	9/10[b]
Female rats	0/10	0/10	0/10	0/10	7/10[b]	9/10[b]
Male mice	0/10	0/10	0/10	1/10	4/10[b]	8/10[b]
Female mice	0/10	0/10	1/10	0/10	1/10	7/10[b]

[a]Incidences of rats or mice with necrosis, 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 5-4. Estimated PODs from the best-fitting models predicting incidences of liver necrosis in F344/N rats or B6C3F$_1$ mice

Data set	Best-fit model	Estimated POD ($BMDL_{10}$ mg/kg-day)
Male rats	Log-logistic	93.4
Female rats	Log-probit	171.4
Male mice	Gamma	131.9
Female mice	Gamma	284.4

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

The $BMDL_{10}$ of 33.8 mg/kg-day for cytomegaly is the POD for the derivation of the subchronic RfD. The POD (derived from data for male mice administered bromobenzene by gavage 5 days/week for 90 days) was duration adjusted to a continuous exposure ($BMDL_{10[ADJ]}$ = 33.8 mg/kg-day × 5 days/7 days = 24.1 mg/kg-day) and divided by a total UF of 1,000. The UF consists of three areas of uncertainty: (1) interspecies extrapolation, (2) interindividual human variability, and (3) database deficiencies.

- A 10-fold UF for laboratory animal-to-human interspecies differences (UF_A) was applied to account for the variability in extrapolating from mice to humans. No information is available on toxicokinetic or toxicodynamic differences or similarities for bromobenzene in animals and humans. In the absence of data to quantify specific toxicokinetic and toxicodynamic differences, a default factor of 10 was applied.

- A 10-fold UF for intraspecies differences (UF_H) was applied 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.

- An UF of 1 for LOAEL-to-NOAEL extrapolation was applied because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% change in the incidence of liver cytomegaly was selected under an assumption that it represents a minimal biologically significant change.

- 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 for bromobenzene. The subchronic 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 hepatotoxic (NTP, 1985a, b). Additionally, bromobenzene and chlorobenzene exhibit 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. In a two-generation reproductive toxicity study in rats, chlorobenzene did not induce developmental effects in the fetuses of pregnant rats exposed to oral dose levels of 100 or 300 mg/kg-day on gestation days 6–15 (IBT, 1977). However, reproductive effects, in particular multi-generational effects, may be important to informing the bromobenzene toxicity database considering the high DNA reactivity of this chemical. 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). Therefore, the lack of a multi-generational study is of particular concern because genetic damage to germ cells of an F1 generation may not be detected until the F2 generation. In the absence of any information concerning reproductive and developmental endpoints following bromobenzene exposure, an UF of 10 was applied.

The subchronic RfD for bromobenzene based on liver cytomegaly was calculated as follows:

$$\begin{aligned} \text{Subchronic RfD} &= BMDL_{10[ADJ]} \div UF \\ &= 24.1 \text{ mg/kg-day} \div 1{,}000 \\ &= 2 \times 10^{-2} \text{ mg/kg-day} \end{aligned}$$

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 studies for repeated oral exposure in laboratory animals that are available for selection of an RfD consist 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. For these reasons, the principal study (NTP, 1985b) and critical effect (cytomegaly) for development of the chronic RfD for bromobenzene is the same as that described for the development of the subchronic RfD (see Section 5.1.1.1).

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 (see Section 5.1.1.2).

5.1.2.3. *RfD Derivation—Including Application of Uncertainty Factors (UFs)*

The $BMDL_{10}$ from the best fitting model for liver cytomegaly of 33.8 mg/kg-day was selected as the POD. The POD (derived from data for male mice administered bromobenzene by gavage 5 days/week for 90 days) was duration adjusted to a continuous exposure ($BMDL_{10[ADJ]}$ = 33.8 mg/kg-day × 5 days/7 days = 24.1 mg/kg-day) and divided by a total UF of 3,000. 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 for laboratory animal-to-human interspecies differences (UF_A) was applied to account for the variability in extrapolating from mice to humans. No information is available on toxicokinetic or toxicodynamic differences or similarities for bromobenzene in animals and humans. In the absence of data to quantify specific toxicokinetic and toxicodynamic differences, a default factor of 10 was applied.

- A 10-fold UF for intraspecies differences (UF_H) was applied 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 factor of 3 UF was applied to account for extrapolating from a subchronic study to chronic exposure scenarios (UF_S). 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 develops 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. Furthermore, as discussed in detail in Section 4.5.4, bromobenzene and chlorobenzene exhibit similarities in structure, toxicokinetic properties, and critical target of toxicity (liver) in rats and mice. In a 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). In a similarly-designed NTP 2-year oral study of chlorobenzene, nonneoplastic lesions attributable to

chlorobenzene were not observed in male and female mice; NTP identified freestanding NOAELs of 60 and 120 mg/kg-day, respectively (NTP, 1985e). These results suggest that the dose-response relationships for liver effects from subchronic and chronic exposure may be similar. It is reasonable to expect such similarities in dose-response relationships for subchronic and chronic exposure to bromobenzene 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).

- An UF of 1 for LOAEL-to-NOAEL extrapolation was applied because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% change in the incidence of liver cytomegaly was selected under an assumption that it represents a minimal biologically significant change.

- A 10-fold UF was applied to account for database deficiencies (UF_D). Subchronic studies in rats and mice are available. As discussed previously (Section 5.1.1.3), the oral database for bromobenzene lacks developmental toxicity and multi-generation reproductive toxicity studies. The subchronic 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 hepatotoxic (NTP, 1985a, b). Additionally, bromobenzene and chlorobenzene exhibit 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. In a two-generation reproductive toxicity study in rats, chlorobenzene did not induce developmental effects in the fetuses of pregnant rats exposed to oral dose levels of 100 or 300 mg/kg-day on gestation days 6–15 (IBT, 1977). However, reproductive effects, in particular multi-generational effects, may be important to informing the bromobenzene toxicity database considering the high DNA reactivity of this chemical. 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). Therefore, the lack of a multi-generational study is of particular concern because genetic damage to germ cells of an F1 generation may not be detected until the F2 generation. In the absence of any information concerning reproductive and developmental endpoints following bromobenzene exposure, an UF of 10 was applied.

The RfD for bromobenzene based on liver cytomegaly was calculated as follows:

$$\begin{aligned} \text{RfD} &= BMDL_{10[ADJ]} \div UF \\ &= 24.1 \text{ mg/kg-day} \div 3{,}000 \\ &= 8 \times 10^{-3} \text{ mg/kg-day} \end{aligned}$$

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.

NTP (1985c, d) conducted comprehensive histopathologic examinations of all major tissues and organs in the subchronic inhalation studies of rats and mice and reported no evidence of exposure-related lesions at sites other than the kidney and liver. NTP (1985c) observed cortical tubular regeneration in the kidneys of all of the control and all but one of the treated male rats. Although the severity was slightly more pronounced in the 300 ppm males (mild severity) compared with controls (minimal severity), no statistically significant effect on the incidence or severity of this kidney lesion was observed. No other effects were noted in the kidneys of male and female rats. Similar renal lesions were observed in male mice exposed to 30 and 100 ppm; female mice did not exhibit renal effects (NTP, 1985d).

Statistically significant increases in liver weight occurred in male and female rats at bromobenzene exposures as low as 100 ppm. Treatment-related, significantly increased liver weights were seen in male mice at 100 ppm (liver weight was not reported for male mice in the 300 ppm group) and in all bromobenzene exposure groups of female mice (≥10 ppm). However, as noted previously in Section 5.1.1.1, while increased liver weight may be considered a part of a continuum related to bromobenzene-induced toxicity in the liver, this effect may also be related to increased metabolism of bromobenzene by the liver. Comprehensive histopathologic examination of the livers of rats exposed to 10–300 ppm bromobenzene did not reveal any significant increase compared with controls in the incidence or severity of treatment-related lesions (NTP, 1985c). Therefore, the NTP (1985c) rat study was not considered further for the derivation of the subchronic RfC.

In the mouse study, hepatic cytomegaly, inflammation, and necrosis were observed; mineralization was also observed in female mice at the highest inhalation concentration (300 ppm) but was not reported for male mice (NTP, 1985d). Hepatic inflammation was observed in 40% of male mice in the 100 ppm group (not statistically significant) compared with 10% of controls, however, the severity of this lesion was scored higher in the control compared with the 100 ppm treated animals. Hepatic inflammation was also observed in 40, 30, 20, 20, and 20% of female mice exposed to 0, 10, 30, 100, and 300 ppm bromobenzene, respectively. Incidences of cytomegaly of 40% (statistically significant) and 20% were observed in the 30 and 100 ppm male mice, respectively. In the 100 and 300 ppm female mice, 20 and 100% (statistically significant) of animals, respectively, exhibited increased incidence and severity of cytomegaly;

controls and 10 and 30 ppm animals showed no signs of cytomegaly. Hepatocellular necrosis was noted in 20, 10, 0, 20, and 50% of female mice exposed to 0, 10, 30, 100, and 300 ppm bromobenzene, respectively, but the incidences of this lesion were not significantly greater than controls. Although the dose-response for hepatocellular necrosis incidence in female mice is irregular (see Table 4-8), it is reasonable to expect that higher exposure levels in the 90-day inhalation studies (NTP, 1985c, d) would have resulted in a statistically significant increased incidence compared to controls.

Liver cytomegaly was selected as the critical effect in the derivation of the inhalation subchronic RfC because this effect represents the most sensitive exposure-related histopathological endpoint in the liver. However, as with the RfD (Section 5.1.1), some of the external peer review panel members suggested that necrosis is the only reliable marker of liver injury following bromobenzene exposure. The treatment-related increased incidence of cytomegaly, compared with frank necrosis, may be a sensitive marker of bromobenzene-induced liver toxicity and may also be considered an adverse effect regardless of a possible association with necrosis. Therefore, EPA concluded that liver cytomegaly is a critical histopathological effect and considered it the most appropriate endpoint for the derivation of the inhalation subchronic RfC for bromobenzene.

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

Available dichotomous models in U.S. EPA BMDS version 1.3.2 were fit to the liver cytomegaly data for female B6C3F$_1$ mice (presented in Table 5-5) from the 90-day inhalation studies (NTP, 1985d). A benchmark response (BMR) of 10% extra risk was selected in the absence of biological information that would warrant a different choice. A 10% increase in incidence relative to controls is considered representative of a minimal biological significant change. Detailed modeling results are presented in Appendix C.

Table 5-5. Incidences of female B6C3F$_1$ mice with cytomegaly in the centrilobular region of the liver following inhalation exposure to bromobenzene vapors 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[a]
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

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

Source: NTP (1985d).

Sigmoidal models (e.g., gamma, probit, logistic, higher degree multistage) and non-sigmoidal models (e.g., quantal quadratic and quantal linear) in the U.S. EPA BMDS (version 1.3.2) were fit to the cytomegaly incidence data in Table 5-5. Modeling results, presented in Table 5-6, show that: (1) all sigmoidal models provide excellent fit to the data; (2) non-sigmoidal models provide poorer fit to the data; and (3) all sigmoidal models provide similar estimates of the benchmark concentration associated with a 10% response level (BMC_{10}) (ranging from about 77 to 97 ppm, a 1.3-fold range) and the 95% lower confidence limit on the BMC_{10} ($BMCL_{10}$) (ranging from about 40 to 60 ppm, a 1.5-fold range). Following U.S. EPA (2000b) guidance for selecting models for POD computation, the model with the best fit and the lowest Akaike's Information Criteria (AIC) was selected to calculate the 95% lower confidence limit on the benchmark concentration (BMCL), which in this case corresponded to both the log-logistic and gamma models (see Table 5-6). The $BMCL_{10}$ values from these best-fitting models (log-logistic and gamma models) were averaged to calculate the POD of 55 ppm (from the log-logistic and gamma models) for liver cytomegaly in female mice.

Table 5-6. BMC modeling results for the incidence of liver cytomegaly in female $B6C3F_1$ mice exposed to bromobenzene vapors 6 hours/day, 5 days/week for 13 weeks

Model	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)	χ^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
Multistage[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 number of dose groups minus 2).

Source: NTP (1985d).

Following U.S. EPA (1994b) RfC methodology, the human equivalent concentration (HEC) for an extrarespiratory effect produced by a category 3 gas, such as bromobenzene (not highly water soluble or reactive in the respiratory tract, with the liver as the critical extrarespiratory target), is calculated by multiplying the duration-adjusted BMCL 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 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 × MW[157] / 24.45 = 353.2 mg/m^3) and adjusted for continuous exposure (353.2 mg/m^3 × 6/24 hours × 5/7 days = 63 mg/m^3). The $BMCL_{10\text{-}ADJ}$ was multiplied by a blood:gas partition coefficient ratio of 1 to obtain the $BMCL_{10\text{-}HEC}$ of 63 mg/m^3.

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

The $BMCL_{10\text{-}HEC}$ of 63 mg/m^3 was used as the POD for the derivation of the subchronic RfC for bromobenzene. The POD 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 applied to account for uncertainties in extrapolating from mice to humans (UF_A). This value is adopted by convention where an adjustment from an animal-specific $BMCL_{ADJ}$ to a $BMCL_{HEC}$ has been incorporated. Application of an UF of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component associated with bromobenzene is mostly addressed by the determination of an HEC as described in the RfC methodology (U.S. EPA, 1994b). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method, and a UF of 3 is retained to account for uncertainty regarding the toxicodynamic differences between mice and humans.

- A default 10-fold UF was applied to account for interindividual toxicokinetic and toxicodynamic variability in humans (UF_H) in the absence of information concerning the extent of variation in sensitivity to bromobenzene within the human population.

- An UF for extrapolation from a LOAEL to NOAEL (UF_L) was not needed because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% increase in the incidence of cytomegaly was selected under an assumption that it represents a minimal biologically significant change.

- A 10-fold UF was applied 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. It should be noted that bromobenzene and chlorobenzene exhibit 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 two-generation reproductive toxicity study in rats, chlorobenzene did not elicit any signs of reproductive toxicity in either generation at an exposure level of 450 ppm (Nair et al., 1987). In the same study, both F0 and F1 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) (IBT, 1977). However, reproductive effects, in particular multi-generational effects, may be important to informing the bromobenzene toxicity database considering the high DNA reactivity of this chemical. 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). Therefore, the lack of a multi-generational study is of particular concern because genetic damage to germ cells of an F1 generation may not be detected until the F2 generation. In the absence of any information concerning reproductive and developmental endpoints following bromobenzene exposure, an UF of 10 was applied.

The subchronic RfC for bromobenzene based on liver cytomegaly was calculated as follows:

$$\begin{aligned} \text{Subchronic RfC} &= BMCL_{10/HEC} \div UF \\ &= 63\ mg/m^3 \div 300 \\ &= 2 \times 10^{-1}\ mg/m^3 \end{aligned}$$

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 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 liver cytomegaly in female mice (NTP, 1985d) was selected as the critical effect for development of the 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 RfC (see Section 5.2.1.2).

5.2.2.3. *RfC Derivation—Including Application of Uncertainty Factors (UFs)*

As described in Section 5.2.1.2, the average $BMCL_{10}$ of 55 ppm was converted to a $BMCL_{10\text{-}HEC}$ of 63 mg/m^3 (see Section 5.2.1.2 for details regarding conversion to the HEC). The $BMCL_{10\text{-}HEC}$ of 63 mg/m^3 was divided by a total UF of 1,000. 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 applied to account for uncertainties in extrapolating from mice to humans (UF_A). This value is adopted by convention where an adjustment from an animal-specific $BMCL_{ADJ}$ to a $BMCL_{HEC}$ has been incorporated. Application of an UF of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component associated with bromobenzene is mostly addressed by the determination of an HEC as described in the RfC methodology (U.S. EPA, 1994b). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method, and an UF of 3 is retained to account for uncertainty regarding the toxicodynamic differences between mice and humans.

- A default 10-fold UF was applied to account for interindividual toxicokinetic and toxicodynamic variability in humans (UF_H) in the absence of information concerning the extent of variation in sensitivity to bromobenzene within the human population.

- A factor of 3 was used to account for extrapolating from a subchronic study to chronic exposure scenarios (UF_S). 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 that 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.

- An UF for extrapolation from a LOAEL to NOAEL (UF_L) was not needed because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% increase in the incidence of cytomegaly was selected under an assumption that it represents a minimal biologically significant change.

- 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. Bromobenzene and chlorobenzene exhibit 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 two-generation reproductive toxicity study in rats, chlorobenzene did not elicit any signs of reproductive toxicity in either generation at an exposure level of 450 ppm (Nair et al., 1987). In the same study, both F0 and F1 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) (IBT, 1977). However, reproductive effects, in particular multi-generational effects, may be important to informing the bromobenzene toxicity database considering the high DNA reactivity of this chemical. 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). Therefore, the lack of a multi-generational study is of particular concern because genetic damage to germ cells of an F1 generation may not be detected until the F2 generation. In the absence of any information concerning reproductive and developmental endpoints following bromobenzene exposure, an UF of 10 was applied.

The RfC for bromobenzene was calculated as follows:

$$\begin{aligned} \text{RfC} &= \text{BMCL}_{10/\text{HEC}} \div \text{UF} \\ &= 63\ \text{mg/m}^3 \div 1{,}000 \\ &= 6 \times 10^{-2}\ \text{mg/m}^3 \end{aligned}$$

5.2.3. Previous RfC Assessment

An RfC was not previously available on IRIS.

5.3. CANCER ASSESSMENT

No studies of cancer risks in humans or cancer bioassays in animals exposed to bromobenzene are available. Bromobenzene is not mutagenic in the Ames assay and does not consistently produce marked cytogenetic effects in vitro with mammalian cells. 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. 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). Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess the carcinogenic potential" of bromobenzene.

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

6.1. HUMAN HAZARD POTENTIAL

No human data are available to ascertain the health effects that could be associated with bromobenzene exposure. No known chronic exposure studies are available in animals. Short-term and subchronic duration bioassays in rats and mice, as well as genotoxicity and metabolism studies, are available for oral or inhaled bromobenzene, as described in Section 4. 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 (1,000 ppm) resulted in lung lesions (Becher et al., 1989). Results from subchronic (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). Renal effects have also been observed following oral exposure but at doses higher than that observed for hepatic effects (see Figure 5-1). Nephrotoxicity has also been observed following inhalation exposure to bromobenzene but occurred only in male rats and mice. No reproductive, developmental toxicity or carcinogenicity studies in animals are available. Following U.S. 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 is not mutagenic in bacterial assays and does not consistently produce marked cytogenetic effects in vitro with mammalian cells. Bromobenzene treatment increased the 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 data, therefore, provide only limited evidence of bromobenzene genotoxicity.

Selecting liver cytomegaly as the critical effect for oral and inhalation noncancer reference value derivation was carefully considered in the assessment. Specifically, histopathological analyses of liver tissue from rats and mice exposed orally or by inhalation to bromobenzene for 90-days revealed a number of lesions to include cytomegaly, inflammation, necrosis, and/or mineralization. Hepatic cytomegaly and inflammation occurred at lower bromobenzene doses than those observed to induce cellular necrosis or mineralization (see Tables 4-2, 4-4, 4-6, and 4-8). Bromobenzene-induced hepatic mineralization occurred typically at the highest exposure levels only, and nonspecific inflammation was observed in the livers of control and low-dose animals. Neither hepatic inflammation nor mineralization was chosen as the critical effect. Necrosis is considered an overt histopathological marker of chemically-

induced toxicity in any organ and altered cellular phenotypes such as cytomegaly observed at doses lower than frank necrosis may be an early marker of injury. Cytomegaly may also be considered an adverse effect regardless of the potential association with necrosis. As such, the histopathological identification of cytomegaly at lower doses of bromobenzene (e.g., 200 mg/kg-day in orally exposed male mice) may be an early indication of altered hepatocyte function, including some state of cell death. Bromobenzene exposure has been shown to induce alterations in cellular physiology that could potentially lead to commitment of hepatocytes to a mixed cell death phenotype, possibly including apoptosis/secondary necrosis, oncotic necrosis, and/or frank necrosis.

Uncertainties associated with deficiencies in the oral and inhalation database include an absence of multi-generational reproductive and developmental studies and chronic duration bioassays. Reproductive effects, in particular multi-generational effects, may be important to informing the bromobenzene toxicity database considering the high DNA reactivity of this chemical. 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). Therefore, the lack of a multi-generational study is of particular concern because genetic damage to germ cells of an F1 generation may not be detected until the F2 generation. Studies to address this aspect of bromobenzene toxicity are currently not available.

6.2. DOSE RESPONSE

Identification of a POD from the dose-response data for both oral and inhalation bromobenzene exposure involved BMD modeling. This approach is advantageous over a NOAEL/LOAEL approach in that all data points within a set are included to develop a dose-response curve from which a modeled POD (e.g., $BMDL_{10}$) is identified. This approach is ideal in estimating PODs since the subsequent derivation of an RfD or RfC comes from a more informed output, as opposed to point estimates such as NOAELs and LOAELs which are dependent upon experimental design (e.g., dose-spacing).

6.2.1. Noncancer/Oral

The liver has been 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 cytomegaly in rats and mice (NTP, 1985a, b) indicate that male mice have a lower POD than female mice or male or female rats (see Table 5-2). An increased incidence of cytomegaly in male mice was selected as the critical effect for deriving the chronic and subchronic RfD.

The lower 95% confidence limit for a BMD of 10% extra risk for liver cytomegaly ($BMDL_{10}$ = 33.8 mg/kg-day) was used as the POD for both the subchronic and chronic RfD. The $BMDL_{10}$ of 33.8 mg/kg-day was duration adjusted to a continuous exposure ($BMDL_{10\text{-}ADJ}$ =

33.8 mg/kg-day × 5 days/7 days = 24.1 mg/kg-day). The subchronic RfD was derived by dividing the $BMDL_{10\text{-}ADJ}$ of 24.1 mg/kg-day by a composite UF of 1,000 to account for three areas of uncertainty (10 for interspecies extrapolation, 10 for interindividual human variability, and 10 for database deficiencies). The resulting subchronic RfD is 24.1 mg/kg-day ÷ 1,000 = 0.02 mg/kg-day.

The overall confidence in the subchronic RfD is medium. The principal study (NTP, 1985b) is an adequate gavage study of subchronic duration and is supported by a similarly-designed study in a second animal species; however, due to a low number of animals per treatment group (10/group), the confidence in the principal study is medium. Confidence in the database is low-to-medium. Studies assessing the developmental toxicity and multi-generation reproductive toxicity of bromobenzene are lacking.

The derivation of the RfD included an additional UF of 3 to account for extrapolation from a subchronic study to chronic exposure scenarios for a composite UF of 3,000. The resulting RfD is 24.1 mg/kg-day ÷ 3,000 = 0.008 mg/kg-day. The overall confidence in the chronic RfD is low-to-medium. Since there are no known chronic duration oral studies available, the RfD is based upon a subchronic duration study (NTP, 1985b). Confidence in this study is medium. Confidence in the database is low-to-medium. Studies assessing the developmental toxicity and multi-generation reproductive toxicity of bromobenzene are lacking.

6.2.2. Noncancer/Inhalation

The NTP 90-day inhalation studies in rats and mice provided evidence of renal and hepatic toxicity following exposure to bromobenzene (NTP, 1985c, d). The liver was selected as the critical target of bromobenzene toxicity. An increased incidence of cytomegaly in female mice was selected as the critical effect for deriving the chronic and subchronic RfD.

The average $BMCL_{10}$ of 55 ppm (from the log-logistic and gamma models) for cytomegaly in female mice was selected as the POD for both the subchronic and chronic RfC. The $BMCL_{10}$ was converted to 353.2 mg/m^3 (55 ppm × MW[157] / 24.45 = 353.2 mg/m^3), which was then adjusted for continuous exposure (353.2 mg/m^3 × 6/24 hours × 5/7 days = 63 mg/m^3) and multiplied by a blood:gas partition coefficient ratio of 1 to obtain the $BMCL_{10\text{-}HEC}$ of 63 mg/m^3. The subchronic RfC was derived by dividing the $BMCL_{10\text{-}HEC}$ 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 overall confidence in the subchronic RfC is medium. The principal study (NTP, 1985d) is an adequate inhalation study of subchronic duration and is supported by a similarly-designed study in a second animal species; however, due to a low number of animals per treatment group (10/group), the confidence in the principal study is medium. Confidence in the

database is low-to-medium. Studies assessing the developmental toxicity and multi-generation reproductive toxicity of bromobenzene are lacking.

The derivation of the RfC includes 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 ÷ 1,000 = 0.06 mg/m^3. The overall confidence in the chronic RfC is low-to-medium. Since there are no known chronic duration inhalation studies available, the RfC is based upon a subchronic duration study (NTP, 1985d). Confidence in this study is medium. Confidence in the database is low-to-medium. Studies assessing the developmental toxicity and multi-generation reproductive toxicity of bromobenzene are lacking.

6.2.3. Cancer/Oral or Inhalation

The lack of cancer studies in humans and cancer bioassays in animals precludes a cancer dose-response assessment for bromobenzene exposure via the oral or inhalation route.

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

The Toxicological Review of bromobenzene has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a, 2000a). The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. EPA did not receive public comments related to the Toxicological Review of Bromobenzene.

On April 10, 2008, EPA introduced revisions to the IRIS process for developing chemical assessments. As part of the revised process, the disposition of peer reviewer and public comments, as found in this Appendix, and the revised IRIS Toxicological Review was provided to the external peer review panel members on February 17, 2009, for an opportunity to comment on how EPA address the panel's recommendations. Any additional comments received as a part of the second review and EPA's responses are included at the end of this Appendix.

EXTERNAL PEER REVIEWER COMMENTS

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

A. General Comments

1. *Is the Toxicological Review logical, clear, and concise? Has EPA accurately, clearly, and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?*

Comments: All reviewers commended the document for general clarity, conciseness, and thoroughness. However, one reviewer offered a number of comments regarding the information found in Section 3.3 (Metabolism) of the assessment document. This reviewer indicated the lack of discussion (and illustration in the figure) of the role of glucuronidation and sulfation of the primary phenolic metabolites as a determinant of the extent of secondary oxidation.

Response: Figure 3-1 has been amended to include an indication of glucuronidation and sulfation of bromophenols. Additional text has been added to Section 3.3 regarding the significance of glucuronidation and sulfation in phase II metabolism of bromobenzene.

Comment: One reviewer stated that it is unclear what is meant by the sentence on page 10, paragraph 3 "Metabolism of bromobenzene in the liver appears to be capacity-limited." Early studies (Zampaglione et al., 1973) showed that the whole body half-life of an intravenous dose of [^{14}C]-bromobenzene was short and not changed by "simultaneous" intraperitoneal administration of bromobenzene in oil.

Response: The statement regarding capacity-limited bromobenzene metabolism is supported by the observations reported by Lertratanangkoon and Horning (1987) that illustrate a significant reduction in the urinary excretion of [^{14}C]-bromobenzene at a hepatotoxic dose (1,200 mg/kg-day) versus a non-hepatotoxic dose (130 mg/kg-day) via the same exposure route. There are three issues with the reviewer's argument in the comment above: (1) the relative contribution of extrahepatic metabolism (e.g., kidney, lung) in vivo is not known; (2) as reported in Zampaglione et al. (1973), the intravenous tracer dose of [^{14}C]-bromobenzene was administered to rats 90 minutes after the intraperitoneal loading dose of unlabeled bromobenzene, not simultaneously as indicated by the reviewer; and (3) the tracer dose of [^{14}C]-bromobenzene reported in the Zampaglione et al. (1973) study was 10 µmol, which is approximately equivalent to a dose of 8 mg/kg body weight. Such a small dose administered intravenously would not be anticipated to provide a significant burden to the overall hepatic load of bromobenzene. Specifically, the metabolic relationship (i.e., half-life) between a "bioavailable" fraction of a presumably hepatotoxic dose of bromobenzene given intraperitoneally and a very low dose of bromobenzene administered via a different route, separated by 90 minutes, is not clear. While there are differences in the interpretation of some of the metabolism studies (e.g., Letratanangkoon and Horning, 1987), it is recognized that other studies such as Zampaglione et al. (1973) suggest an alternative conclusion regarding capacity-limitation (or not) at different bromobenzene doses. As such, the text in question on page 11 of the Toxicological Review has been removed as suggested.

Comment: One reviewer questioned the citation for Lertratanangkoon and Horning (1987), stating that the paper deals predominantly with the pathways of elimination of premercapturic acids of bromobenzene rather than of bromobenzene itself.

Response: The reference provided on page 10, paragraph 3, lines 22–26 of the external review draft is Lertratanangkoon and Horning (1987). There is another study from the same group and same year (i.e., Lertratanangkoon, Horning, and Horning [1987]).

2. *Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of bromobenzene.*

Comments: None of the reviewers suggested additional studies. One reviewer suggested further laboratory experimentation to supplement the available bromobenzene database; specifically, the reviewer thought that in vitro cytotoxicity assay data from rat and human hepatocyte cultures would be useful.

Response: Additional studies regarding bromobenzene toxicity would be highly desirable.

Chemical-Specific Charge Questions

B. Oral Reference Dose (RfD) Values

1. *A subchronic and chronic RfD for bromobenzene have been derived from the 90-day oral gavage study (NTP, 1985b) in mice. Please comment on whether the selection of this study as the principal study has been scientifically justified and transparently and objectively described in the document. Please identify and provide the rationale for any other studies that should be selected as the principal study.*

Comments: All reviewers agreed with the selection of NTP (1985b) as the principal study. One reviewer indicated that there is concern for the number of animals used per treatment group in the study (n = 10) and stated that the design, implementation, and interpretation of data from NTP studies conducted in the mid-1980s is rather poor. Another concern was that the majority of the animals receiving the 600 mg/kg-day dose died.

Response: The NTP (1985b) study employed 10 animals/sex/treatment group (a total of 20 animals/treatment group). While the qualitative evaluations were divided by sex, 10 animals/sex/treatment group allowed for analysis of statistical and biological significance of bromobenzene toxicity via the oral route (see Table 4-4). Observations at the high dose are taken as a qualitative identification of an apparent upper bound of what is tolerable in the experimental animal model. As can be seen in the derivation of the bromobenzene oral RfD values, the 600 mg/kg-day dose group has virtually no influence on the interpretation of data in the low-dose region where the POD (i.e., BMDL) is identified.

2. *Liver toxicity (including increased liver weight and liver lesions) was selected as the most appropriate critical effect. Please comment on whether the selection of this critical effect has*

been scientifically justified and transparently and objectively described in the document. Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Comment: None of the reviewers disagreed with the selection of liver toxicity as the critical effect. One reviewer agreed with the combined liver lesion index; however this reviewer did not agree with inclusion of increased liver weight in any analysis. Three reviewers disagreed with the use of a combined index of liver injury (combination of cytomegaly, necrosis, inflammation and mineralization); one of these reviewers stated that the only meaningful indicator of toxicity is hepatocellular necrosis and that increased liver weight is potentially adaptive and therefore not appropriate for consideration. Another reviewer recommended a new BMD analysis based only on liver necrosis for identification of a POD, and a third reviewer noted that cytomegaly, inflammation, and mineralization may be more or less constantly related to centrilobular necrosis although this reviewer believed the data in F344/N rats (see Table 4-2) and $B6C3F_1$ mice (see Table 4-4) do not seem to support such a relationship. This reviewer added that the mild to modest inflammation seen in the controls and at high doses is unlikely related to the significant inflammation observed when frank necrosis was present, and that the cytomegaly and the associated increase in liver weight are nonspecific responses and are normally considered to be adaptive changes rather than pathological responses. Another reviewer asserted that chemicals can produce increases in liver weight in the absence of toxicity and suggested consideration of selecting toxic endpoint(s) more closely related to the mode of action of toxicants that generate reactive intermediates, such as changes in gene expression of stress genes and markers of oxidative stress.

Response: The combined liver lesion index of cytomegaly, inflammation, mineralization, and necrosis has been removed from the assessment. In consideration of the reviewers' suggestions regarding the selection of hepatocellular necrosis as the critical effect, two approaches for deriving the subchronic RfD were presented in Section 5.1.1. One approach utilized an increased incidence of cytomegaly as the critical effect and the second approach was based on an increased incidence of necrosis as the critical effect. Detailed discussion related to both of these approaches was included in Section 5.1. Ultimately, EPA selected liver cytomegaly as the critical effect as it may represents a sensitive precursor effect leading to more overt bromobenzene-induced liver injury, including necrosis, of bromobenzene-induced liver injury. In addition, in EPA's judgment, cytomegaly may be considered an adverse effect regardless of its potential association with necrosis.

Comment: One reviewer noted that the importance of distinguishing between adaptive and toxic responses is illustrated by the studies of Heijne et al (2004). As discussed in Section 4.5.3,

bromobenzene was administered to rats and time- and dose-related genomic changes at the transcriptional level were examined. Numerous alterations in gene expression were observed but none could be related to the pathological sequence of events leading up to cell death.

Response: The Heijne et al. (2004) study identified genes that may indicate early signs of bromobenzene-induced liver toxicity. For example, a single oral dose of bromobenzene (0.5 mmol/kg) in rats induced an increase in the transcriptional activity of genes involved in oxidative stress (e.g., heme oxygenase-1) and a decrease in genes involved in cytoprotection against xenobiotic-induced liver injury (e.g., glutathione-S-transferase-1 theta, heat shock protein 70). Bromobenzene has also been shown to induce alterations in mitochondrial bioenergetics and Ca^{2+} homeostasis and increase the expression of p53. Any or all of these bromobenzene-induced alterations in transcriptional expression or cellular functionality could potentially lead to commitment of hepatocytes to cell death.

Comment: One reviewer was concerned with the use of the combined index based on what is known about the mechanism underlying bromobenzene-induced hepatocellular necrosis stating that while the precise steps that lead to cell death are still undefined, there is ample evidence for the crucial role played by glutathione in protecting the liver cell against the toxic metabolite and that the toxic "hit" occurs only after glutathione has been depleted from the liver cell. It was stated that this "threshold' nature of the toxic mechanism is well accepted for bromobenzene and explains the very sharp dose-response curve seen in acute animal studies.

Response: The Agency agrees that the specific steps involved in bromobenzene-induced liver cell death (apoptotic or necrotic) have not been characterized in the available literature. However a significant body of evidence exists to suggest that potential key events in addition to (and likely related to) GSH depletion may be involved in the changes observed. The changes include alterations in Ca^{2+} homeostasis, oxidative stress, and alterations in mitochondrial respiration/bioenergetics (e.g., ATP synthesis). However, cytomegaly, described in Section 5.1.1.1 as an "enlargement of both the cell and the nucleus", may be a critical histopathological indicator of adverse cellular events that ultimately result in the manifestation of necrotic foci. It should be noted that necrotic cell death is commonly referred to as "oncosis" or "oncotic" necrosis; oncotic meaning "pertaining to, caused by or marked by swelling." As such, the morphometric identification of "cytomegaly" at lower doses of bromobenzene (e.g., 200 mg/kg-day in male mice) may indeed be an early indication of altered hepatocyte function. Thus, while GSH depletion may play an important role in the observed dose-response for oral bromobenzene-induced liver toxicity, it may not be the only molecular/cellular mechanism involved in the mode of action responsible for the observed lesions.

3. *The subchronic and chronic RfDs have been derived utilizing BMD modeling to define the point of departure (POD). All available models were fit to the data for the combined incidence of animals with one or more of the histopathologic liver lesions (centrilobular cytomegaly, necrosis, inflammation, mineralization), liver weight, and SDH levels. Please comment on the appropriateness and scientific justification presented for combining the incidence of liver effects to obtain a data set for BMD modeling. Please provide comments with regards to whether BMD modeling is the best approach for determining the point of departure. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the benchmark response selected for use in deriving the POD been scientifically justified and transparently and objectively described? Please comment on the appropriateness of averaging the BMDs for increased liver weight and liver lesions to derive the POD. Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.*

Comment: Four reviewers disagreed with combining the incidence of liver effects; two of these reviewers recommended using only the incidence of hepatocellular necrosis. One reviewer recommended additional discussion for BMD modeling of data that included animals exhibiting mortality or moribundity.

Response: As stated in response to the comment under charge question B.2, the combined index of liver injury as the critical effect was deleted and the data were reevaluated. BMD modeling approaches for both liver cytomegaly and necrosis are presented in Sections 5.1.1.2 and 5.1.1.3. EPA selected cytomegaly as the critical effect and the quantitative derivations and associated BMD modeling were revised accordingly. Mortality or moribundity of animals was considered. Considering that the BMD modeling approach involves identification of a BMR, BMD, and 95% lower confidence limit on the BMD (BMDL) in the low dose region of a given dose response, the mortality and moribundity in animals in the high dose groups has virtually no bearing on the shape of the dose-response curve for liver toxicity in the low dose region.

Comment: One reviewer concurred with the BMD approach overall but had some concerns related to parameterization of the continuous dose-response models used to assess liver weight changes. Specifically, this reviewer commented that there was no discussion or reference provided to support the choice of a one standard deviation shift in the mean for the BMD and that no statement is explicitly presented that the BMD_{1sd}= BMD_{10}. The reviewer suggested that the choice of a one standard deviation shift in the mean for an effect measured on a continuous scale needs to be presented. The reviewer also suggested that a weighted average of BMDLs across models could have been used, or alternatively, for a specific biological effect, BMDLs

could be averaged across studies (e.g., male and female rats and mice) in order to obtain a more representative value. Furthermore, the reviewer asserted that there is no logic for averaging BMDLs across different biological effects (i.e., liver lesions and increased liver weight).

Response: The reviewers were unanimous in dismissing increased liver weight as a significant effect following bromobenzene exposure. As such, the revised assessment document does not present continuous BMD modeling of liver weight. The reviewer's suggestion of averaging weighted BMDLs across models or studies was considered but was not used in the analysis because choosing a sensitive endpoint and the best fitting model is the preferred approach. For example, the POD, in this assessment a $BMDL_{10}$, is selected from the best fitting model from the most sensitive species. The *p*-value of 1.33 in Table 5-8 is incorrect in the external review draft. The correct *p*-value of 0.16 has been inserted into the table.

4. *Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfDs. For instance, are they scientifically justified and transparently and objectively described in the document?*

Comments: Four reviewers agreed with the selection of the areas of uncertainty and three of these reviewers stated the uncertainty factors were appropriately applied. One reviewer agreed with the applied uncertainty factors with the exception of a factor of 10 for interindividual variability. This reviewer stated a reduction of the intraspecies uncertainty factor of 10 to 5 would adequately protect sensitive subpopulations.

Response: Based on the absence of any information regarding the relative differences in kinetics (and dynamics) among the human population, the 10-fold UF for interindividual variability was retained.

5. *EPA used the data available for chlorobenzene to inform the selection of the subchronic to chronic uncertainty factor for the derivation of the chronic RfD for bromobenzene. Please comment on the scientific justification for this use of data from chlorobenzene. Has the scientific justification for this selection been transparently and objectively presented?*

Comments: None of the reviewers objected to the inclusion of chlorobenzene data to inform the selection of a subchronic to chronic UF for oral bromobenzene exposure.

Response: No response needed.

C. Inhalation Reference Concentration (RfC) Values

1. *A subchronic and chronic RfC for bromobenzene has been derived from the 13 week inhalation study (NTP, 1985d) in mice. Please comment on whether the selection of this study as the principal study has been scientifically justified and transparently and objectively described in the document. Please identify and provide the rationale for any other studies that should be selected as the principal study.*

Comments: None of the reviewers disagreed with the selection of the principal study. One reviewer expressed concern for the number of animals used per treatment group in the study (n = 10) and suggested adding a discussion of NTP's statistical power analysis to the assessment.

Response: The NTP (1985d) study employed 10 animals/sex/treatment group (a total of 20 animals/treatment group). While the qualitative evaluations were divided by sex, data from the 10 animals/sex/treatment group allowed for analysis of statistical and biological significance of bromobenzene toxicity via the inhalation route (see Table 4-8).

2. *Liver cytomegaly in female mice was selected as the critical toxicological effect. Please comment on whether the selection of this critical effect has been scientifically justified and transparently and objectively described in the document. Specifically, please address whether the selection of increased incidence of cytomegaly as the critical effect instead of increased liver weight has been adequately and transparently described. Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.*

Comments: Three reviewers disagreed with the selection of liver cytomegaly, and suggested using liver necrosis, instead, as the critical effect. One review stated that there was inadequate justification provided for the choice of critical effect.

Response: The critical effect has been changed to liver cytomegaly and the accompanying text has been revised Please refer to the responses provided to the comments under Charge Questions B.2 and B.3.

3. *The subchronic and chronic RfCs have been derived utilizing BMD modeling to define the point of departure. Please provide comments with regards to whether BMD modeling is the best approach for determining the point of departure. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the benchmark response selected for use in deriving the POD been scientifically justified and transparently and objectively*

described? Please comment on the justification for not utilizing the 100 ppm dose identified in the NTP (1985d) study as a NOAEL. Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.

Comment: None of the reviewers disagreed with the BMD modeling approach. One reviewer stated that the 100 ppm dose in the NTP (1985d) study was not a NOAEL based on the statistically significant increase in liver weight of female mice.

Response: The text was revised to identify the statistically significant increase in liver weight, compared with controls, at the 100 ppm exposure level as a LOAEL rather than a NOAEL.

4. *Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfCs. For instance, are they scientifically justified and transparently and objectively described in the document.*

Comments: None of the reviewers disagreed with the application of the UFs. One reviewer suggested the factor of 10 for interindividual variability overestimates the intraspecies uncertainty.

Response: As noted in response to comment under Charge Question B.4, the interindividual uncertainty of 10 is retained in the absence of information regarding the relative differences in kinetics (and dynamics) among the human population.

5. *EPA used the data available for chlorobenzene to inform the selection of the subchronic to chronic uncertainty factor for the derivation of the chronic RfC for bromobenzene. Please comment on the scientific justification for this use of data from chlorobenzene. Has the scientific justification for this selection been transparently and objectively presented?*

Comments: None of the reviewers disagreed with the use of data from chlorobenzene.

Response: No response needed.

Carcinogenicity of Bromobenzene

Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment *(www.epa.gov/iris/backgr-d.htm), data are inadequate for an assessment of the human carcinogenic potential of bromobenzene. Please comment on the scientific justification for the cancer weight of the evidence characterization. A quantitative cancer assessment was not derived for bromobenzene. Has the scientific justification for not deriving a quantitative cancer assessment been transparently and objectively described?*

Comments: None of the reviewers disagreed with the cancer assessment.

Response: No response needed.

ADDITIONAL EXTERNAL PEER REVIEW PANEL COMMENTS IN RESPONSE TO REVISIONS

(Note that comments were received from one reviewer.)

Comment: One reviewer suggested a more thorough discussion of the contribution of glucuronidation and sulfation in bromobenzene metabolism, as it relates to nephrotoxicity. Specifically, the reviewer asked that more descriptive text from the Chadwick et al. (1997) reference be incorporated into the Toxicological Review.

Response: The Chadwick et al. (1997) study was reexamined and additional text was added to Section 3.3. (Metabolism) to provide a more descriptive explanation of the results.

Comment: One reviewer disagreed with text in Section 3.3., characterizing bromobenzene metabolism at hepatotoxic doses as capacity-limited. The reviewer suggested revision or removal of such text from the Toxicological Review.

Response: After further review of the original published reference upon which the opinion of capacity-limited bromobenzene metabolism was based (Lertratanangkoon and Horning, 1987), the text identified by the reviewer was deleted.

Comment: The reviewer reiterated their concern that frank hepatic necrosis is the only manifestation of bromobenzene exposure suitable for consideration as a critical effect. The reviewer further commented that hepatic cytomegaly (the selected critical effect in the revised toxicological review) is not justifiable as a critical effect and should be reconsidered.

Response: Based on the reviewer's comment, two approaches for deriving the subchronic RfD are included in Section 5.1.1; one based on liver cytomegaly and the other based on necrosis as a critical effect. Discussion of these approaches and additional supporting text to clarify the selection of cytomegaly as the critical effect is included in Section 5.1. Please also see responses to comments under Charge Questions B.2, B.3, and C.2.

APPENDIX B. BENCHMARK DOSE CALCULATIONS FOR THE RfD

All available dichotomous models in the EPA's BMDS (version 1.4.1) were fit to the incidence data for liver cytomegaly in male and female F344/N rats and male and female $B6C3F_1$ mice from the 90-day gavage studies conducted by the NTP (1985a, b). The cytomegaly incidence data modeled are presented in Table 5-1 of the Toxicological Review. As a point of comparison, all dichotomous models were fit to the incidence data for liver necrosis in male and female rats and mice from the same NTP studies (NTP, 1985a, b). The incidence data for liver necrosis are presented later in this appendix. An incidence rate that was 10% above the rate in controls was selected as the BMR in the absence of biological information that would warrant a different choice. A 10% increase in incidence relative to controls is considered representative of a minimal biologically significant change.

Cytomegaly

Table B-1. BMD modeling results for the incidence of liver cytomegaly in male F344/N rats exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10} and $BMDL_{10}$ values (mg/kg-day)		χ^2 *p*-value	AIC
	BMD_{10}	$BMDL_{10}$		
Log-logistic[a]	132.5	87.5	0.29	28.42
Log-probit[a]	128.6	84.9	0.29	28.93
Multistage[c]	99.3	57.1	0.25	30.32
Gamma[b]	122.4	73.5	0.14	30.36
Weibull	103.2	57.4	0.13	32.30
Logistic	129.4	86.3	0.0005	33.61
Probit	123.9	81.9	0.003	35.08
Quantal linear	32.6	22.7	0.08	38.48

[a]Slope restricted to >1.
[b]Restrict power ≥1.
[c]Restrict betas ≥0; degree of polynomial = 2.

The log-logistic model provided the best fit to the male rat data as illustrated by the lowest AIC value of 28.42. Based on the log-logistic model, a $BMDL_{10}$ of 87.5 mg/kg-day represents the dose level at which a 10% increase in the incidence of liver cytomegaly occurs in bromobenzene-treated male rats relative to controls.

Table B-2. BMD modeling results for the incidence of liver cytomegaly in female F344/N rats exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10} and $BMDL_{10}$ values (mg/kg-day)		χ^2 *p*-value	AIC
	BMD_{10}	$BMDL_{10}$		
Log-logistic[a]	185.5	130.2	1.0	14.22
Gamma[b]	164.0	119.3	1.0	14.32
Multistage[c]	147.9	102.0	1.0	14.76
Weibull[b]	182.5	112.5	1.0	16.22
Probit	185.3	116.0	1.0	16.22
Logistic	192.4	123.4	1.0	16.22
Log-probit[a]	184.0	126.0	1.0	16.22
Quantal linear	31.0	21.5	0.03	34.68

[a]Slope restricted to >1.
[b]Restrict power ≥1.
[c]Restrict betas ≥0; degree of polynomial = 4.

The log-logistic model provided the best fit to the female rat data as illustrated by the lowest AIC value of 14.22. Based on the log-logistic model, a $BMDL_{10}$ of 130.2 mg/kg-day represents the dose level at which a 10% increase in the incidence of liver cytomegaly occurs in bromobenzene-treated female rats relative to controls.

Table B-3. BMD modeling results for the incidence of liver cytomegaly in male $B6C3F_1$ mice exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10} and $BMDL_{10}$ values (mg/kg-day)		χ^2 *p*-value	AIC
	BMD_{10}	$BMDL_{10}$		
Log-logistic[a]	57.6	33.8	0.21	56.34
Gamma[b]	74.3	49.7	0.12	57.24
Weibull[b]	74.3	49.7	0.12	57.24
Multistage[c]	74.3	49.7	0.12	57.24
Quantal linear	74.3	49.7	0.12	57.24
Log-probit[a]	110.4	78.9	0.06	58.15
Probit	173.3	123.0	0.01	63.98
Logistic	185.9	129.8	0.01	64.37

[a]Slope restricted to >1.
[b]Restrict power ≥1.
[c]Restrict betas ≥0; degree of polynomial = 1.

The log-logistic model provided the best fit to the male mouse data as illustrated by the lowest AIC value of 56.34. Based on the log-logistic model, a $BMDL_{10}$ of 33.8 mg/kg-day

represents the dose level at which a 10% increase in the incidence of liver cytomegaly occurs in bromobenzene-treated male mice relative to controls.

Table B-4. BMD modeling results for the incidence of liver cytomegaly in female B6C3F$_1$ mice exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10} and $BMDL_{10}$ values (mg/kg-day)		χ^2 *p*-value	AIC
	BMD_{10}	$BMDL_{10}$		
Multistage[a]	84.3	47.2	0.98	29.95
Log-probit[b]	104.5	68.3	0.99	31.27
Gamma[c]	102.3	60.3	0.99	31.33
Log-logistic[b]	107.2	68.0	0.98	31.52
Weibull[c]	95.8	53.5	0.96	31.73
Probit	107.3	70.3	0.81	32.89
Logistic	110.8	73.5	0.76	33.20
Quantal linear	27.8	19.4	0.28	38.49

[a]Restrict betas ≥0; Degree of polynomial = 2.
[b]Slope restricted to >1.
[c]Restrict power ≥1.

The multistage model provided the best fit to the female mouse data as illustrated by the lowest AIC value of 29.95. Based on the multistage model, a $BMDL_{10}$ of 47.2 mg/kg-day represents the dose level at which a 10% increase in the incidence of liver cytomegaly occurs in bromobenzene-treated female mice relative to controls.

Among the BMD modeled datasets, male mice provided the lowest $BMDL_{10}$ of 33.8 mg/kg-day for increased incidence in liver cytomegaly. This $BMDL_{10}$ is selected as the POD for derivation of a subchronic and chronic oral RfD (see Sections 5.1.1.3 and 5.1.2.3). A plot of the log-logistic BMD modeling fit and associated output for the male mouse data follow:

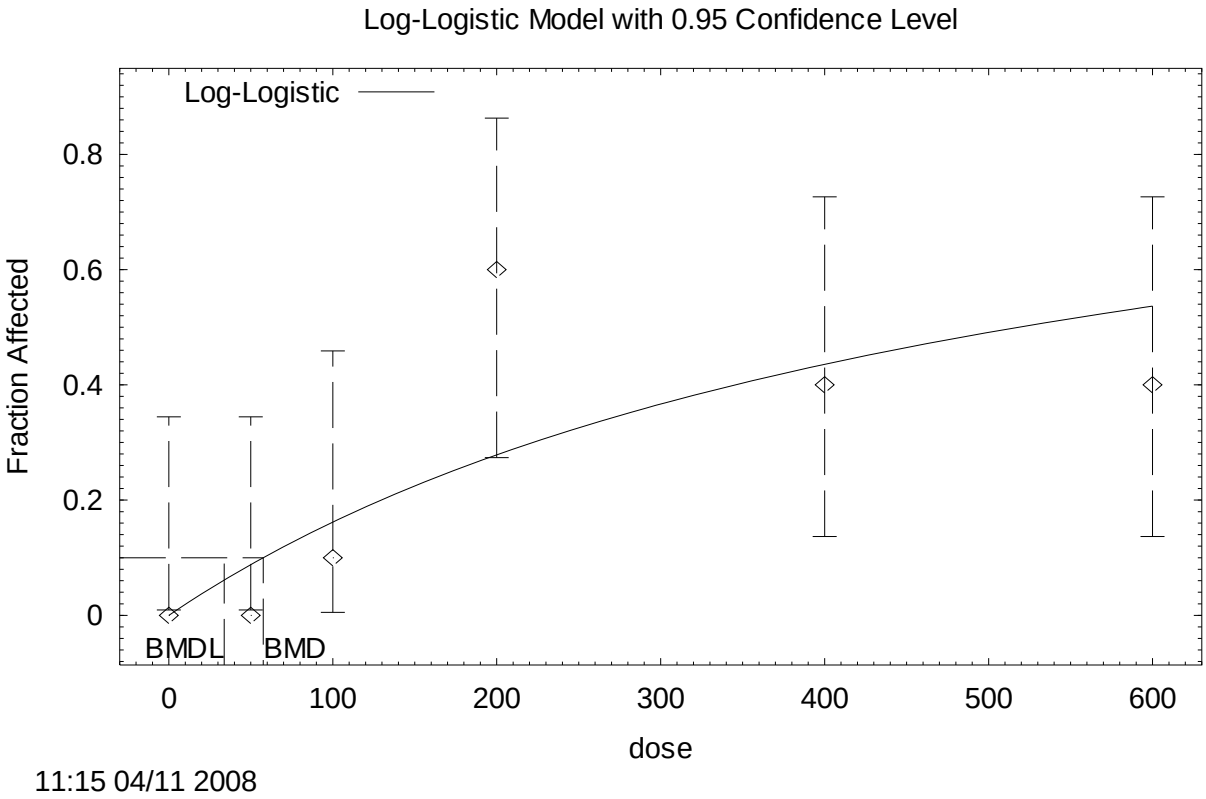

Figure B-1. Observed and log-logistic model-predicted incidences of male B6C3F$_1$ mice exhibiting bromobenzene-induced liver cytomegaly following gavage treatment 5 days/week for 90 days.

The form of the probability function is:

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

Dependent variable = Response
Independent variable = Dose
Slope parameter is restricted as slope $\geq$ 1

Total number of observations = 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

User has chosen the log transformed model

Default Initial Parameter Values

background = 0
intercept = -7.16677
slope = 1.15199

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

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-6.25085	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	*p*-value
Full model	-23.4412	6			
Fitted model	-27.1679	1	7.45353	5	0.189
Reduced model	-33.7401	1	20.5979	5	0.0009647

AIC: 56.3359

Goodness of Fit

Dose	Est.Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	10	0.000
50.0000	0.0880	0.880	0	10	-0.982
100.0000	0.1617	1.617	1	10	-0.530
200.0000	0.2784	2.784	6	10	2.269
400.0000	0.4355	4.355	4	10	-0.227
600.0000	0.5365	5.365	4	10	-0.865

Chi-square = 7.19 d.f. = 5 *p*-value = 0.2066

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 57.6058

BMDL = 33.8014

Necrosis

Table B-5. Incidences of male and female F344/N rats and B6C3F$_1$ mice with liver necrosis[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	0/10	0/10	0/10	3/10	9/10[b]	9/10[b]
Female rats	0/10	0/10	0/10	0/10	7/10[b]	9/10[b]
Male mice	0/10	0/10	0/10	1/10	4/10[b]	8/10[b]
Female mice	0/10	0/10	1/10	0/10	1/10	7/10[b]

[a]Incidences of rats or mice with necrosis, 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 B-6. BMD modeling results for the incidence of liver necrosis in male F344/N rats exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10} and $BMDL_{10}$ values (mg/kg-day)		χ^2 *p*-value	AIC
	BMD_{10}	$BMDL_{10}$		
Log-logistic[a]	144.4	93.4	0.82	30.82
Log-probit[a]	143.0	93.3	0.8	30.89
Multistage[b]	110.5	66.7	0.69	31.39
Gamma[c]	138.5	83.3	0.63	31.83
Weibull[c]	122.8	69.2	0.49	33.23
Logistic	149.9	100.2	0.14	34.78
Probit	144.1	95.5	0.15	35.25
Quantal linear	38.3	26.5	0.14	39.74

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

The log-logistic model provided the best fit to the male rat necrosis data as illustrated by the lowest AIC value of 30.82. Based on the log-logistic model, a $BMDL_{10}$ of 93.4 mg/kg-day represents the dose level at which a 10% increase in the incidence of liver necrosis occurs in bromobenzene-treated male rats relative to controls.

Table B-7. BMD modeling results for the incidence of liver necrosis in female F344/N rats exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10} and $BMDL_{10}$ values (mg/kg-day)		χ^2 *p*-value	AIC
	BMD_{10}	$BMDL_{10}$		
Log-probit[a]	248.9	171.4	0.91	23.88
Log-logistic[a]	252.9	171.1	0.91	23.90
Gamma[b]	245.6	163.2	0.84	24.39
Weibull[b]	221.9	141.3	0.65	25.91
Probit	243.3	163.3	0.57	25.91
Logistic	251.0	172.4	0.51	25.91
Multistage[c]	211.3	139.3	0.66	26.07
Quantal-linear	57.6	38.5	0.05	37.88

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

The log-probit model provided the best fit to the female rat necrosis data as illustrated by the lowest AIC value of 23.88. Based on the log-probit model, a $BMDL_{10}$ of 171.4 mg/kg-day represents the dose level at which a 10% increase in the incidence of liver necrosis occurs in bromobenzene-treated female rats relative to controls.

Table B-8. BMD modeling results for the incidence of liver necrosis in male $B6C3F_1$ mice exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10} and $BMDL_{10}$ values (mg/kg-day)		χ^2 *p*-value	AIC
	BMD_{10}	$BMDL_{10}$		
Gamma[a]	220.9	131.9	0.99	34.29
Weibull[a]	223.7	129.4	0.99	34.32
Multistage[b]	227.6	116.4	0.99	34.36
Log-probit[c]	217.3	134.1	0.98	34.37
Log-logistic	224.9	134.7	0.98	34.40
Probit	242.7	165.3	0.97	34.74
Logistic	255.2	176.1	0.94	35.16
Quantal-linear	79.5	51.5	0.34	39.92

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

The gamma model provided the best fit to the male mouse necrosis data as illustrated by the lowest AIC value of 34.29. Based on the gamma model, a $BMDL_{10}$ of 131.9 mg/kg-day

represents the dose level at which a 10% increase in the incidence of liver necrosis occurs in bromobenzene-treated male mice relative to controls.

Table B-9. BMD modeling results for the incidence of liver necrosis in female B6C3F$_1$ mice exposed to bromobenzene by gavage 5 days/week for 90 days

Model	BMD_{10} and $BMDL_{10}$ values (mg/kg-day)		χ^2 *p*-value	AIC
	BMD_{10}	$BMDL_{10}$		
Gamma[a]	394.3	284.4	0.5	32.20
Multistage[b]	376.9	157.3	0.46	32.44
Logistic	319.4	224.8	0.15	33.48
Log-Probit[c]	412.4	294.6	0.38	34.07
Log-logistic	415.1	288.5	0.38	34.08
Weibull[a]	418.9	271.7	0.38	34.09
Probit	286.0	200.1	0.15	34.25
Quantal-linear	128.4	77.3	0.16	37.30

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

The gamma model provided the best fit to the female mouse necrosis data as illustrated by the lowest AIC value of 32.20. Based on the gamma model, a $BMDL_{10}$ of 284.4 mg/kg-day represents the dose level at which a 10% increase in the incidence of liver necrosis occurs in bromobenzene-treated female mice relative to controls.

Among the BMD modeled datasets for necrosis in rats or mice, male rats provided the lowest $BMDL_{10}$ of 93.4 mg/kg-day. This $BMDL_{10}$ for liver necrosis is approximately three-fold greater than the lowest $BMDL_{10}$ identified for liver cytomegaly in male mice. A plot of the log-logistic BMD modeling fit and associated output for the male rat data follow:

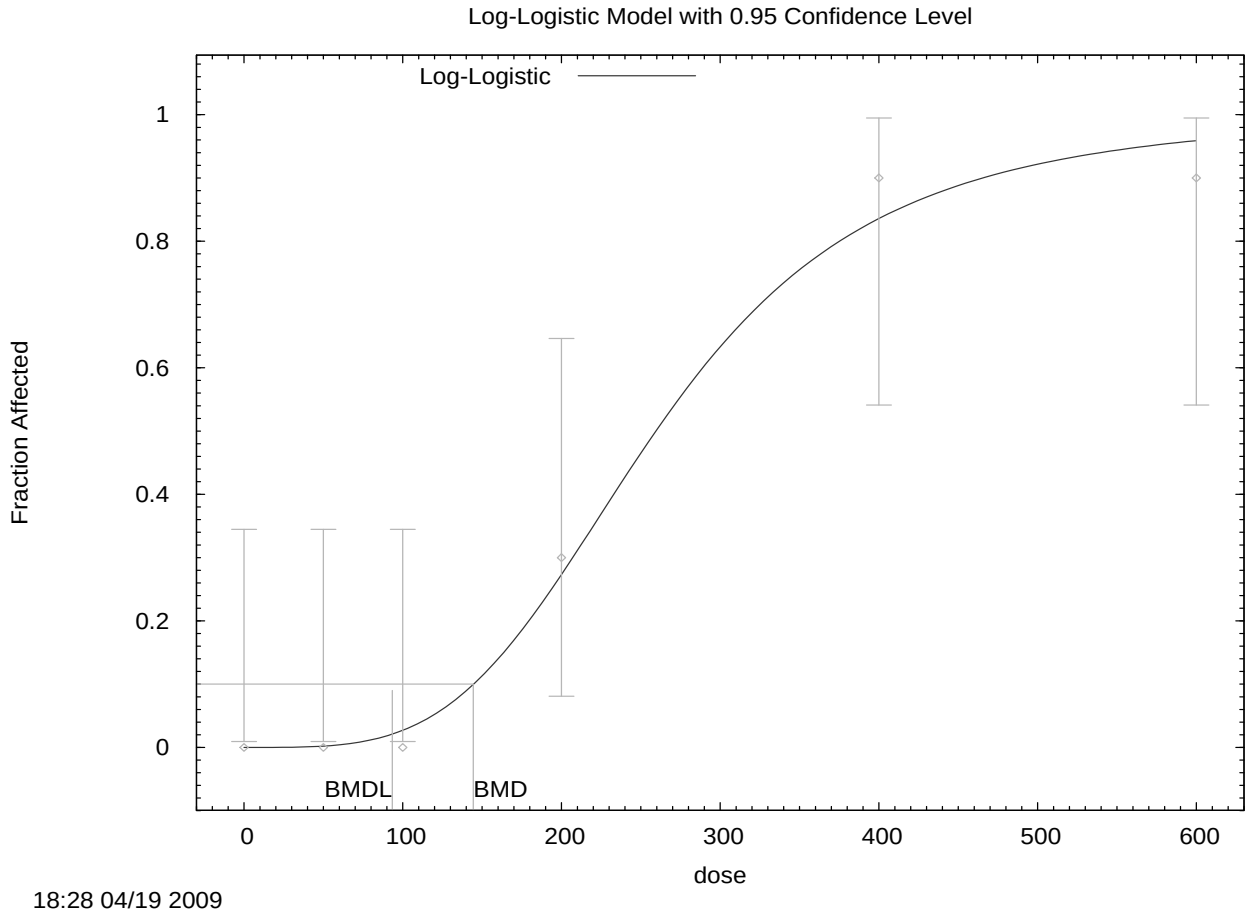

Figure B-2. Observed and log-logistic model-predicted incidences of male F344/N rats exhibiting bromobenzene-induced liver necrosis following gavage treatment 5 days/week for 90 days.

The form of the probability function is:

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

Dependent variable = Response
Independent variable = DOSE
Slope parameter is restricted as slope ≥1

Total number of observations = 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

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = -13.5315
slope = 2.48497

Asymptotic Correlation Matrix of Parameter Estimates

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

	intercept	slope
intercept	1	-1
slope	-1	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-20.913	*	*	*
slope	3.7636	*	*	*

* Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	*p*-value
Full model	-12.6103	6			
Fitted model	-13.412	2	1.60346	4	0.8082
Reduced model	-38.8468	1	52.473	5	<.0001

AIC: 30.8241

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	10	0.000
50.0000	0.0020	0.020	0.000	10	-0.143
100.0000	0.0271	0.271	0.000	10	-0.528
200.0000	0.2744	2.744	3.000	10	0.181
400.0000	0.8371	8.371	9.000	10	0.539
600.0000	0.9594	9.594	9.000	10	-0.952

Chi-square = 1.53 d.f. = 4 *p*-value = 0.8217

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 144.436

BMDL = 93.4059

APPENDIX C. BENCHMARK DOSE CALCULATIONS FOR THE RfC

Incidence data for centrilobular cytomegaly in the liver of female $B6C3F_1$ mice were selected to serve as the basis for the derivation of the RfC, based on the results from the 13-week NTP inhalation study indicating that female mice have a lower POD for bromobenzene hepatotoxicity than male mice or male or female rats. The data considered for BMD modeling are shown in Table 5-6. 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).

Sigmoidal models (e.g., gamma, probit, logistic, higher degree multistage) and non-sigmoidal models (e.g., quantal quadratic and quantal linear) in the U.S. EPA BMDS (version 1.3.2) were fit to the data in Table 5-6. Modeling results are presented in Table C-1 showing that (1) all sigmoidal models provided excellent fit to 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).

Table C-1. BMC modeling results for the incidence of liver cytomegaly in female $B6C3F_1$ mice exposed to bromobenzene vapors 6 hours/day, 5 days/week for 13 weeks

Model	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)	χ^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
Multistage[c]	77.09	40.33	1.00	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 number of dose groups minus 2).

Following U.S. EPA (2000b) guidance for selecting models for POD 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 (see Table C-1). The

$BMCL_{10}$ values from these best-fitting models (log-logistic and gamma models) were averaged (55 ppm) to arrive at the POD for deriving the subchronic and chronic RfC. Figures C-1 and C-2 are visual 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 follow:

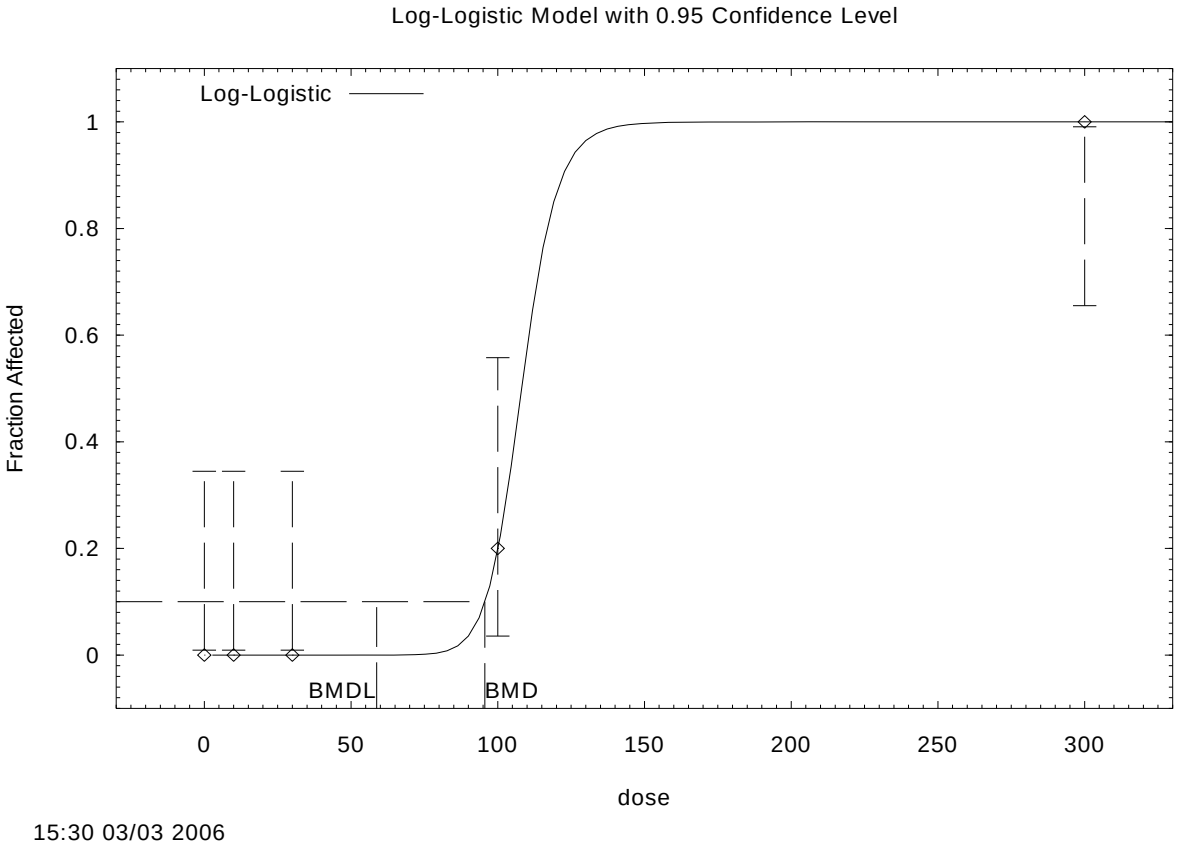

Figure C-1. Observed and log-logistic model-predicted incidences of liver cytomegaly in female $B6C3F_1$ mice exposed to bromobenzene vapors 6 hours/day, 5 days/week for 13 weeks.

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	Observed	Size	Scaled 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

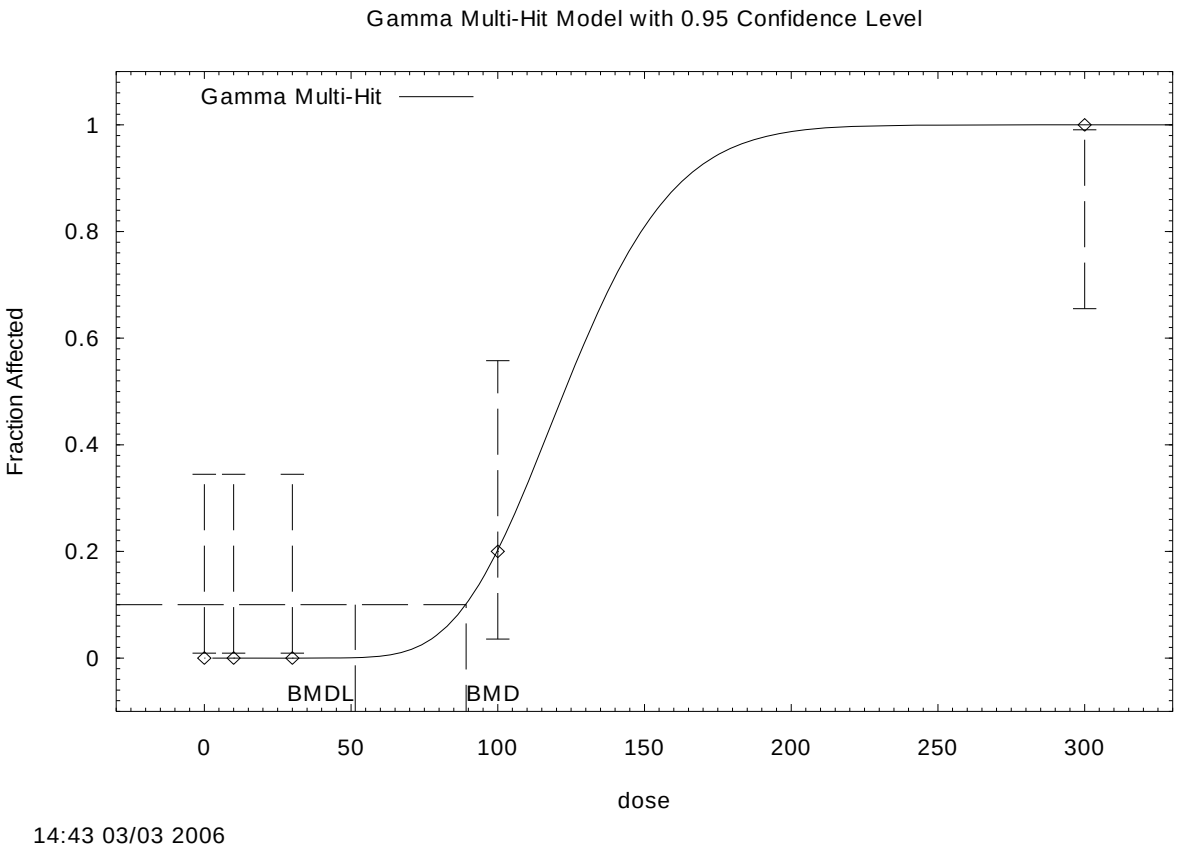

Figure C-2. Observed and gamma model-predicted incidences of liver cytomegaly in female B6C3F$_1$ mice exposed to bromobenzene vapors 6 hours/day, 5 days/week for 13 weeks.

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	Observed	Size	Scaled 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