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LIST OF ABBREVIATIONS AND ACRONYMS

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

FOREWORD

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

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

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

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1. INTRODUCTION

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

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

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are derived from the application of a low-dose extrapolation procedure, and are presented in two ways to better facilitate their use. First, route-specific risk values are presented. The “oral slope factor” is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a “unit risk” is an upper bound on the estimate of risk per unit of concentration, either per µg/L drinking water or per µg/m³ air breathed. Second, the estimated concentration of the chemical substance in drinking water or air when associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000 is also provided.

Development of these hazard identification and dose-response assessments for bromobenzene has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). U.S. Environmental Protection Agency (EPA) guidelines and Risk Assessment Forum Technical Panel Reports that were used in the development of this assessment include the following: *Guidelines for Developmental Toxicity Risk Assessment* (U.S.

1 EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines*
2 *for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Guidelines for Carcinogen Risk*
3 *Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-*
4 *Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Recommendations for and Documentation of*
5 *Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for*
6 *Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods*
7 *for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*
8 (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA,
9 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a, 2005c),
10 *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose*
11 *Technical Guidance Document* (U.S. EPA, 2000c), and *A Review of the Reference Dose and*
12 *Reference Concentration Processes* (U.S. EPA, 2002).

13 The literature search strategy employed for this compound was based on the Chemical
14 Abstract Service Registry Number (CASRN) and at least one common name. Any pertinent
15 scientific information submitted by the public to the IRIS Submission Desk was also considered
16 in the development of this document. The relevant literature was reviewed through February,
17 2007.

18

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

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

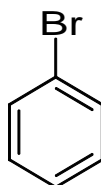


Figure 2-1. Chemical structure of bromobenzene

CASRN:	108-86-1 (Lide, 2000)
Molecular weight:	157.01 (Budavari, 2001)
Chemical formula:	C ₆ H ₅ Br (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:	4.46x10 ² mg/L at 30°C (Chiou et al., 1977)
Other solubility:	Miscible with chloroform, benzene, and petroleum hydrocarbons. Solubility in alcohol (0.045 g/100 g at 25°C), in ether (71.3 g/100 g at 25°C) (Budavari, 2001)
Partition coefficient:	log K _{ow} = 2.99 (Hansch et al., 1995)
Flash point:	51°C (Budavari, 2001)
Heat of combustion:	-1.98x10 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:	2.47x10 ⁻³ atm m ³ /mol at 25°C (Shiu and Mackay, 1997)
OH reaction rate constant:	7.70x10 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

1 carrier. Bromobenzene was produced in quantities less than 10,000 pounds (4.5×10^3 kg) in 1986,
2 1990, 1994, 1998, and 2002 (U.S. EPA, 2002). U.S. imports of bromobenzene were 2.00×10^3 kg
3 in 1984 (HSDB, 2003). Bromobenzene is used for organic synthesis, especially in the
4 production of the synthetic intermediate phenyl magnesium bromide (Budavari, 2001; Lewis,
5 1997). Bromobenzene is also used as an additive to motor oils and a crystallizing solvent.

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

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

14 Bromobenzene is expected to have moderate to high mobility in soil, based on a soil
15 sorption constant (K_{oc}) of 150 and an octanol/water partition coefficient ($\log K_{ow}$) of 2.99
16 (Hansch et al., 1995; U.S. EPA, 1987; Swann et al., 1983). Volatilization of bromobenzene from
17 moist soil surfaces may be significant, based on its Henry's Law constant of 2.47×10^{-3} atm
18 m^3/mol at 25°C (Shiu and Mackay, 1997; Lyman et al., 1990).

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

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

29 Bromobenzene has been detected in water samples from the Delaware River basin, the
30 Mississippi River, the Hudson River, and Lake Michigan (U.S. EPA, 1987). The average
31 concentration of bromobenzene from eight observations in stream water reported in 1976 was
32 12.75 $\mu g/L$, with a range of 3-38 $\mu g/L$, according to the STORET database (U.S. EPA, 1987).
33 Bromobenzene was identified with a maximum concentration of 10 ng/L in a contaminated
34 plume of groundwater near Falmouth, MA over 3500 meters long (Barber et al., 1988). The
35 plume resulted from the long-term disposal of secondary treated sewage effluent into a shallow,

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

3 Bromobenzene can be formed in small quantities during water chlorination (HSDB,
4 2003). For example, it has been detected (albeit infrequently) at low concentrations in finished
5 water in the lower Mississippi River area. During a groundwater supply survey (Westrick et al.,
6 1984), finished water samples were collected from public water systems located across the
7 United States that serve both greater than 10,000 persons and fewer than 10,000 persons.
8 Bromobenzene was detected above 0.5 µg/L (quantitation limit) in 3 out of 280 random sample
9 sites serving fewer than 10,000 persons with a median of positives of 1.9 µg/L and a maximum
10 value of 5.8 µg/L. It was also detected in 1 out of 186 random sample sites serving greater than
11 10,000 persons at 1.7 µg/L. In 2 of 321 nonrandom sample sites serving fewer than 10,000
12 persons, bromobenzene was detected with a median of positives of 0.97 µg/L and a maximum
13 value of 1.2 µg/L. Bromobenzene was not detected above the quantitation limit in 158
14 nonrandom sample sites serving more than 10,000 persons. In 0.13% of 24,125 public water
15 systems tested in a 20-state cross-section survey conducted for the U.S. EPA Office of Water
16 between 1993 and 1997 (U.S. EPA, 2003), bromobenzene was detected. The overall median
17 concentration of the detections was 0.5 µg/L. Detection frequency was higher in public water
18 systems using surface water (0.23% of 2664 surface water systems) than those using
19 groundwater (0.12% of 21,461 groundwater systems).

20 Bromobenzene has been detected at low concentrations in air samples collected near
21 unidentified emission sources (U.S. EPA, 1987; Brodzinsky and Singh, 1982). In 35 air samples
22 from El Dorado, AR collected from 1976 to 1978, bromobenzene concentrations ranged from
23 0.83 to 2100 ppt, with a mean concentration of 210 ppt. In 28 air samples from Magnolia, AR
24 collected in 1977, bromobenzene concentrations ranged from 0 to 8.3 ppt, with a mean
25 concentration of 1.5 ppt. Bromobenzene was not detected in seven air samples from Grand
26 Canyon, AZ or in one air sample from Edison, NJ.

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

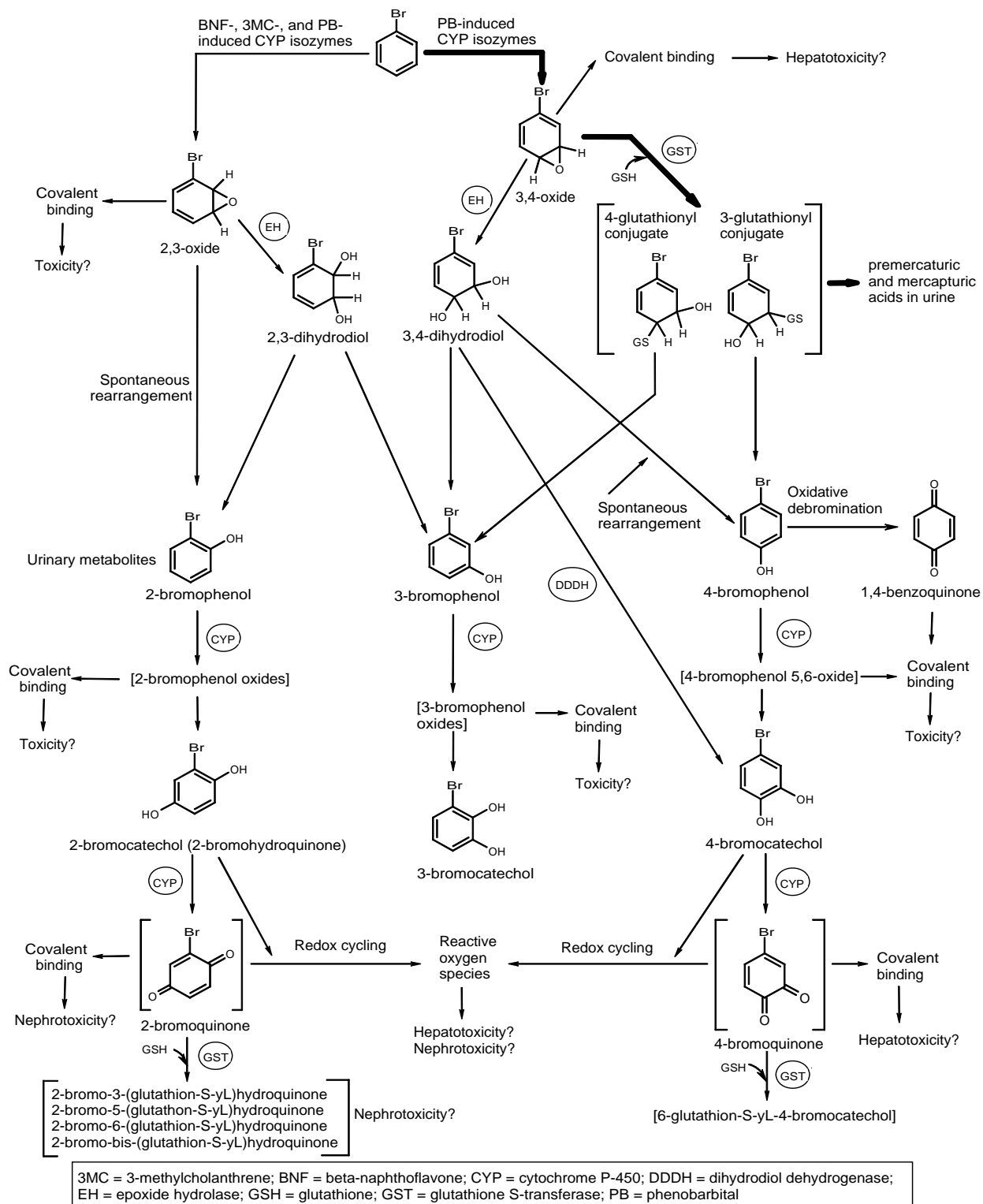
1 In another study, concentrations of bromobenzene in tissues from rats 10 hours after
2 intraperitoneal injection of 5 mg of bromobenzene were highest in adipose tissue (3.38 µg/g),
3 followed by liver (0.18 µg/g), seminal fluid (0.15 µg/g), blood (0.12 µg/g), brain (0.08 µg/g), and
4 pectoral muscle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

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

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

27 28 **3.3. METABOLISM**

29 The metabolism of bromobenzene has been extensively studied in *in vivo* and *in vitro*
30 mammalian systems (see Lau and Monks, 1997a,b; Lertratanangkoon et al., 1993; Lau and
31 Monks, 1988). Based on available data, a proposed metabolic scheme for bromobenzene is
32 illustrated in Figure 3-1. There are two initial competing steps involving conversion of
33 bromobenzene to either the 3,4-oxide derivative catalyzed by phenobarbital-induced cytochrome
34 isozymes CYP 450 1A2, 2A6, 2B6, and 3A4 or the 2,3-oxide derivative catalyzed by
35 3-methylcholanthrene and β-naphthoflavone-induced CYP isozymes, CYP 450 1A1, 1A2, and
36



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2
3
4

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

1 1B1, as well as phenobarbital-induced CYP isozymes (Girault et al., 2005; Krusekopf et al.,
2 2003; Lau and Zannoni, 1979, 1981a; Reid et al., 1971).

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

11 Both the 3,4- and 2,3-oxide derivatives may be converted to the corresponding
12 dihydrodiols by epoxide hydrolase (EH). The subsequent formation of bromophenols (2-, 3-,
13 and 4-bromophenol) from the oxide derivatives includes several proposed pathways
14 (Lertratanangkoon et al., 1993; Lau and Monks, 1988). The chemical instability of the 2,3-oxide
15 derivative and its relatively short biological half-life indicate that spontaneous rearrangement is
16 the predominant pathway to the formation of 2-bromophenol in the rat and guinea pig *in vivo*
17 (Lertratanangkoon et al., 1993), although it has been suggested that both 2- and 3-bromophenol
18 may also be formed by rearrangement of the 2,3-dihydrodiol (Lertratanangkoon et al., 1987,
19 1993; see also Figure 3-1). Other pathways to the formation of 3-bromophenol may include
20 rearrangement of the 3,4-dihydrodiol or the 4-S-glutathione conjugate of the 3,4-oxide derivative
21 (Lertratanangkoon et al., 1987, 1993). Spontaneous rearrangement of the 3,4-dihydrodiol is
22 thought to be the major pathway leading to the formation of 4-bromophenol in the rat, whereas
23 the pathway leading through the 3-S-glutathione conjugate of the 3,4-oxide derivative is thought
24 to predominate in the guinea pig (Lertratanangkoon et al., 1987, 1993).

25 The bromophenol metabolites may be subsequently oxidized by CYP to their respective
26 bromocatechols (2-, 3-, or 4-bromocatechol, Figure 3-1), likely involving bromophenol oxide
27 intermediates. The 4-bromocatechol may also be formed via dihydrodiol dehydrogenase
28 (DDDH)-catalyzed conversion of the 3,4-dihydrodiol, the pathway that appears to predominate
29 in the rat *in vivo* (Miller et al., 1990). The 4-bromophenol may undergo oxidative debromination
30 to form 1,4-benzoquinone (Slaughter and Hanzlik, 1991; Zheng and Hanzlik, 1992). Redox
31 cycling of 2- and 4-bromocatechol and conjugation by glutathione S-transferase (GT) produce
32 2-bromo-3-(glutathion-S-yL)hydroquinone and 6-glutathion-S-yL-4-bromocatechol, respectively
33 (Lau and Monks, 1988).

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

1 detected (using GC-MS) as mercapturic acids, derived from the 3,4-oxide pathway, in the
2 24-hour urine of rats given bromobenzene parenterally at doses ranging from 7.9 to 158
3 mg/kg-day (Chakrabarti and Brodeur, 1984; Zampaglione et al., 1973). Following oral
4 administration of bromobenzene (10 mg/rat, 1 mg/mouse, 10 mg/rabbit), approximately 50-60%
5 of the 96-hour urinary recovery of bromobenzene metabolites was in the form of
6 4-bromophenylmercapturic acid (Ogino, 1984a). Other metabolites that have been measured in
7 the urine of rats include the phenolic compounds, dihydrodiols, catechols, and hydroquinones
8 (Miller et al., 1990; Lertratanangkoon and Horning, 1987; Chakrabarti and Brodeur, 1984; Lau et
9 al., 1984a; Monks et al., 1984a,b; Jollow et al., 1974; Zampaglione et al., 1973).

10 Animal studies have elucidated species-specific differences in urinary excretion of the
11 bromophenols (2-, 3-, and 4-bromophenol) following exposure to bromobenzene. For example,
12 in the 96-hour urine of mice that had been administered a nontoxic oral dose of bromobenzene (1
13 mg/mouse; approximately 33 mg/kg-day), 2-bromophenol accounted for 12.1% of the dose,
14 3-bromophenol accounted for 8.8%, and 4-bromophenol accounted for 3.1% (Ogino, 1984a). In
15 similarly-treated rats (10 mg/rat; approximately 56 mg/kg-day), however, 2-bromophenol
16 accounted for only 2.6% of the dose, while 3-bromophenol accounted for 19.2% and
17 4-bromophenol accounted for 13.1%. In the urine of the mice, 2-bromophenol was 4 times more
18 prevalent than 4-bromophenol, whereas 4-bromophenol was 5 times more prevalent than
19 2-bromophenol in the urine of the rats. This metabolic difference between rats and mice has
20 been associated with a difference in susceptibility to bromobenzene acute nephrotoxicity (Reid,
21 1973; see also Section 4.5.3).

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

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

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

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

3 Recent studies have noted that intraperitoneal injection of bromobenzene into rats can
4 induce many different types of enzymes. In a toxicogenomics approach, Heijne et al. (2005,
5 2004, 2003) noted induction of more than 20 liver proteins (including γ -glutamylcysteine
6 synthetase, a key enzyme in glutathione biosynthesis) and transient changes in the transcriptional
7 expression of numerous genes involved in drug metabolism, oxidative stress, glutathione
8 depletion, the acute phase response, metabolism, and intracellular signaling following
9 intraperitoneal administration of bromobenzene to rats. Other studies (Minami et al., 2005;
10 Stierum et al., 2005; Waters et al., 2006) have utilized toxicogenomics to characterize the
11 relationship between bromobenzene hepatotoxicity and hepatic gene expression profiles.

12 13 **3.4. ELIMINATION**

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

19 In rats, mice, and rabbits given bromobenzene in single oral doses of approximately 3-30
20 mg/kg-day, detection of metabolites in urine collected for 4 days accounted for 60-70% of the
21 administered dose, most of which had been recovered within 8 hours following administration
22 (Ogino, 1984a). Small amounts of parent compound were detected in the urine and feces of all
23 three species. Approximately 85% of an intraperitoneally injected dose (250 mg/kg-day) of
24 ^{14}C -bromobenzene was excreted within 24 hours as metabolites in the urine of rats (Reid et al.,
25 1971). In other rat studies, metabolites detected in the urine collected for 48 hours accounted for
26 more than 90% of administered doses of 8 mg/kg-day (intravenous) or 1570 mg/kg-day
27 (intraperitoneal) (Zampaglione et al., 1973).

28 Biliary excretion of bromobenzene-glutathione conjugate has been demonstrated in rats;
29 the rate of biliary excretion can be used as an index of *in vivo* activation of bromobenzene
30 (Madhu and Klaassen, 1992). Additional information regarding biliary excretion of
31 bromobenzene metabolites was demonstrated in bile-cannulated rats that were administered a
32 non-hepatotoxic dose (20 mg/kg-day) of ^{14}C -bromobenzene in the femoral vein (Sipes et al.,
33 1974). Cumulative excretion of radioactivity in the bile was 56% of administered radioactivity
34 during 3 hours after dosing. Combined with demonstrations that, in normal non-cannulated rats,
35 elimination of bromobenzene predominantly occurs via urinary excretion of metabolites (Ogino,
36 1984a; Zampaglione et al., 1973; Reid et al., 1971) and not via fecal excretion (Ogino, 1984a), it

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

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

14 15 **3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS (PBTK)**

16 No PBTK models have been developed for bromobenzene.
17

4. HAZARD IDENTIFICATION

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

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

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

4.2.1. Oral Exposure

4.2.1.1. Subchronic Toxicity

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

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

1 blood, gross lesions, and tissue masses) were performed on all control rats and all rats from the
2 400- and 600-mg/kg-day dose groups.

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

18 In the basic study, treatment-related clinical signs were observed only at the 600 mg/kg-
19 day dose level and included ruffled fur (9/10 rats of each sex), emaciation (9/10 rats of each sex),
20 tremors (2/10 males and 1/10 females), ataxia (1/10 of each sex), hypoactivity (5/10 males and
21 7/10 females), and ocular discharge (2/10 of each sex). Observations of similar clinical signs
22 were made in rats of the supplementary study, but distinguishing between treatment-related
23 clinical signs and symptoms that may have resulted from repeated anesthesia, blood sample
24 collection, and prolonged housing in metabolism cages was difficult.

25 Treatment-related mortality was observed in male and female rats at 600 mg/kg-day (9/10
26 males and 8/10 females in the basic study and 3/5 males and 1/5 females in the supplementary
27 study). By the end of week 7, mortality rates in high-dose male and female rats were 7/10 and
28 3/10, respectively. Occasional deaths at lower doses were attributed to gavage error.
29 Statistically significantly reduced mean body weight (approximately 7-11% lower than controls)
30 was observed in 400-mg/kg-day male rats from week 5 until study end. At 600 mg/kg-day, both
31 male and female rats were visibly emaciated. Table 4-1 presents terminal body and liver weights
32 and serum levels of selected liver enzymes in male and female rats of the basic study. Dose-
33 related statistically significantly increased mean liver and kidney weights (absolute, relative-to-
34 body weight) were observed at doses ≥ 100 mg/kg-day in male rats and at all dose levels
35 (including 50 mg/kg-day) in female rats. Changes in the 600 mg/kg-day males were similar in
36 magnitude to changes in the 400 mg/kg-day males, but could not be assessed for statistical

1

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

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

2 ^aHigh 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
3 weight data or serum enzyme changes

4 ^bStatistically significantly increased from controls ($p < 0.05$) based on student's two-tailed t-test

5 ^cOutside 3 standard deviations from the control mean

6 ^dChange relative to controls

7 Source: NTP (1985a)

8

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

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

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

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

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

^aIncidence = number of animals in which lesion was found/number of animals in which organ was examined.

^bMost male and female rats of the 600 mg/kg-day dose level died during the study, which may have affected incidences of selected lesions.

^cIncidences of rats with one or more of the liver lesion types (cytomegaly, necrosis, inflammation, mineralization), extracted from individual animal histopathologic results provided to Syracuse Research Corporation by NTP.

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

Severity: Average severity score: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.

Source: NTP (1985a)

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

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

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

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

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

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

15 In the mouse study (NTP, 1985b), groups of 10 male and 10 female B6C3F1 mice were
16 administered 0, 50, 100, 200, 400, or 600 mg/kg-day of bromobenzene by gavage in corn oil 5
17 days/week for 90 days; supplementary groups of 10 mice/sex were similarly treated with 0, 50,
18 200, or 600 mg/kg-day and housed in pairs in metabolism cages throughout the study. Blood
19 samples were collected on days 2, 4, 24, and 95 for hematology and clinical chemistry. Urine
20 and clinical chemistry samples were collected from these mice on days 1, 3, 17, and 94. Other
21 details of study design were the same as those described for the rats (NTP, 1985a), with the
22 exception of histopathologic examination of kidney tissues, which was not performed in 50 or
23 100 mg/kg-day mice.

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

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

1 mg/kg-day (but not 600 mg/kg-day) males. The 600 mg/kg-day males in the supplemental study
2 exhibited approximately 12% lower terminal body weight, relative to controls. Consistent
3 treatment-related effects on body weight were not seen in female mice. Table 4-3 presents
4 terminal body and liver weights and serum levels of selected liver enzymes in male and female
5 mice of the basic study. In male mice, absolute liver weight was significantly increased at dose
6 levels ≥ 200 mg/kg-day, while the liver:body weight ratio was significantly increased at dose
7 levels ≥ 100 mg/kg-day and the liver:brain weight ratio was significantly increased at dose levels
8 ≥ 400 mg/kg-day. In female mice, all three measures of liver weight were significantly increased
9 at all dose levels, relative to controls. The effect on absolute liver weight increased with dose,
10 ranging from approximately 12% in the 50 mg/kg-day group to greater than 50% in the 600
11 mg/kg-day group. Statistically significantly increased serum SDH activity (indicative of
12 hepatotoxicity) was observed in both sexes at dose levels ≥ 200 mg/kg-day, relative to sex-
13 matched controls, but the magnitude only approached a 2-fold increase (a biologically significant
14 level) at ≥ 200 mg/kg-day in males and ≥ 400 mg/kg-day in females. Activities of AST or ALT
15 were not elevated in any exposed mouse group, compared with control values. Results of
16 urinalysis and serum chemistry did not indicate clear evidence of bromobenzene-induced effects
17 on the renal system. Hematological results were generally unremarkable.

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

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

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

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

^aStatistically significantly increased from controls ($p < 0.05$) based on Student's two-tailed t-test

^bChange relative to controls

Source: NTP (1985b)

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

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

^aIncidence = number of animals in which lesion was found/number of animals in which organ was examined.

^bCytomegaly and mineralization were not diagnosed in 5 high-dose male mice that died on treatment day 1

^cIncidences of mice with one or more of the liver lesion types (cytomegaly, necrosis, inflammation, mineralization), extracted from individual animal histopathologic results provided to Syracuse Research Corporation by NTP.

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

Severity: Average severity score: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.

NE = Not examined.

Source: NTP (1985b)

14

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

4 The most prominent toxicological effects observed in B6C3F1 mice treated with
5 bromobenzene by oral gavage for 90 days (NTP, 1985b) were observed in the liver.
6 Significantly increased incidences of hepatocellular necrosis (a clear indicator of an adverse
7 effect) were observed at doses of 400 and 600 mg/kg-day in male mice and the 600 mg/kg-day
8 dose level in female mice. Significantly increased incidences of cytomegaly were noted at doses
9 ≥ 200 mg/kg-day in male and female mice. Significant increases in mean liver weight were
10 observed at doses as low as 50 mg/kg-day in female mice and 100 mg/kg-day in male mice.
11 Treatment-related increased occurrence of cytomegaly (i.e., hypertrophy) and increased liver
12 weight may provide indication of liver toxicity from higher levels of exposure, but the
13 toxicological significance of these effects by themselves is questionable.

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

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

31 **4.2.1.2. Chronic Toxicity**

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

1 **4.2.2. Inhalation Exposure**

2 **4.2.2.1. Subchronic Toxicity**

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

8 Groups of 10 male and 10 female Fischer 344/N rats were exposed to bromobenzene
9 vapors through whole body exposure at 0, 10, 30, 100, or 300 ppm (0, 64.2, 192.6, 642, or 1926
10 mg/m³) for 6 hours/day, 5 days/week for 13 weeks. Rats were observed twice daily for
11 morbidity and mortality. Clinical observations and body weight measurements were performed
12 weekly. Blood samples for hematologic examination (erythrocyte and leukocyte counts;
13 hemoglobin concentrations; red blood cell indices of MCV, MCH, and MCHC; leukocyte
14 differential counts) were collected from all surviving rats at terminal sacrifice. Terminal body
15 and organ (liver, brain, testis, kidney, lung, heart, and thymus) weights were recorded; organ-to-
16 body weight and organ-to-brain weight ratios were calculated for each sex. Complete gross
17 necropsy was performed on all rats. Complete histopathologic examinations of all major tissues
18 and organs (including liver, kidney, urinary bladder, spleen, pancreas, brain, spinal cord [if
19 neurologic signs were present], heart, lung, trachea, nasal cavity, larynx, esophagus, stomach,
20 small intestine, cecum, colon, skin, uterus, ovaries, preputial or clitoral glands, testes, prostate,
21 sternbrae, adrenals, pituitary, thyroid, parathyroids, salivary gland, mandibular lymph node,
22 thymus, mammary gland, blood, and gross lesions, and tissue masses) were performed on all
23 control rats and all rats from the 300-ppm groups. Kidney tissue was examined
24 histopathologically in all male rats of the lower exposure concentrations (10, 30, and 100 ppm).

25 No mortality was observed during the study. Clinical signs were unremarkable except for
26 tearing, facial grooming, and listlessness in 300-ppm rats on the first day of exposure. Terminal
27 body weights did not differ significantly from controls. Liver and kidney weights (absolute,
28 relative-to-body weight, and relative-to-brain weight) were significantly increased at
29 concentrations ≥ 100 ppm in both sexes. Liver and kidney weight data are reported in Table 4-5.
30 In males, absolute liver weights increased 13% at 100 ppm and 20% at 300 ppm. In females,
31 absolute liver weights increased 12% at 100 ppm and 22% at 300 ppm. MCH and MCV were
32 statistically significantly decreased in males at concentrations ≥ 10 ppm and in females at 300
33 ppm, but the changes were small and considered not to be biologically significant. There was no
34 histopathological evidence of bromobenzene-induced liver lesions, although livers were
35 examined only from control rats and rats of the highest exposure level (100 ppm in males and
36 300 ppm in females) (see Table 4-6).

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

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

2 ^aChange relative to controls

3 ^bStatistically significantly different from controls ($p < 0.05$) based on student's two-tailed t-test

4 ^cOutside 3 standard deviations from the control mean

5 Source: NTP (1985d)

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

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

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

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

5 Source: NTP (1985c)

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7

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

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

13 Since increased liver weight at the 100 ppm and 300 ppm dose groups in the NTP
14 (1985c) study were not accompanied by bromobenzene induced liver lesions these effects were
15 considered to be of questionable toxicological significance and not considered to be a LOAEL;
16 therefore, the highest dose level tested (300 ppm) is considered to be a NOAEL in this study.

17 In the mouse study, groups of 10 male and 10 female B6C3F1 mice were exposed to 0,
18 10, 30, 100, or 300 ppm (females only) and (0, 64.2, 192.6, 642, or 1926 mg/m³) of
19 bromobenzene 6 hours/day, 5 days/week for 13 weeks (NTP, 1985d). No rationale for excluding
20 a 300-ppm exposure level for the male mice was included in the available study report. Clinical
21 observations and body weight measurements were performed weekly. Blood samples for
22 hematologic examination (erythrocyte and leukocyte counts; hemoglobin concentrations; red
23 blood cell indices of MCV, MCH, and MCHC; leukocyte differential counts) were collected
24 from all surviving mice at terminal sacrifice. Terminal body and organ (liver, brain, testis,
25 kidney, lung, heart, and thymus) weights were recorded; organ-to-body weight and organ-to-
26 brain weight ratios were calculated for each sex. Complete gross necropsy was performed on all
27 mice. Histopathologic examinations of all major tissues and organs (including liver, kidney,
28 urinary bladder, spleen, pancreas, gall bladder, brain, spinal cord [if neurologic signs were
29 present], heart, lung, trachea, nasal cavity, larynx, esophagus, stomach, small intestine, cecum,
30 colon, skin, uterus, ovaries, preputial or clitoral glands, testes, prostate, sternbrae, adrenals,
31 pituitary, thyroid, parathyroids, salivary gland, mandibular lymph node, thymus, mammary
32 gland, blood, gross lesions, and tissue masses) were performed on all control, 100-ppm male and
33 300-ppm female mice. Liver and kidney tissues were examined histopathologically in all other
34 groups of mice.

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

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

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

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

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

1

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

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

^aChange relative to controls

^bStatistically significantly different from controls ($p < 0.05$) based on Student's two-tailed t-test

^cOutside 3 standard deviations from the control mean

Source: NTP (1985d)

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

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

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

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

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

^dKidney tubular degeneration could not be distinguished from artifacts of fixation or staining.

^eMineralization was not reported in male mice.

^fNo histopathologic renal lesions were identified in any group of female mice.

Source: NTP (1985d)

15

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

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

13 **4.2.2.2. Chronic Toxicity**

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

17 **4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION**

18 **4.3.1. Reproductive Toxicity Studies**

19 No reproductive toxicity studies were located for bromobenzene.

21 **4.3.2. Developmental Toxicity Studies**

22 No developmental toxicity studies were located for bromobenzene.

24 **4.4. OTHER STUDIES**

25 **4.4.1. Acute Toxicity Studies**

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

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

1 glutathione was reduced at a dose of 157 mg/kg-day, but no other renal effects were noted at
2 doses up to 628 mg/kg-day. Other acute oral studies reported hepatic necrosis in rats (Heijne et
3 al., 2004) or mice (Patrick and Kennedy, 1964) administered bromobenzene at doses in the range
4 of 500-700 mg/kg; reduced renal glutathione levels, increased BUN levels, and severe tubular
5 necrosis in mice given 2355 mg/kg-day (Casini et al., 1986); extensive vacuolization and
6 necrosis in Clara cells in the lungs of mice given 785 mg/kg-day (Forkert, 1985); and increased
7 LDH levels in lung lavage fluid accompanied by bronchiolar damage in the lungs of mice given
8 2355 mg/kg-day (Casini et al., 1986).

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

14 15 **4.4.2. Genotoxicity Studies**

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

26 Bromobenzene was observed to increase formation of micronucleated erythrocytes, in
27 femoral polychromatic mouse bone marrow cells *in vivo* (Mohtashamipur et al., 1987) and
28 actively bind to rat and mouse DNA, RNA, and proteins both *in vivo* and *in vitro* (Prodi et al.,
29 1986; Colacci et al., 1985). Following intraperitoneal injection of ¹⁴C-bromobenzene (6.35
30 µmol/kg; lower than a minimally hepatotoxic dose) in rats and mice, the degree of binding in
31 liver, kidney, and lung tissues of both species was RNA > proteins > DNA (Colacci et al., 1985).
32 Mouse kidney exhibited a much greater degree of binding to macromolecules than rat kidney. In
33 both rats and mice, the relative order of binding to DNA in the various organs was liver > kidney
34 > lung. Bromobenzene was second only to 1,2-dibromoethane in its relative *in vivo* reactivity
35 with rat liver DNA, exhibiting higher reactivity than 1,2-dichloroethane, chlorobenzene,
36 epichlorohydrin, and benzene (Prodi et al., 1986). Microsomal enzyme-catalyzed the *in vitro*

Table 4-9. Results of bromobenzene genotoxicity testing

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

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

4

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

6 Reactive metabolites of bromobenzene are produced *in vivo* as discussed in Section 3.3
7 and could be expected to interact with DNA. The central pathway for the mammalian
8 metabolism of bromobenzene appears to be the production of bromocatechols via bromophenols,
9 as depicted in Figure 3-1 (Lertratanangkoon et al., 1993; Lau and Monks, 1988). Although
10 reactive metabolites, 2,3-oxide and 3,4-oxide, are formed as precursors in the predominant
11 pathway in bromobenzene's metabolism, 2,3-oxide has a very short biological half-life,
12 indicating spontaneous rearrangement to the formation of 2-bromophenol in the rat and pig
13 (Lertratanangkoon et al., 1993). Another reactive intermediate, 2,3-dihydrodiol, also rapidly
14 rearranges to form both 2-bromophenol and 3-bromophenol in the detoxification bromocatechol
15 pathway (Lertratanangkoon et al., 1987). Furthermore, spontaneous rearrangement of the
16 3,4-dihydrodiol is considered to be the major pathway in bromobenzene's metabolism, leading to
17 the formation of 4-bromophenol in the rat, while a pathway leading through an S-glutathione
18 conjugate to 4-bromophenol is predominant in the guinea pig (Lertratanangkoon et al., 1987,
19 1993). The bromophenols are subsequently oxidized by CYP to their respective bromocatechols
20 in a detoxification pathway (Miller et al., 1990; Lau and Monks, 1988). While these
21 toxicokinetic events are expected to elicit a toxicity response from liver tissue, the reactive
22 metabolites generated in the process may be too transient and reactive to elicit measurable
23 responses in Salmonella mutagenicity assays and other genotoxicity assays involving external rat
24 liver S-9 metabolic activation.

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

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

4 4.4.3. Tumor Promotion Studies

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

22 4.5. MECHANISTIC STUDIES

23 4.5.1. Mechanistic Studies of Liver Effects

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

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

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

13
Table 4-10. The effect of CYP inhibition on the hepatotoxicity and metabolism of single intraperitoneal doses of bromobenzene

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

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

17
18
19 Chemically reactive metabolites of bromobenzene may damage cellular macromolecules,
20 leading to cytotoxicity. These metabolites include the 2,3- and 3,4-oxides of bromobenzene, the
21 oxides of the bromophenols, the 1,4-benzoquinone, and the radicals and quinones derived from
22 redox cycling of the 2- and 4-bromocatechols (Slaughter and Hanzlik, 1991; Lau and Monks,
23 1988). The 3,4-epoxide binds covalently to microsomal protein at the site of synthesis while the
24 2,3- epoxide binds to the soluble protein, i.e., hemoglobin β chain (Lau and Zannoni, 1981b).
25 The bromobenzene 3,4-oxide alkylates histidine and lysine side chains in rat liver proteins *in*
26 *vivo* (Bambal and Hanzlik, 1995). Phenolic metabolites of bromobenzene are activated to toxic
27 metabolites, which deplete cellular glutathione and have caused cell death in isolated hepatocytes
28 (Lau and Monks, 1997a). Hydroquinone metabolites of bromobenzene have been indicated as
29 subcellular targets of nephrotoxicity in the rat, causing changes in proximal tubular brush border,

1 nuclei, and endoplasmic reticulum (Lau and Monks, 1997b). Slaughter et al. (1993)
2 demonstrated that bromobenzene-derived oxides, quinones, and bromoquinones are capable of
3 alkylating protein sulfhydryl groups, the major adduct arising from the 1,4-benzoquinone
4 electrophilic metabolite. Quinone-derived protein adducts appear to be formed to a greater
5 extent than those derived from the epoxides (Bambal and Hanzlik, 1995; Slaughter and Hanzlik,
6 1991). Several liver proteins have been identified as targets for reactive metabolites of
7 bromobenzene (Koen and Hanzlik, 2002; Koen et al., 2000; Rombach and Hanzlik, 1997, 1998,
8 1999; Aniya et al., 1988). While electrophilic metabolites of bromobenzene have the ability to
9 interact with tissue macromolecules, a causal role for this binding in hepatotoxicity has yet to be
10 demonstrated (Koen and Hanzlik, 2002; Lau and Monks, 1997a).

11 Results of mechanistic studies further indicate that hepatotoxicity is primarily elicited via
12 the metabolic pathway that involves the 3,4-oxide derivative of bromobenzene, and that the toxic
13 effect is likely mediated via covalent binding of one or more reactive metabolites with
14 hepatocellular macromolecules (Monks et al., 1984a; Jollow et al., 1974; Reid and Krishna,
15 1973; Zampaglione et al., 1973; Brodie et al., 1971). Supporting evidence includes the findings
16 that: (1) induction of β -naphthoflavone- or 3-methylcholanthrene-induced CYP isozymes
17 (possibly cytochrome P-488) results in increased urinary excretion of 2-bromophenol (formed
18 via the 2,3-oxide pathway) and decreased hepatotoxicity (Lau et al., 1980; Lau and Zannoni,
19 1979; Jollow et al., 1974; Zampaglione et al., 1973), whereas (2) induction of phenobarbital-
20 induced CYP isozymes results in increased urinary excretion of 4-bromophenol (formed via the
21 3,4-oxide pathway), as well as increases in both severity of hepatocellular necrosis and the extent
22 of covalent binding of radioactivity from ^{14}C -bromobenzene to hepatocellular macromolecules in
23 the region of observed hepatocellular necrosis (Brodie et al., 1971).

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

1 from the liver. CYP-inhibition (by SKF 525A) significantly retarded bromobenzene metabolism
2 and reduced the rate of glutathione depletion; these rats exhibited no histopathologic signs of
3

1

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

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

2 ^aCriteria of Brodie et al. (1971) (minimal = a few degenerating parenchymal cells; extensive =
3 central veins surrounded by several rows of dead or degenerating cells; massive = necrosis of
4 extensive liver areas).

5 ^bHalf-time of clearance of radioactivity from the whole body of rats administered

6 ¹⁴C-bromobenzene.

7 ^cSignificantly different from the values of rats treated with bromobenzene only; $p < 0.05$.

8 Source: Jollow et al. (1974)

9

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

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

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

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

3 As noted in the proposed metabolic scheme for bromobenzene (Section 3.3, Figure 3-1),
4 candidates for reactive metabolites of the 3,4-oxide pathway that may elicit hepatotoxicity
5 include the 3,4-oxide itself, the oxide derivative of 4-bromophenol, the quinone
6 (4-bromoquinone) formed from 4-bromocatechol, and reactive oxygen species formed via redox
7 cycling of 4-bromoquinone. The relative importance of these metabolites to bromobenzene
8 hepatotoxicity is uncertain. There is some evidence that 4-bromophenol and its further
9 metabolites may not be involved in hepatotoxicity since centrilobular hepatic necrosis was
10 observed in rats that were administered bromobenzene (400 mg/kg-day) intraperitoneally, but not
11 in other rats administered 4-bromophenol (up to 440 mg/kg-day) or 4-bromocatechol (up to 485
12 mg/kg-day) (Monks et al., 1984a).

14 **4.5.2. Mechanistic Studies of Kidney Effects**

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

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

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

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

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

16

17 **4.5.3. Genomic/Proteomic Responses of the Liver to Bromobenzene**

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

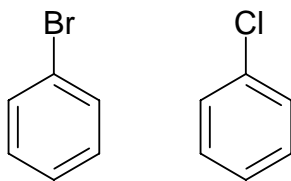
24 Heijne and coworkers (Stierum et al., 2005; Heijne et al., 2004, 2003) used these
25 techniques to identify changes in gene expression in the rodent liver in response to
26 bromobenzene. As previously discussed, bromobenzene undergoes CYP-mediated epoxidation
27 to form the electrophilic 3,4-epoxide,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

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

4 Heijne et al. (2004) demonstrated dose- and time-related changes in bromobenzene-
5 induced liver gene expression profiles by administering bromobenzene to groups of rats by oral
6 gavage at doses of 0.5, 2.0, or 4.0 mM/kg and assessing changes in the liver transcriptome at 6,
7 24, and 48 hours posttreatment. Dose- and time-related changes were observed in the
8 transcriptional expression of numerous genes involved in GSH depletion, drug metabolism,
9 intracellular signaling, metabolism (cholesterol, fatty acid, and protein metabolism), and the
10 acute phase response. At the highest dose, the time-course of altered gene expression coincided
11 with that of histopathological evidence of bromobenzene-induced liver lesions, with few signs of
12 adverse effects at 6 hours and increased evidence of histopathologic liver lesions and altered
13 transcriptional expression at 24 and 48 hours. Although histopathologic liver lesions were not
14 observed at the two lower doses, dose-related altered transcriptional expression was noted and
15 recovery was apparent in the mid-dose group at 48 hours posttreatment. Results of available
16 toxicogenomics assessments provide suggestive evidence for the involvement of some genes in
17 particular aspects of bromobenzene hepatotoxicity. However, the toxicogenomics studies
18 available do not establish key events in the mode of action for bromobenzene-induced
19 hepatotoxicity.

21 4.5.4. Similarities Between Bromobenzene and Chlorobenzene

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



29
30 **Figure 4-1. Chemical structure of bromobenzene and chlorobenzene**

31
32 Independent *in vivo* and *in vitro* studies indicate that bromobenzene and chlorobenzene
33 have similar toxicokinetic properties and share the same critical target of toxicity (liver).

1 Bromobenzene and chlorobenzene each exhibit the ability to enter the systemic circulation of
2 laboratory animals following inhalation or oral exposure (see Section 3.1 for a detailed
3 discussion of the toxicokinetics of bromobenzene and Hellman (1993) for a summary of
4 toxicokinetic information for chlorobenzene). Results of parenteral injection studies in animals
5 indicate that, following absorption, bromobenzene and its metabolites are widely distributed,
6 with highest levels found in adipose tissue (Ogino, 1984b; Zampaglione et al., 1973; Reid et al.,
7 1971). Similar distribution of chlorobenzene has been observed in rats following inhalation
8 exposure to radiolabeled chlorobenzene (Sullivan et al., 1983). Metabolic schemes for both
9 bromobenzene and chlorobenzene include initial CYP-catalyzed epoxidation to reactive epoxide
10 intermediates and subsequent formation of corresponding dihydrodiol derivatives, phenols,
11 glutathione conjugates, catechols, and quinones. Elimination is mainly accomplished via the
12 urinary excretion of bromobenzene- and chlorobenzene-derived metabolites.

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

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

27
28 The database for bromobenzene does not include reproductive or developmental toxicity
29 studies. However, chlorobenzene was assessed for reproductive toxicity in a two-generation
30 study of rats exposed to chlorobenzene vapor concentrations of 0, 50, 150, or 450 ppm daily, 6
31 hours/day for 10 or 11 weeks prior to mating and throughout mating, gestation, and lactation
32 (Nair et al., 1987). Statistically significantly increased incidences of rats with histopathologic
33 liver and kidney lesions were observed in F₀ and F₁ male rats at exposure levels ≥ 150 ppm. The
34 NOAEL for hepatic effects in this study was 50 ppm. The highest exposure level (450 ppm) did
35 not elicit any clear signs of reproductive toxicity in either generation. Furthermore,

1 chlorobenzene did not induce developmental effects in the fetuses of pregnant rats exposed to
2 vapor concentrations as high as 590 ppm for 6 hours/day on gestation days 6-15 (John et al.,
3 1984).

4 The oral database for chlorobenzene includes one developmental study in which Charles
5 River albino rat dams were administered chlorobenzene at oral dose levels of 100 or 300 mg/kg-
6 day on gestation days 6-15 (IBT, 1977). Although no developmental toxicity was elicited, it is
7

1

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

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

2 ^aSignificantly decreased survival in the 750 mg/kg-day dose groups may have influenced
3 observed incidences of animals with liver and/or kidney lesions.

4 ^bStatistically significantly different from control groups according to Fisher's exact test ($p < 0.05$),
5 performed by Syracuse Research Corporation.

6 Source: NTP (1985e)

7

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

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

8 ^aSignificantly decreased survival in the 500 and 750 mg/kg-day dose groups may have
9 influenced observed incidences of animals with liver and/or kidney lesions.

10 ^bStatistically significantly different from control groups according to Fisher's exact test ($p < 0.05$),
11 performed by Syracuse Research Corporation.

12 NE = not examined, due to the absence of lesions at the next higher dose

13 Source: NTP (1985e)

14

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

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

14 Although no chronic-duration oral or inhalation animal studies are available for
15 bromobenzene, a 2-year toxicity and carcinogenicity study is available for chlorobenzene (NTP,
16 1985e). Groups of male and female F344/N rats and B6C3F1 mice (50/sex/species) were
17 administered chlorobenzene by oral gavage at doses of 0, 60, or 120 mg/kg-day (0, 30, or 60
18 mg/kg-day for male mice), 5 days/week for 2 years. There was no evidence of treatment-related
19 increased incidences of nonneoplastic liver lesions in female rats or male or female mice,
20 including the highest dose level tested (120 mg/kg-day for female rats and mice, 60 mg/kg-day
21 for male mice). There was equivocal evidence for treatment-related increased incidence of
22 hepatocellular necrosis in high-dose (120 mg/kg-day) male rats. The original pathology report
23 noted necrosis in 7/50 high-dose males (0/50 in vehicle controls). However, an independent
24 pathological review resulted in a diagnosis of hepatocellular necrosis in one vehicle control male
25 rat (1/50) and a single high-dose male rat (1/50). The NTP 2-year oral study of chlorobenzene
26 identified a free-standing no-observed-adverse-effect level (NOAEL) of 120 mg/kg-day in
27 female rats and equivocal evidence of a lowest-observed-adverse-effect level (LOAEL) of 120
28 mg/kg-day for hepatocellular necrosis in male rats. In male and female mice, free-standing
29 NOAELs were 60 and 120 mg/kg-day, respectively, for nonneoplastic liver effects. In a
30 similarly-designed subchronic (90-day) oral toxicity study in mice, a NOAEL of 125 mg/kg-day
31 was identified in both males and females; the LOAEL was 250 for chlorobenzene-induced liver
32 lesions (NTP, 1985e). These results suggest the development of some degree of tolerance to
33 chlorobenzene during chronic exposure (i.e., dose-response relationships for subchronic and
34 chronic exposure appear to be similar). It is reasonable to expect such similarities in dose-
35 response relationships for subchronic and chronic exposure to bromobenzene as well because

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

4 **4.6. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS**

5 **4.6.1. Oral**

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

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

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

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

35 **4.6.2. Inhalation**

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

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

21 The liver was shown to be a target of bromobenzene toxicity in mice following a single
22 4-hour exposure to bromobenzene vapor concentrations as low as 250 ppm (Becher et al., 1989;
23 Brondeau et al., 1983). Necrosis was also noted in the lungs of mice following a single 4-hour
24 exposure to bromobenzene at a vapor concentration of 1000 ppm (Becher et al., 1989).
25 However, lung lesions were not seen in rats or mice repeatedly exposed to bromobenzene vapors
26 at concentrations up to 300 ppm (NTP, 1985c,d).

29 **4.6.3. Mode of Action Information**

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

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

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

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

9 Supporting evidence includes the findings that:

- 10 • Induction of β -naphthoflavone- or 3-methylcholanthrene-induced CYP isozymes
11 results in increased urinary excretion of 2-bromophenol (formed via the 2,3-oxide
12 pathway) and decreased hepatotoxicity (Jollow et al., 1974; Lau and Zannoni, 1979;
13 Lau et al., 1980; Zampaglione et al., 1973), whereas
- 14 • Induction of phenobarbital-induced CYP isozymes results in increased urinary
15 excretion of 4-bromophenol (formed via the 3,4-oxide pathway) as well as increases
16 in severity of hepatocellular necrosis and increases in the extent of covalent binding
17 of radioactivity from ^{14}C -bromobenzene to hepatocellular macromolecules in the
18 region of observed hepatocellular necrosis (Brodie et al., 1971).

19 Candidates for reactive metabolites of the 3,4-oxide pathway that may elicit
20 hepatotoxicity include the 3,4-oxide itself, the oxide derivative of 4-bromophenol, the quinone
21 (4-bromoquinone) formed from 4-bromocatechol, and reactive oxygen species formed via redox
22 cycling of 4-bromoquinone. The relative importance of these metabolites to bromobenzene
23 hepatotoxicity is uncertain. There is some evidence that 4-bromophenol and its further
24 metabolites may not be involved in hepatotoxicity since centrilobular hepatic necrosis was
25 observed in rats that were administered bromobenzene (400 mg/kg) intraperitoneally but not in
26 other rats administered 4-bromophenol (up to 440 mg/kg) or 4-bromocatechol (up to 485 mg/kg)
27 (Monks et al., 1984a).

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

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

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

10 **4.7. EVALUATION OF CARCINOGENICITY**

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

21 **4.8. SUSCEPTIBLE POPULATIONS**

22 **4.8.1. Possible Childhood Susceptibility**

23 Limited data were located regarding age-related susceptibility to bromobenzene. Single
24 intraperitoneal injection of bromobenzene at concentrations that produced extensive centrilobular
25 necrosis in the liver of adult rats failed to produce similar lesions in neonatal rats (Green et al.,
26 1984; Mitchell et al., 1971). The lack of hepatotoxicity in the neonatal rats was presumably the
27 result of a generally low level of hepatic microsomal enzymes observed in early neonatal stages
28 of development (Kato et al., 1964).

30 **4.8.2. Possible Gender Differences**

1 Available information regarding gender-related susceptibility to bromobenzene is
2 restricted to animal studies. In rats (NTP, 1985a), results of subchronic-duration oral exposure to
3 bromobenzene indicate that males are somewhat more susceptible than females to hepatocellular
4 effects such as centrilobular necrosis and cytomegaly (see Table 4-2). Male-female differences
5 were not as apparent following subchronic-duration oral exposure in mice (see Table 4-4) (NTP,
6 1985b).

8 **4.8.3. Other**

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

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE

5.1.1. Subchronic Oral RfD

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

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

The liver appears to be the principal target organ for bromobenzene toxicity in rodents. Significantly increased incidences of hepatocellular necrosis (a clear indicator of an adverse effect) were observed at doses of 400 and 600 mg/kg-day in male and female B6C3F1 mice and male Fischer 344/N rats (600 mg/kg-day in female Fischer 344/N rats) (NTP, 1985a,b). These dose levels also resulted in greater than 3-fold increases (statistically and biologically significant) in serum concentrations of SDH, an enzyme indicative of liver damage. Significantly increased incidences of cytomegaly were observed at doses ≥ 200 mg/kg-day in male and female mice and male rats (≥ 400 mg/kg-day in female rats). Significantly increased mean liver weights were observed at doses as low as 50 mg/kg-day in female rats and mice and 100 mg/kg-day in male rats and mice.

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

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

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

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

11 12 **5.1.1.2. Methods of Analysis - Including Models (PBPK, BMD, etc.)**

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

20 The modeled liver lesion data are shown in Table 5-1. Results of the best fitting models
21 (lowest Akaike Information Criterion [AIC]) for incidences of male and female rats and mice
22 with liver lesions are presented in Table 5-2. The female mouse liver lesion data produced the
23 lowest BMDL₁₀ (24.8 mg/kg-day), indicating that female mice have a lower point of departure
24 for bromobenzene hepatotoxicity (BMDs and BMDLs for 10, 5, and 1% extra risk are shown in
25 Table 5-3). The conventional BMR of 10% extra risk (U.S. EPA, 2000c) was selected because
26 the small group sizes (n=10) in the principal study preclude selecting a lower benchmark risk
27 level.

28 The modeled data for absolute liver weight and liver-to-body weight ratio (relative liver
29 weight) for rats and mice are shown in Table 5-4. Dose-related statistically significantly
30 increased mean liver weights (absolute, relative-to-body weight) were observed in male rats at
31 doses of 100-400 mg/kg-day and at all dose levels in female rats. Changes in the 600 mg/kg-day
32 males were similar in magnitude to changes in the 400 mg/kg-day males, but could not be
33 assessed for statistical significance because only one survivor remained in this group at study
34 termination. In male mice, absolute liver weight was significantly increased at dose levels ≥ 200
35 mg/kg-day, while the liver-to-body weight ratio was significantly increased at dose levels ≥ 100
36 mg/kg-day. In female mice, both measures of liver weight were significantly increased in a
37

1

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

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

2 ^aIncidences of rats with one or more of the liver lesion types (cytomegaly, necrosis,
3 inflammation, mineralization), extracted from individual animal histopathologic results provided
4 to Syracuse Research Corporation by NTP. Liver lesions were not seen in 2/10 male rats of the
5 200 mg/kg-day dose level that died early due to gavage error.

6 ^bStatistically different from control groups according to Fisher's exact test ($p < 0.05$), performed
7 by Syracuse Research Corporation.

8 Source: NTP (1985a,b)

9

10

Table 5-2. Benchmark doses (BMD₁₀s and BMDL₁₀s) from best fitting models predicting combined incidences of Fischer 344/N rats or B6C3F1 mice with liver lesions (see Appendix B)

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

11

12

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

BMDs and BMDLs (mg/kg-day)					
10% Extra risk		5% Extra risk		1% Extra risk	
BMD ₁₀	BMDL ₁₀	BMD ₀₅	BMDL ₀₅	BMD ₀₁	BMDL ₀₁
56.1	24.8	36.0	12.7	13.2	2.8

13 Source: NTP (1985b)

14

15

1

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

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

2 *Statistically significantly different from controls ($p < 0.05$) based on Student's two-tailed t-test.

3 Source: NTP (1985a,b)

4

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

Table 5-5. Benchmark doses (BMD₁₀ and BMDL₁₀) from best fitting models for increased absolute liver weight and liver-to-body weight ratio in Fischer 344/N rats and B6C3F1 mice administered bromobenzene by gavage 5 days/week for 90 days

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

8 Source: NTP (1985a,b)

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

BMDs and BMDLs (mg/kg-day)			
BMD _{1sd}	BMDL _{1sd}	BMD _{0.5sd}	BMDL _{0.5sd}
34.78	25.79	16.43	12.34

11 Source: NTP (1985b)

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

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

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

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

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

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

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

13
 14
 15 In summary, female mice have a lower point of departure for hepatotoxicity of
 16 bromobenzene than male mice or male or female rats as indicated by the BMDLs in Tables 5-2
 17 (liver lesions) and 5-5 (absolute liver weight and liver-to-body weight ratio). The increase in
 18 SDH levels in male and female mice was a less sensitive effect and was highly variable. The
 19 lowest BMDL_{1sd} from the best fitting model for liver weight changes was 25.8 mg/kg-day, which
 20 was very similar to the lowest BMDL₁₀ from the best fitting model for combined liver lesions of
 21 24.8 mg/kg-day. For this reason, liver toxicity in female mice, as defined by an increase in liver
 22 weight and liver lesions was selected as the critical effect for deriving the subchronic RfD. The
 23 average BMDL of 25 mg/kg-day was selected as the point of departure to derive the subchronic
 24 RfD for bromobenzene.

1 **5.1.1.3. Subchronic RfD Derivation - Including Application of Uncertainty Factors (UFs)**

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

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

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

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

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

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

2 The subchronic RfD for bromobenzene was calculated as follows:

$$\begin{aligned} \text{Subchronic RfD} &= (\text{average BMDL} \times 5/7) \div \text{UF} \\ &= (25 \text{ mg/kg-day} \times 5/7) \div 1000 \\ &= 17.8 \text{ mg/kg-day} \div 1000 \\ &= 0.02 \text{ mg/kg-day (rounded to one significant figure)} \end{aligned}$$

9 **5.1.2. Chronic Oral RfD**

10 **5.1.2.1. Choice of Principal Study and Critical Effect - with Rationale and Justification**

11 As discussed in Section 4.1, there are no human studies available for development of a
12 chronic RfD. The toxicity database for repeated oral exposure in laboratory animals that are
13 available for selection of a chronic RfD consists of two 90-day gavage studies: one in rats (NTP,
14 1985a) and one in mice (NTP, 1985b). No chronic-duration, reproductive toxicity, or
15 developmental toxicity studies are available.

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

24 **5.1.2.2. Methods of Analysis - Including Models (PBPK, BMD, etc.)**

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

28 **5.1.2.3. Chronic RfD Derivation - Including Application of Uncertainty Factors (UFs)**

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

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

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

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

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

21 Although chronic oral or inhalation animal studies are not available for bromobenzene, a
22 chronic oral toxicity study is available for chlorobenzene. As discussed in detail in Section
23 4.5.4, bromobenzene and chlorobenzene exhibit striking similarities in structure, toxicokinetic
24 properties, and critical target of toxicity (liver) in rats and mice. Mice appear to be more
25 sensitive than rats to nonneoplastic hepatotoxicity induced by either bromobenzene or
26 chlorobenzene. The NTP 2-year oral study of chlorobenzene concluded that, nonneoplastic
27 lesions clearly attributable to chlorobenzene were not observed, and identified free-standing
28 NOAELs of 60 and 120 mg/kg-day in male and female mice, respectively (NTP, 1985e). In a
29 similarly-designed subchronic (90-day) oral toxicity study in mice, a NOAEL of 125 and a
30 LOAEL of 250 mg/kg-day were identified in both males and females for chlorobenzene-induced
31 liver lesions (NTP, 1985e). These results suggest that the dose-response relationships for liver
32 effects from subchronic and chronic exposure are similar. It is reasonable to expect such
33 similarities in dose-response relationships for subchronic and chronic exposure to bromobenzene
34 as well, due to the similarity between the two chemicals with respect to chemical reactivity and
35 structure, including similar Pauling electronegativities of chlorine (3.16) and bromine (2.96)
36 (Loudon, 1988). In addition, a study by Chan et al. 2007 suggests that halobenzene congeners

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

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

11 The chronic RfD for bromobenzene was calculated as follows:

$$\begin{aligned} \text{Chronic RfD} &= (\text{average BMDL} \times 5/7) \div \text{UF} \\ &= (25 \text{ mg/kg-day} \times 5/7) \div 3000 \\ &= 17.8 \text{ mg/kg-day} \div 3000 \\ &= 0.006 \text{ mg/kg-day (rounded to one significant figure)} \end{aligned}$$

18 **5.1.3. Previous Oral Assessment**

19 An RfD was not previously available on IRIS.

21 **5.2. INHALATION REFERENCE CONCENTRATION (RfC)**

22 **5.2.1. Subchronic Inhalation RfC**

23 **5.2.1.1. Choice of Principal Study and Critical Effect - with Rationale and Justification**

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

29 An adverse effect level was not identified in the 13-week inhalation study in male and
30 female Fischer 344/N rats repeatedly exposed to bromobenzene vapor concentrations as high as
31 300 ppm (NTP, 1985c). Significantly increased mean liver weights in 100- and 300-ppm male
32 and female rats may be indicators of an adaptive liver effect of questionable toxicological
33 significance in the absence of more overt toxicity, e.g., liver lesions or necrosis. It should be
34 noted also that this finding is in general agreement with the available oral studies in rats (NTP,
35 1985a) indicating that, unlike mice, this species does not exhibit overt liver toxicity following
36 bromobenzene exposure. Cortical tubular regeneration in the kidney of male rats appeared to be

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

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

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

1 **5.2.1.2. Methods of Analysis - Including Models (PBPK, BMD, etc.)**

2 Available models in U.S. EPA BMDS version 1.3.2 were fit to the liver lesion
3 (cytomegaly) data for female B6C3F1 mice and to absolute liver weight and liver-to-body
4 weight data for male and female B6C3F1 mice from the 90-day inhalation studies (NTP,
5 1985c,d). Modeling results are presented in Appendix C.

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

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

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

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

25
26
27 Consideration was given to using a NOAEL/LOAEL approach for the cytomegaly data
28 set since there is little change in effect until a dose of 100 ppm. However, it was decided that the
29 use of the entire dataset in a BMD modeling approach would be a more sound method since the

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

5 Sigmoidal models and two non-sigmoidal models (quantal quadratic and quantal linear)
6 in the U.S. EPA BMDS (version 1.3.2.) were fit to the data in Table 5-9. Modeling results,
7 presented in Table 5-10, show that: (1) all sigmoidal models provided excellent fit to the data (as
8 expected due to the nature of the data); (2) the non-sigmoidal models provided poorer fits to the
9 data; and (3) all sigmoidal models provided similar estimates of BMC_{10S} (ranging from about 77
10 ppm to 97 ppm, a 1.3-fold range) and $BMCL_{10S}$ (ranging from about 40 ppm to 60 ppm, a
11 1.5-fold range). The conventional BMR of 10% extra risk (U.S. EPA, 2000c) was selected
12 because the small group sizes ($n=10$) in the principal study preclude selecting a lower benchmark
13 risk level. Following U.S. EPA (2000c) guidance for selecting models for point of departure
14 computation, the model with the best fit and the lowest AIC is selected to calculate the BMCL
15 which in this case corresponds to the log-logistic and gamma models (Table 5-10). The
16 $BMCL_{10S}$ from these best-fitting models (from the log-logistic and gamma models) were
17 averaged (55 ppm) to arrive at the point of departure for deriving the RfC, as per U.S. EPA
18 (2000c) guidance. Table 5-11 shows BMCs and BMCLs associated with 10, 5, and 1% extra
19 risk levels.

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

25 All continuous variable models in the U.S. EPA BMDS (version 1.3.2.) were fit to the
26 absolute and relative liver weight data for female mice. As shown in Table 5-13, all models
27 provided adequate fits to the data for absolute liver weight and liver-to-body weight ratio in
28 female B6C3F1 mice as assessed by a chi-square goodness-of-fit test. Second-degree
29 polynomial models provided the best fits for both variables as determined by the AIC (Table
30 5-13). One standard deviation change from the control mean corresponds to an excess risk of
31 approximately 10% for the proportion of individuals above the 98th percentile (or below the 2nd
32 percentile) of the control distribution for normally distributed effects (see Appendix C).
33 Predicted BMC_{1sd} values were 52.38 ppm for absolute liver weight and 52.42 ppm for relative
34 liver weight; associated 95% lower confidence limits ($BMCL_{1sdS}$) were 33.51 ppm for absolute
35 liver weight and 33.90 ppm for relative liver weight (see Table 5-13). A 0.5 standard deviation

1

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

Model	BMC ₁₀ (ppm)	BMCL ₁₀ (ppm)	χ^2 <i>p</i> -value	AIC
Log-logistic ^a	95.59	58.73	1.00	12.01
Gamma ^b	89.24	51.42	1.00	12.01
Multi-stage ^c	77.09	40.33	0.999	12.17
Weibull ^b	92.34	47.08	1.00	14.01
Log-probit ^a	92.95	57.45	1.00	14.01
Logistic	96.75	59.75	1.00	14.01
Probit	93.71	54.94	1.00	14.01
Quantal quadratic	55.15	40.15	0.87	14.05
Quantal linear	21.38	13.18	0.16	22.78

2 ^aSlope restricted to >1

3 ^bRestrict power > = 1

4 ^cRestrict betas > = 0; degree of polynomial = 3 (maximum degree restricted to #dose groups
5 minus 2)

6 Source: NTP (1985d)

7

8

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

10% Extra risk		5% Extra risk		1% Extra risk	
BMC ₁₀	BMCL ₁₀	BMC ₀₅	BMCL ₀₅	BMC ₀₁	BMCL ₀₁
Log-logistic model					
95.59	58.73	91.71	46.09	83.67	26.47
Gamma model					
89.24	51.42	80.98	38.52	66.93	20.53

9 Source: NTP (1985d)

10

1

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

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

2 ^aStatistically significantly different from controls ($p < 0.05$) based on Student's two-tailed t-test

3 ^bOutside 3 standard deviations from the control mean

4 Source: NTP (1985d)

5

6

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

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

7 ^aStatistical tests indicated that variances were not constant across exposure groups. Model
8 results are for non-homogeneous variance, with the exception of the linear and 3-degree
9 polynomial models for liver-to-body weight ratio.

10 ^bModeled as a continuous variable using one standard deviation as the BMR.

11 Source: NTP (1985d)

12

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

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

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

Source: NTP (1985d)

The BMDL₁₀ for absolute and relative liver weight changes in female mice was 34 ppm. The BMDL₁₀ for the incidence of cytomegaly was 55 ppm derived from an average of the BMDL₁₀s from the two best-fitting models. There is some uncertainty associated with the choice of the critical effect and the point of departure. Although cytomegaly in the absence of necrosis or other indicators of degenerative effects may represent an adaptive hepatic effect rather than an adverse effect, necrosis and mineralization observed in livers of some of the 300-ppm female mice was considered by the Pathology Working Group (NTP, 1986b) to be an exposure-related effect. Therefore, the 300-ppm exposure level may represent an effect level in female mice that is near the threshold of significantly detectable bromobenzene hepatotoxicity. For this reason, the average BMCL₁₀ of 55 ppm (from the log-logistic and gamma models) for cytomegaly in female mice was selected as the point of departure to derive the subchronic RfC for bromobenzene. There is less uncertainty in choosing this endpoint over the increase in liver weight due to the lack of directly observable statistically significant toxicity at higher doses.

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

Following U.S. EPA (1994b) methodology, the human equivalent concentration (HEC) for an extra respiratory effect produced by a category 3 gas, such as bromobenzene (not highly water soluble or reactive in the respiratory tract, the liver as the critical extrarespiratory target), is calculated by multiplying the duration-adjusted BMCL or NOAEL by the ratio of the blood:gas partition coefficients in animals and humans $[(H_{b/g})_A / (H_{b/g})_H]$. Because bromobenzene blood:gas partition coefficients are not available for humans or mice, a default value of 1 is used for this ratio. The BMCL₁₀ of 55 ppm for hepatocellular cytomegaly in female mice was converted to 353.2 mg/m³ (55 ppm x MW[157] / 24.45 = 353.2 mg/m³), which was then converted to a

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

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

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

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

23 The subchronic RfC for bromobenzene was calculated as follows:

$$\begin{aligned} \text{Subchronic RfC} &= \text{BMCL}_{10\text{HEC}} \div \text{UF} \\ &= 63 \text{ mg/m}^3 \div 300 \\ &= 0.2 \text{ mg/m}^3 \text{ (rounded to one significant figure)} \end{aligned}$$

29 **5.2.2. Chronic Inhalation RfC**

30 **5.2.2.1. Choice of Principal Study and Critical Effect - with Rationale and Justification**

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

1 The choices of principal study and critical effect for development of the chronic RfC for
2 bromobenzene are the same as those described for the development of a subchronic RfC (see
3 Section 5.2.1.1). The increase in incidence of cytomegaly and the increase in absolute and
4 relative liver weight in female mice (NTP, 1985d) were considered in the selection of the critical
5 effect for development of the subchronic RfC for bromobenzene.
6

7 **5.2.2.2. Methods of Analysis - Including Models (PBPK, BMD, etc.)**

8 The methods of analysis used to derive the subchronic RfC for bromobenzene apply to
9 the derivation of the chronic RfC as well (see Section 5.2.1.2).
10

11 **5.2.2.3. Chronic RfC Derivation - Including Application of Uncertainty Factors (UFs)**

12 As described in detail in Section 5.2.1.3, the average BMCL₁₀ of 55 ppm for cytomegaly
13 in female mice was selected as the point of departure to derive the subchronic RfC for
14 bromobenzene. The same point of departure was used to derive the chronic RfC. The BMCL₁₀
15 of 55 ppm for hepatocellular cytomegaly in female mice was converted to a BMCL_{10HEC} of 63
16 mg/m³ (see Section 5.2.1.3 for details regarding conversion to the HEC). The BMCL_{10HEC} of 63
17 mg/m³ was divided by a total UF of 1000. The UF consists of four areas of uncertainty: (1)
18 interspecies extrapolation, (2) interindividual human variability, (3) extrapolation from
19 subchronic- to chronic-duration exposure, and (4) database deficiencies.

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

27 A 10-fold UF was selected to account for interindividual toxicokinetic and
28 toxicodynamic variability in humans (UF_H). Although hepatotoxicity was observed only in
29 female mice, a 300-ppm (1926 mg/m³) group of male mice was not included in the study. Due to
30 the lack of conclusive information concerning gender-specific differences in bromobenzene
31 hepatotoxicity following inhalation exposure, as well as the lack of data concerning the extent of
32 variation in sensitivity to bromobenzene within the human population, the default value of 10
33 was not reduced.

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

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

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

10 The chronic RfC for bromobenzene was calculated as follows:

$$\begin{aligned} \text{Chronic RfC} &= \text{BMCL}_{10\text{HEC}} \div \text{UF} \\ &= 63 \text{ mg/m}^3 \div 1000 \\ &= 0.06 \text{ mg/m}^3 \text{ (rounded to one significant figure)} \end{aligned}$$

16 **5.3. CANCER ASSESSMENT**

17 No studies of cancer risks of humans or cancer bioassays in animals exposed to
18 bromobenzene were located. Bromobenzene was not mutagenic in the Ames assay and did not
19 consistently produce marked cytogenetic effects *in vitro* with mammalian cells, even in the
20 presence of rat liver S-9 preparations. Bromobenzene induced micronuclei in bone marrow of
21 mice given acute oral doses of 125 mg/kg and was bound to DNA and RNA following
22 intraperitoneal injection. Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,
23 2005a), there is "inadequate information to assess the carcinogenic potential" of brombenzene.

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

3
4
5 **6.1. HUMAN HAZARD POTENTIAL**

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

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

25 Following EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is
26 "inadequate information to assess the carcinogenic potential" of bromobenzene due to the lack of
27 data on the possible carcinogenicity of bromobenzene in humans or animals. Bromobenzene was
28 not mutagenic in bacterial assays and did not consistently produce marked cytogenetic effects *in*
29 *vitro* with mammalian cells, even in the presence of rat liver metabolizing preparations.
30 Bromobenzene increased formation of micronucleated polychromatic erythrocytes in bone
31 marrow of mice given acute oral doses of 125 mg/kg and was bound to DNA and RNA
32 following intraperitoneal injection. The available genotoxicity data, therefore, provide only
33 limited evidence of bromobenzene genotoxicity.
34

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

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

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

6.2.2. Noncancer/Inhalation

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

The average $BMCL_{10}$ of 55 ppm (from the log-logistic and gamma models) for cytomegaly in female mice was selected as the point of departure. The $BMCL_{10}$ was converted to 353.2 mg/m³ (55 ppm \times MW[157] / 24.45 = 353.2 mg/m³), which was then converted to a

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

9 **6.2.3. Cancer/Oral**

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

12

13 **6.2.4. Cancer/Inhalation**

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

16

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

[to be provided]

1 **APPENDIX B. BENCHMARK DOSE CALCULATIONS FOR THE RfD**

2
3
4 All available models in the EPA BMDS (version 1.3.2) were fit to incidence data for
5 histopathologic liver lesions in male and female Fischer 344/N rats and male and female B6C3F1
6 mice from the 90-day oral gavage studies (NTP, 1985a,b). The data that were modeled are
7 shown in Table 5-1.

8 All models provided adequate fits to the data for histopathologic liver lesions
9 (centrilobular inflammation, cytomegaly, mineralization, or necrosis; combined) in the NTP
10 (1985a,b) studies, as assessed by a chi-square goodness-of-fit test (see Tables B-1, B-2, B-3, and
11 B-4 below and respective plots of observed and predicted values from the various models
12 [Figures B-1, B-2, B-3, and B-4]).

13
Table B-1. BMD modeling results for the incidence of combined liver effects in male Fischer 344/N rats exposed to bromobenzene by gavage 5 days/week for 90 days

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

14 ^aSlope restricted to >1

15 ^bRestrict power >=1

16 ^cRestrict betas >=0; Degree of polynomial = 5

1
2

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

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

3 ^aSlope restricted to >14 ^bRestrict power >=15 ^cRestrict betas >=0; Degree of polynomial = 5

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

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

8 ^aRestrict power >=19 ^bRestrict betas >=0; Degree of polynomial = 510 ^cSlope restricted to >1

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

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

2 ^aRestrict power >=1

3 ^bSlope restricted to >1

4 ^cRestrict betas >=0; Degree of polynomial = 5

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The log-logistic model provided the best fit to the male rat data (see Table B-1), and was thus selected to estimate a BMD for the male rats from the NTP (1985a) data. The BMD₁₀ associated with a 10% extra risk for histopathologic liver lesions in male rats was 172.1 mg/kg-day and its lower 95% confidence limit (BMDL₁₀) was 69.2 mg/kg-day (see Figure B-1 for a plot of observed and predicted values). The form and parameters of the log-logistic model for male rat liver effects (NTP, 1985a) are:

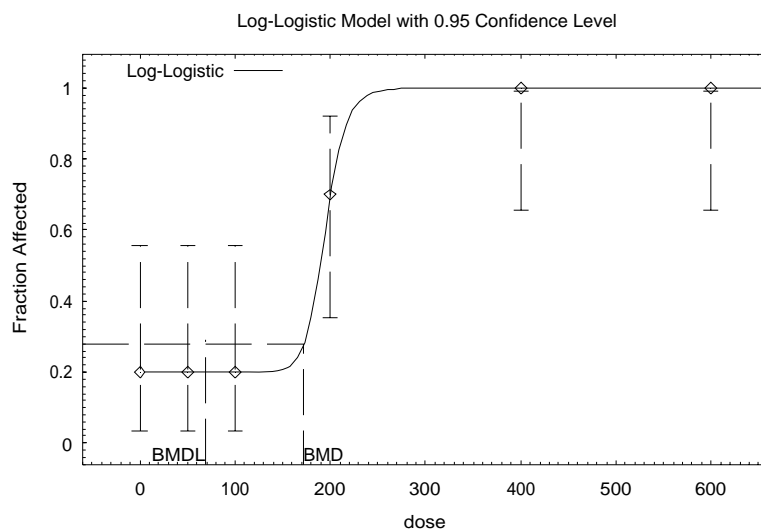
$$P(d) = B_0 + (1 - B_0) / [1 + \exp(-\text{intercept} - \text{slope} * \log(d))] \quad (\text{Eq. B-1})$$

d = exposure dose

B₀ = 0.199998 (se = 0.0730298)

intercept = -94.8589 (se = 0.786551)

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



13:13 11/10 2003

1 **Figure B-1. Observed and predicted incidences of male Fischer 344/N rats**
 2 **exhibiting bromobenzene-induced combined liver lesions following gavage**
 3 **treatment 5 days/week for 90 days. BMD=ED₁₀; BMDL=LED₁₀**
 4
 5

6 The log-logistic model provided the best fit to the female rat data (see Table B-2 and
 7 Figure B-2) and was thus selected to estimate a BMD for the female rats from the NTP (1985a)
 8 data. The BMD₁₀ associated with a 10% extra risk for histopathologic liver lesions in female rats
 9 was 184.7 mg/kg-day and its lower 95% confidence limit (BMDL₁₀) was 66.1 mg/kg-day (see
 10 Figure B-2 for a plot of observed and predicted values). The form and parameters of the log-
 11 logistic model for female rat liver effects are as follows:

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

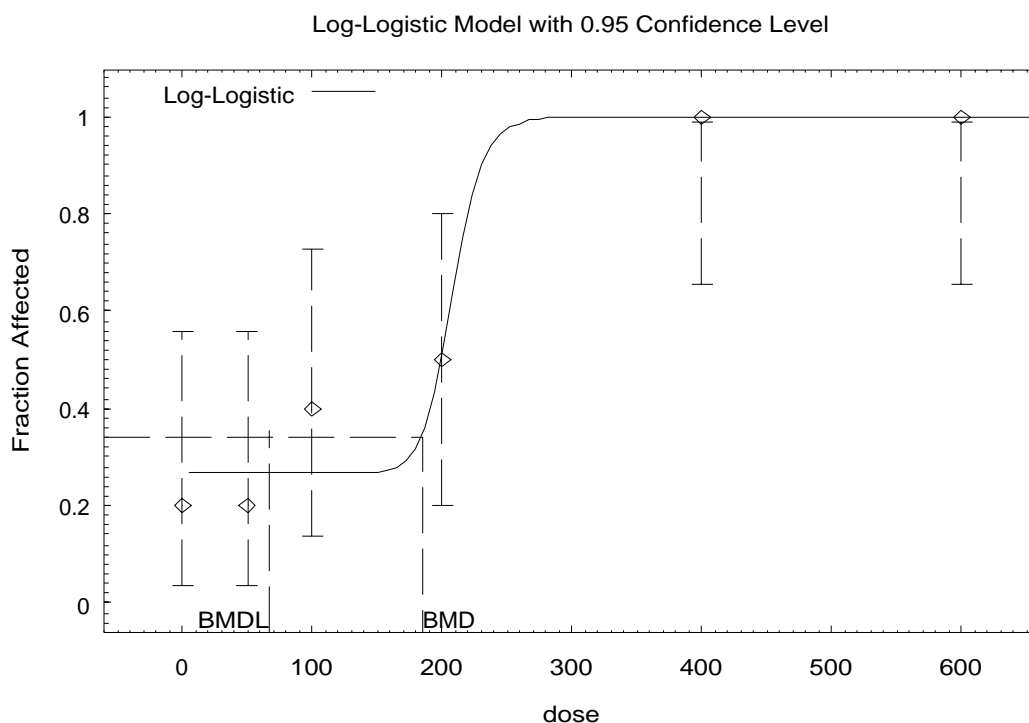
13 d = exposure dose

14 B₀ = 0.266665 (se = 0.0807368)

15 intercept = -96.1318 (se = 1.05229)

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

17



14:29 11/10 2003

1 **Figure B-2. Observed and predicted incidences of female Fischer 344/N rats**
 2 **exhibiting bromobenzene-induced combined liver lesions following gavage**
 3 **treatment 5 days/week for 90 days. BMD=ED₁₀; BMDL=LED₁₀**
 4
 5

6 The multi-stage model provided the best fit to the male mouse liver lesion data (see Table
 7 5-1), and was thus selected to estimate a BMD for the male mice from the NTP (1985a) data.
 8 The BMD₁₀ associated with a 10% extra risk for histopathologic liver lesions in male mice was
 9 97.99 mg/kg-day and its lower 95% confidence limit (BMDL₁₀) was 38.82 mg/kg-day (see
 10 Figure B-3 for a plot of observed and predicted values). The form of the multi-stage model for
 11 male mouse liver effects are as follows:

12
$$P(d) = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose} - \beta_2 * d^2 - \beta_3 d^3 - \beta_4 d^4)] \quad (\text{Eq. B-3})$$

13 background = 0

14 $\beta_1 = 1.94919e+017$

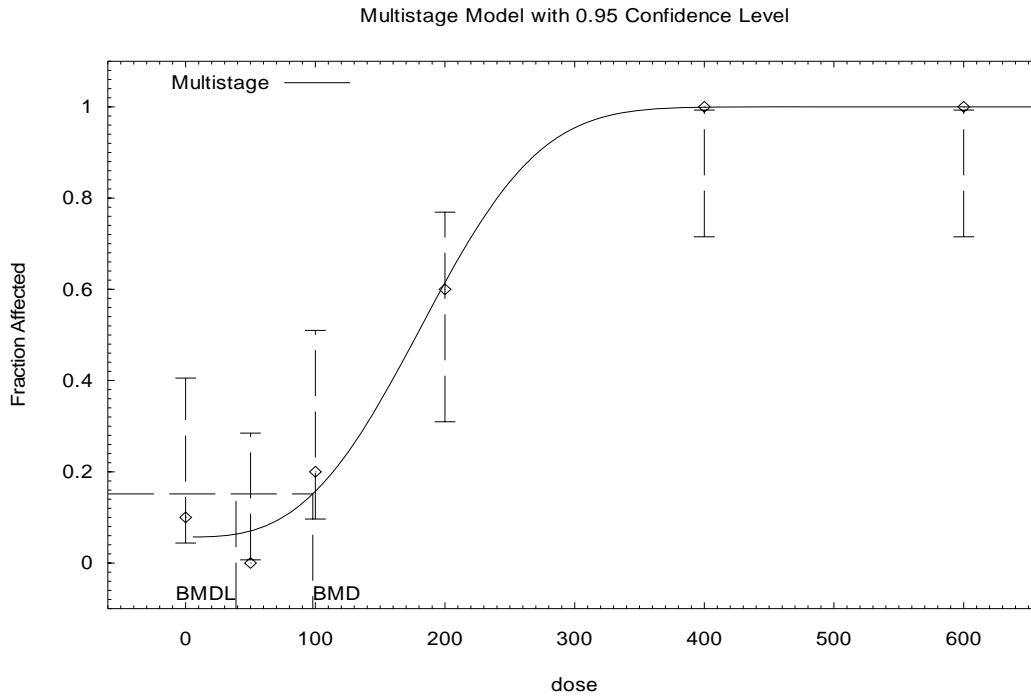
15 $\beta_2 = 1.63151e+013$

16 $\beta_3 = 0$

17 $\beta_4 = 0.$

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Figure B-3. Observed and predicted incidences of male B6C3F1 mice exhibiting bromobenzene-induced combined liver lesions following following gavage treatment 5 days/week for 90 days. BMD=ED₁₀; BMDL=LED₁₀

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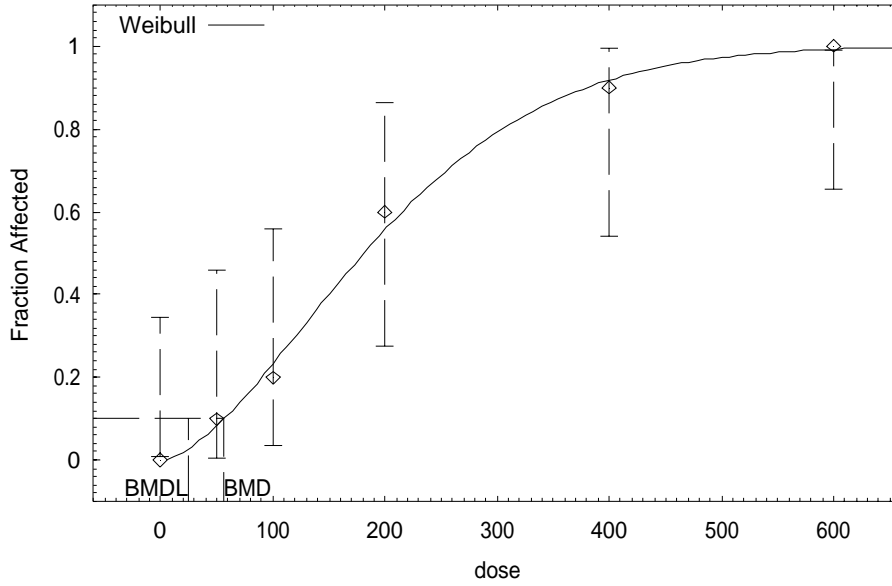
The Weibull model provided the best fit to the female mouse liver lesion data (see Table B-4), and was thus selected to estimate a BMD for liver lesions in female mice from the NTP (1985b) data. The BMD₁₀ associated with a 10% extra risk for histopathologic liver lesions in female mice was 56.1 mg/kg-day and its lower 95% confidence limit (BMDL₁₀) was 24.8 mg/kg-day (see Figure B-4 for a plot of observed and predicted values). Estimated BMDs and BMDLs associated with 5% and 1% extra risk are presented in Table 5-3 (see Figures B-5 and B-6 for a plot of observed and predicted values associated with 5% and 1% extra risk, respectively). The form and parameters of the Weibull model for female mouse liver effects are as follows:

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

- d = exposure dose
- B₀ = 0
- slope = 0.00152103 (se = 0.000322079)
- power = 1.62425 (se = 0.383589)

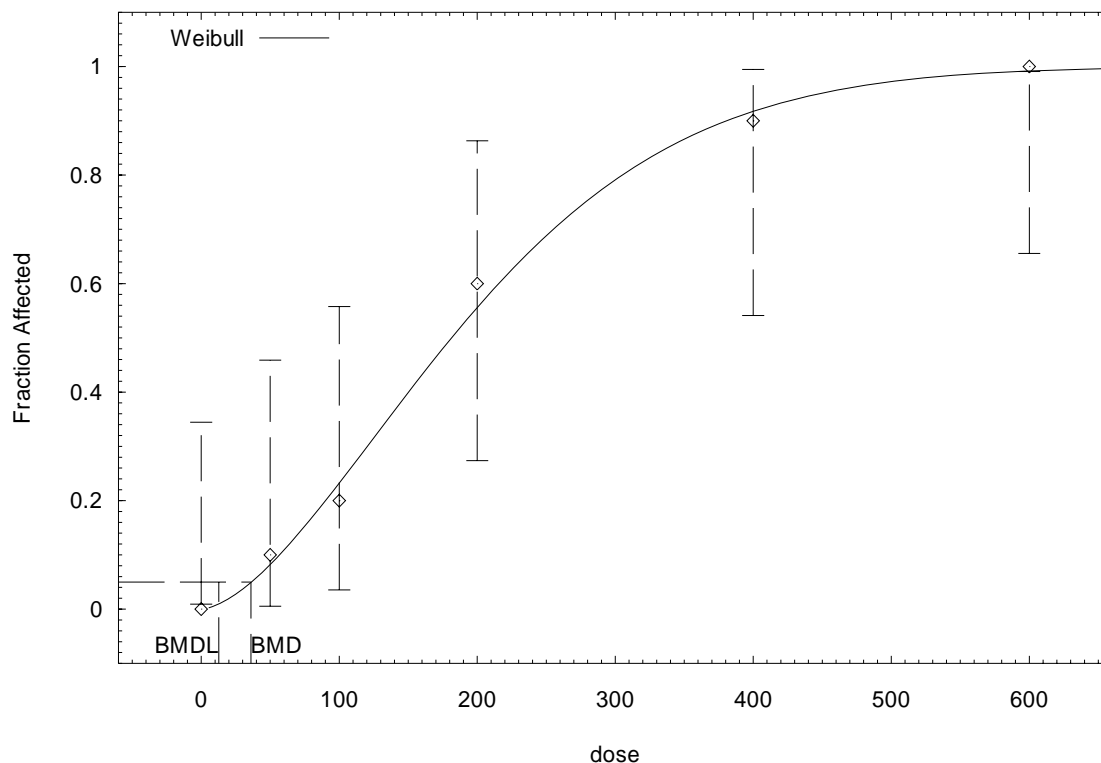
Weibull Model with 0.95 Confidence Level



11:41 11/10 2003

Figure B-4. Observed and predicted incidences of female B6C3F1 mice exhibiting bromobenzene-induced 10% extra risk for liver lesions following gavage treatment 5 days/week for 90 days. BMD=ED₁₀; BMDL=LED₁₀

Weibull Model with 0.95 Confidence Level



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

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

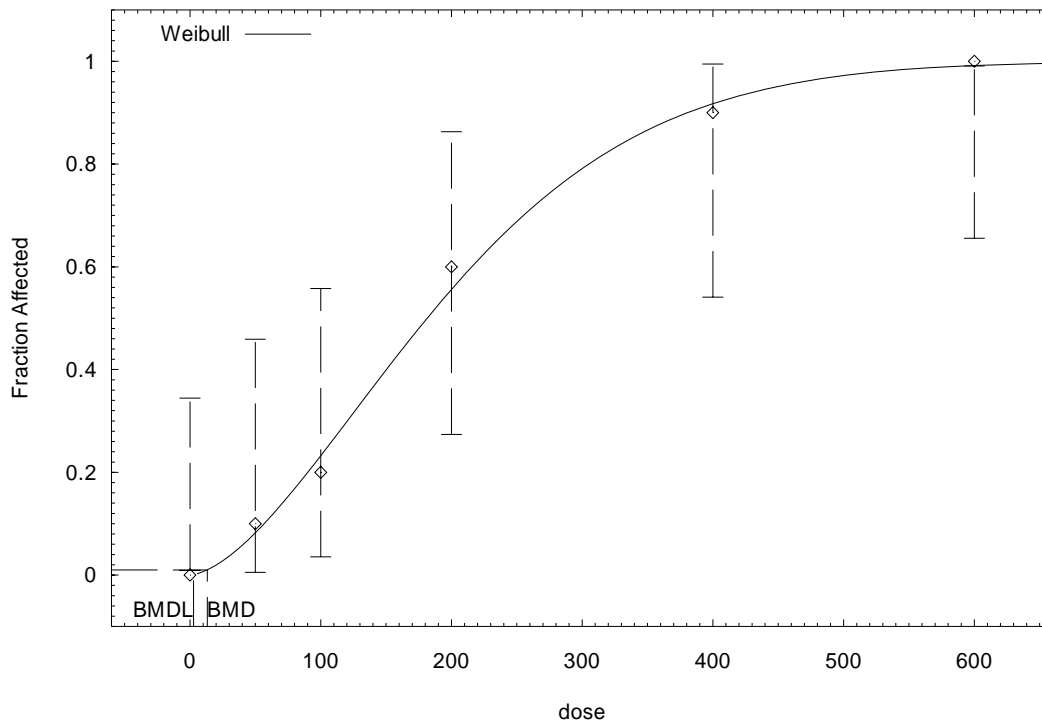
$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{slope} * \text{dose}^{\text{power}})] \quad (\text{Eq. B-5})$$

$$\text{background} = 0$$

$$\text{slope} = 0.000152103 \text{ (se} = 0.000322079\text{)}$$

$$\text{power} = 1.62425 \text{ (se} = 0.383589\text{)}$$

Weibull Model with 0.95 Confidence Level



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1 **Figure B-6. Observed and predicted incidences of female B6C3F1 mice**
2 **exhibiting bromobenzene-induced 1% extra risk for liver lesions following**
3 **gavage treatment 5 days/week for 90 days**
4

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

7 $P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{slope} * \text{dose}^{\text{power}})]$ (Eq. B-6)

8 background = 0

9 slope = 0.000152103 (se = 0.000322079)

10 power = 1.62425 (se = 0.383589)

11

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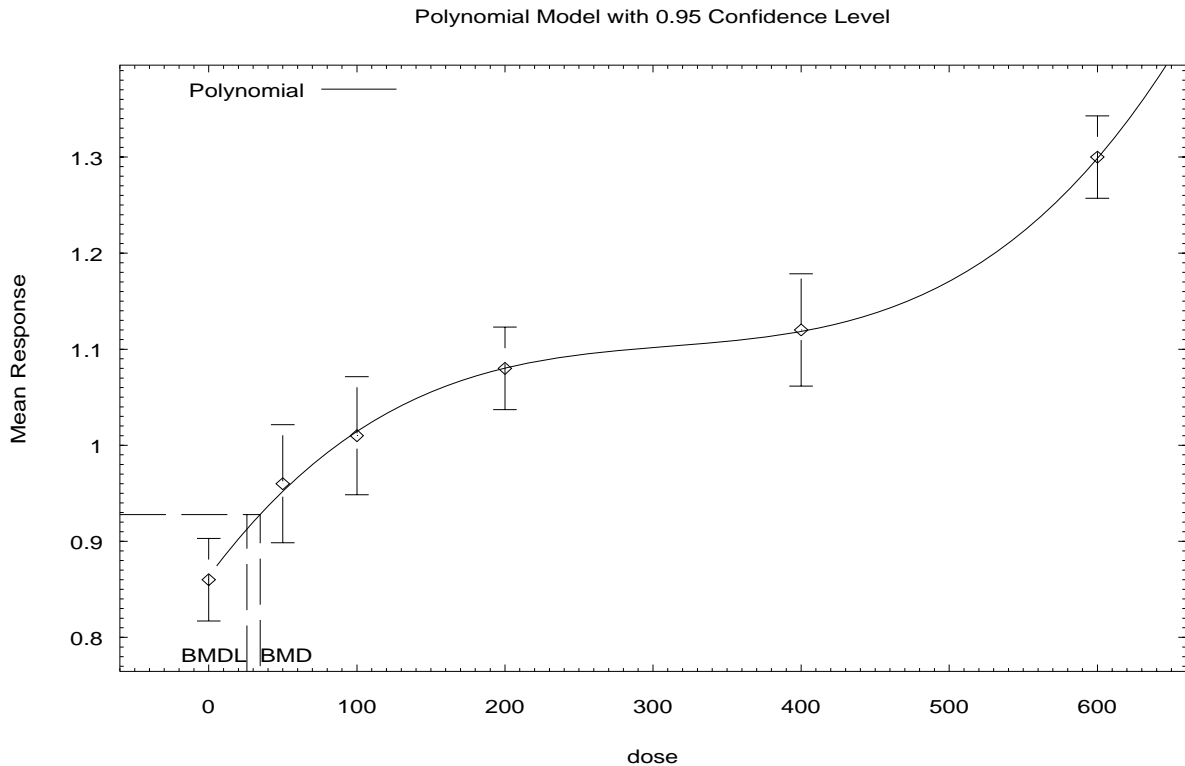
13 All available models in the EPA BMDS (version 1.3.2) were fit to absolute liver weight
14 and liver-to-body weight data in male and female Fischer 344/N rats and male and female
15 B6C3F1 mice from the 90-day oral gavage studies (NTP, 1985a,b). The data that were modeled
16 are shown in Table 5-4.

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

7
 8 The 3-degree polynomial model form of the response function for the female mice
 9 absolute liver weight ratio data is:

$$11 \quad Y[\text{dose}] = \text{beta}_0 + \text{beta}_1 * \text{dose} + \text{beta}_2 * \text{dose}^2 + \dots \quad (\text{Eq. B-7})$$

12
 13 A constant variance was assumed.



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14 **Figure B-7. Observed and predicted 1 standard deviation extra risk for**
 15 **absolute liver weight changes in female B6C3F1 mice administered**
 16 **bromobenzene by gavage 5 days/week for 90 days. BMD=ED₁₀;**
 17 **BMDL=LED₁₀**

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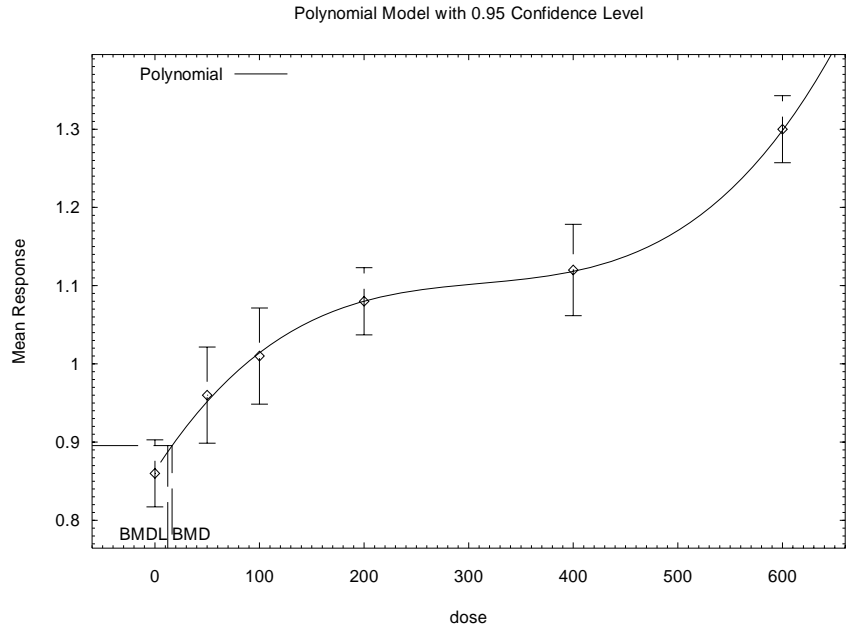


Figure B-8. Observed and predicted 0.5 standard deviation extra risk for absolute liver weight changes in female B6C3F1 mice administered bromobenzene by gavage 5 days/week for 90 days

The form of the response function is:

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

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

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

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

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

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

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

4
5
6 Serum levels for sorbital dehydrogenase (SDH) for male and female mice were modeled
7 using the linear, polynomial, power and hill models. The power model results for female mice
8 provided the best fit and the results of that model follow.

9
10 **POWER MODEL FOR SDH FEMALE MICE**

11
12 The form of the response function is:

$$13 \quad Y[\text{dose}] = \text{control} + \text{slope} * \text{dose}^{\text{power}} \quad (\text{Eq. B-9})$$

14
15
16
17 Dependent variable = MEAN

18 Independent variable = Dose

19 The power is restricted to be greater than or equal to 1

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

21
22 Total number of dose groups = 6

23 Total number of records with missing values = 0

24 Maximum number of iterations = 250

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

26 Parameter Convergence has been set to: 1e-008

27
28 **Default Initial Parameter Values**

29 alpha = 68.6796

30 rho = 0

31 control = 12

32 slope = 0.00131174

33 power = 1.53281

Asymptotic Correlation Matrix of Parameter Estimates

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

Parameter Estimates

95.0% Wald Confidence Interval

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

Table of Data and Estimated Values of Interest

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

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

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

Explanation of Tests

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

Tests of Interest

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

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

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

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

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

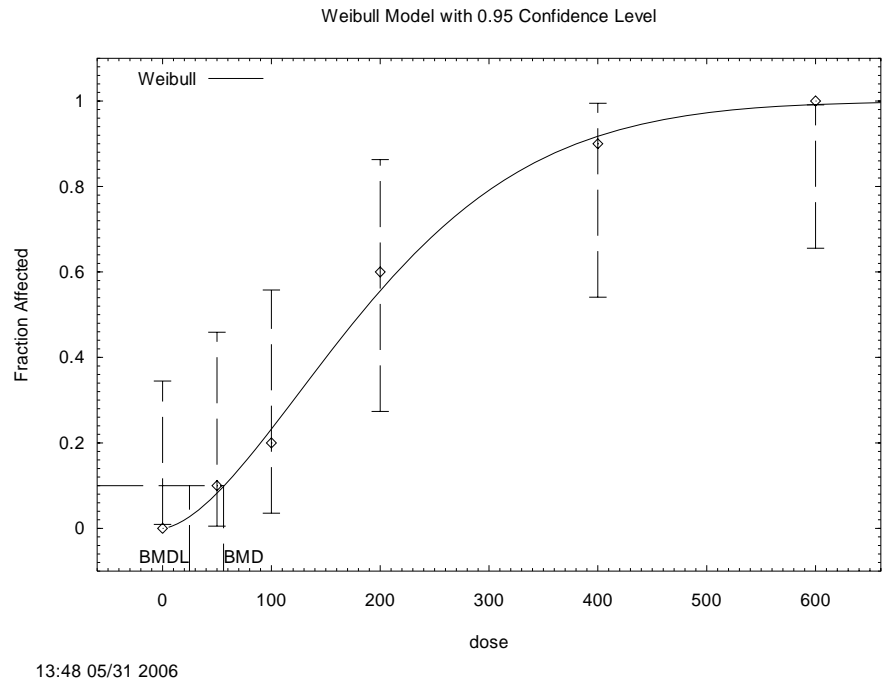
Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

1 Confidence level = 0.95
 2 BMD = 196.474
 3 BMDL = 145.789
 4

5 The lowest BMDL_{1sd} from the best fitting model for liver weight changes was 25.8
 6 mg/kg-day, which was very similar to the lowest BMDL₁₀ from the best fitting model for
 7 combined liver lesions of 24.8 mg/kg-day. For this reason, liver toxicity in female mice, as
 8 defined by an increase in liver weight and liver lesions was selected as the critical effect for
 9 deriving the subchronic RfD. The average BMDL₁₀ of 25 mg/kg-day was selected as the point
 10 of departure to derive the chronic and subchronic RfD for bromobenzene. Full modeling results
 11 for 10% extra risk for combined liver lesions in the Weibull model in female B6C3F1 mice are
 12 presented after Figure B-9.
 13
 14



15 **Figure B-9. Full modeling results for 10% extra risk for combined liver**
 16 **lesions in the Weibull model in female B6C3F1 mice treated by oral gavage**
 17 **that were used to estimate the RfD**
 18

19 The form of the probability function is:
 20 $P[\text{response}] = \text{background} + (1-\text{background}) * [1-\text{EXP}(-\text{slope} * \text{dose}^{\text{power}})]$ (Eq. B-10)
 21 background = 0
 22 slope = 0.000152103 (se = 0.000322079)
 23 power = 1.62425 (se = 0.383589)

APPENDIX C. BENCHMARK DOSE CALCULATIONS FOR THE RfC

Liver Lesion Data

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

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

1

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

Model	BMC₁₀ (ppm)	BMCL₁₀ (ppm)	x² p-value	AIC
Log-logistic ^a	95.59	58.73	1.00	12.01
Gamma ^b	89.24	51.42	1.00	12.01
Multi-stage ^c	77.09	40.33	0.999	12.17
Weibull ^b	92.34	47.08	1.00	14.01
Log-probit ^a	92.95	57.45	1.00	14.01
Logistic	96.75	59.75	1.00	14.01
Probit	93.71	54.94	1.00	14.01
Quantal quadratic	55.15	40.15	0.87	14.05
Quantal linear	21.38	13.18	0.16	22.78

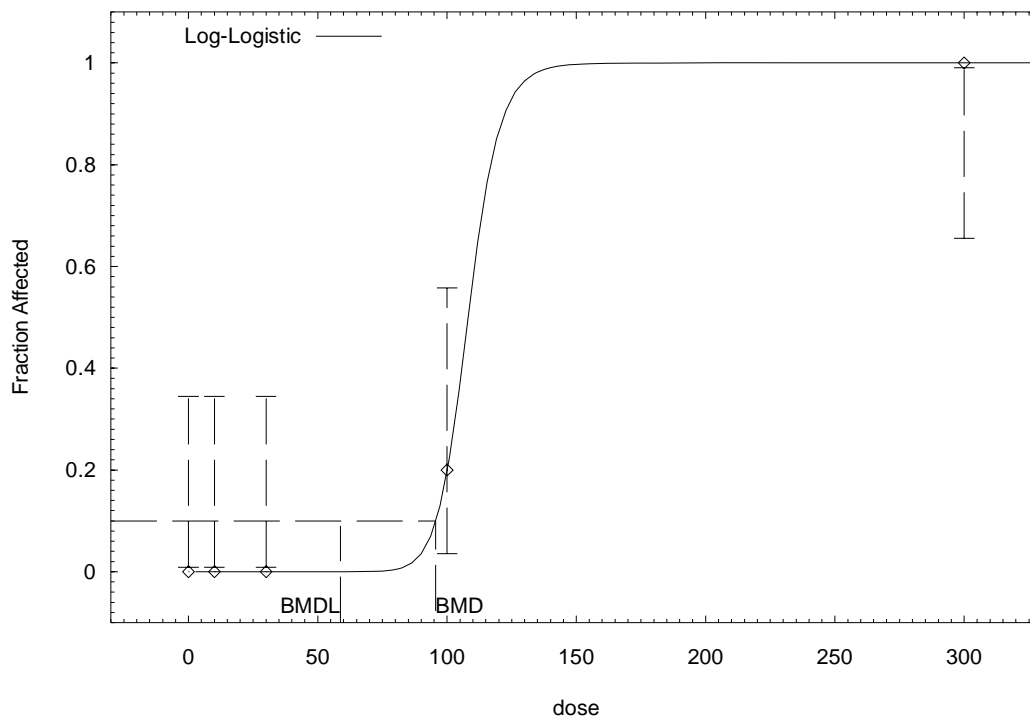
2 ^aSlope restricted to >1

3 ^bRestrict power > = 1

4 ^cRestrict betas > = 0; degree of polynomial = 3 (maximum degree restricted to #dose groups
5 minus 2)

6

Log-Logistic Model with 0.95 Confidence Level



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4 **Figure C-1. Observed and predicted incidences of female B6C3F1 mice**
5 **exhibiting 10% extra risk of bromobenzene-induced hepatocellular**
6 **cytomegaly following inhalation exposure for 6 hours/day, 5 days/week for 13**
7 **weeks. Log-logistic model predictions. dose=concentration in ppm.**
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11 The form and parameters of the log-logistic model for the incidence of female mouse
12 cytomegaly are as follows:

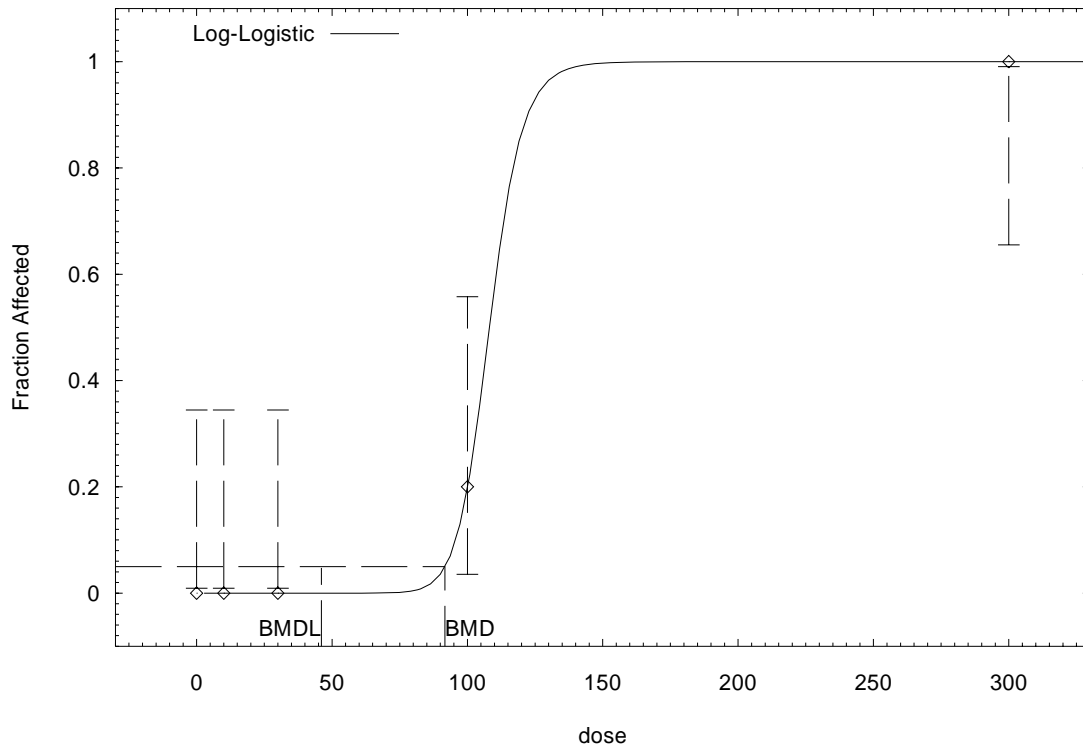
13
$$P[\text{response}] = \text{background} + (1 - \text{background}) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))]$$
 (Eq. C-1)

14 background = 0

15 intercept = -84.2793 (se = 0.790565)

16 slope = 18
17

Log-Logistic Model with 0.95 Confidence Level



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

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

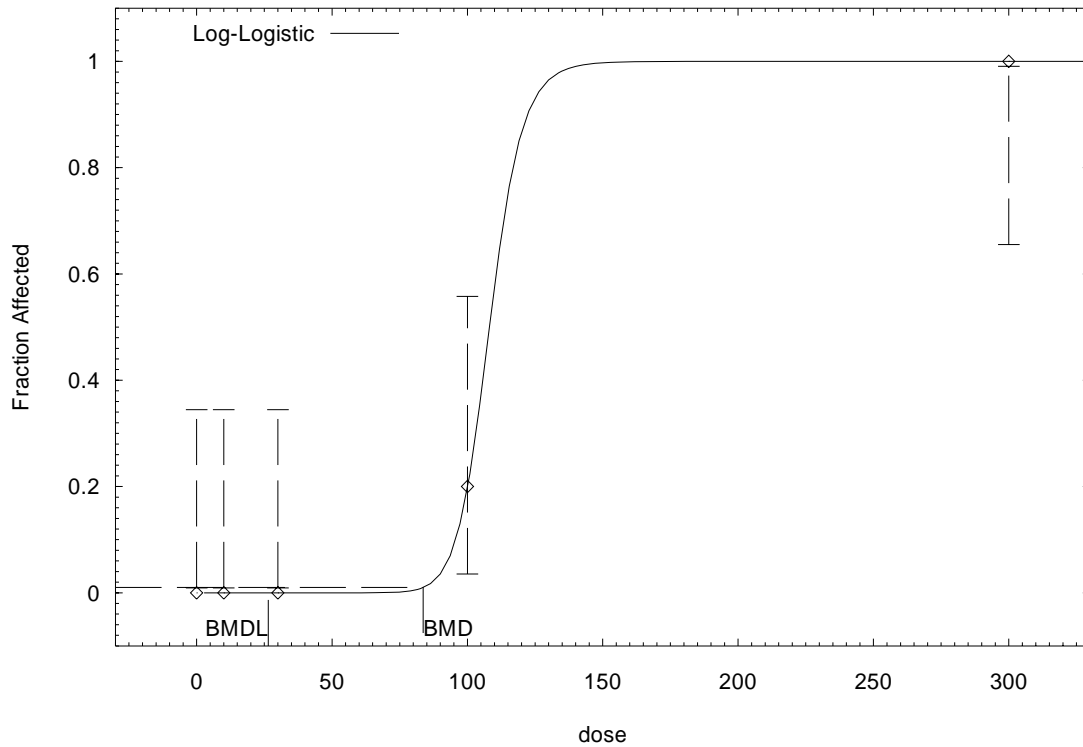
$$P[\text{response}] = \text{background} + (1 - \text{background}) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))] \quad (\text{Eq. C-2})$$

$$\text{background} = 0$$

$$\text{intercept} = -84.2793 \text{ (se} = 0.790565\text{)}$$

$$\text{slope} = 18$$

Log-Logistic Model with 0.95 Confidence Level



14:13 02/02 2006

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

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

9 $P[\text{response}] = \text{background} + (1 - \text{background}) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))]$ (Eq. C-3)

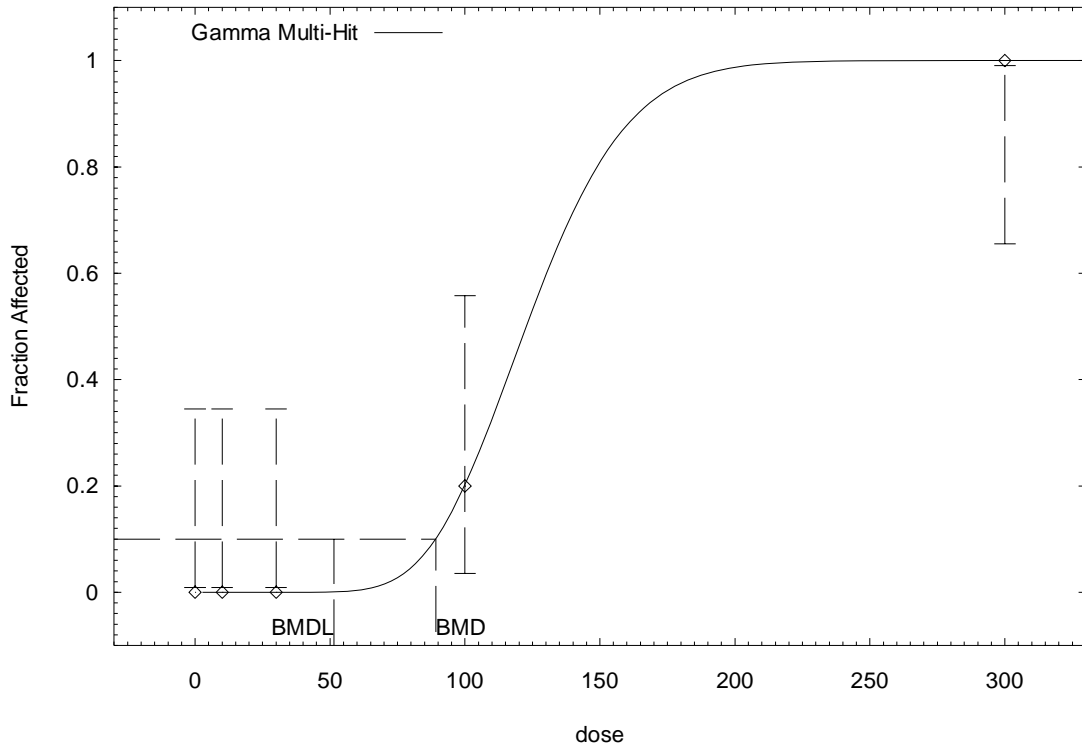
10 background = 0

11 intercept = -84.2793 (se = 0.790565)

12 slope = 18

13

Gamma Multi-Hit Model with 0.95 Confidence Level



14:43 03/03 2006

2
3

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

8

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

$$11 \quad P[\text{response}] = \text{background} + (1 - \text{background}) * \text{CumGamma}[\text{slope} * \text{dose}, \text{power}] \quad (\text{Eq. C-4})$$

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

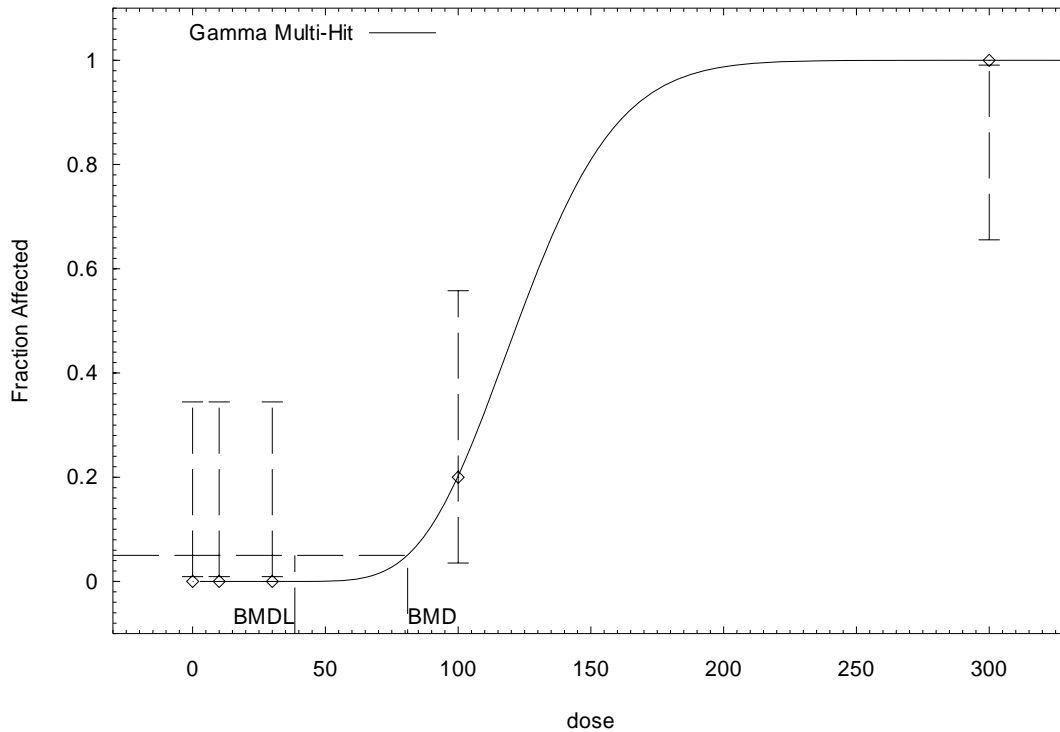
$$13 \quad \text{background} = 0$$

$$14 \quad \text{slope} = 0.143677 \quad (\text{se} = 0.0164918)$$

$$15 \quad \text{power} = 18$$

16

Gamma Multi-Hit Model with 0.95 Confidence Level



14:08 02/02 2006

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

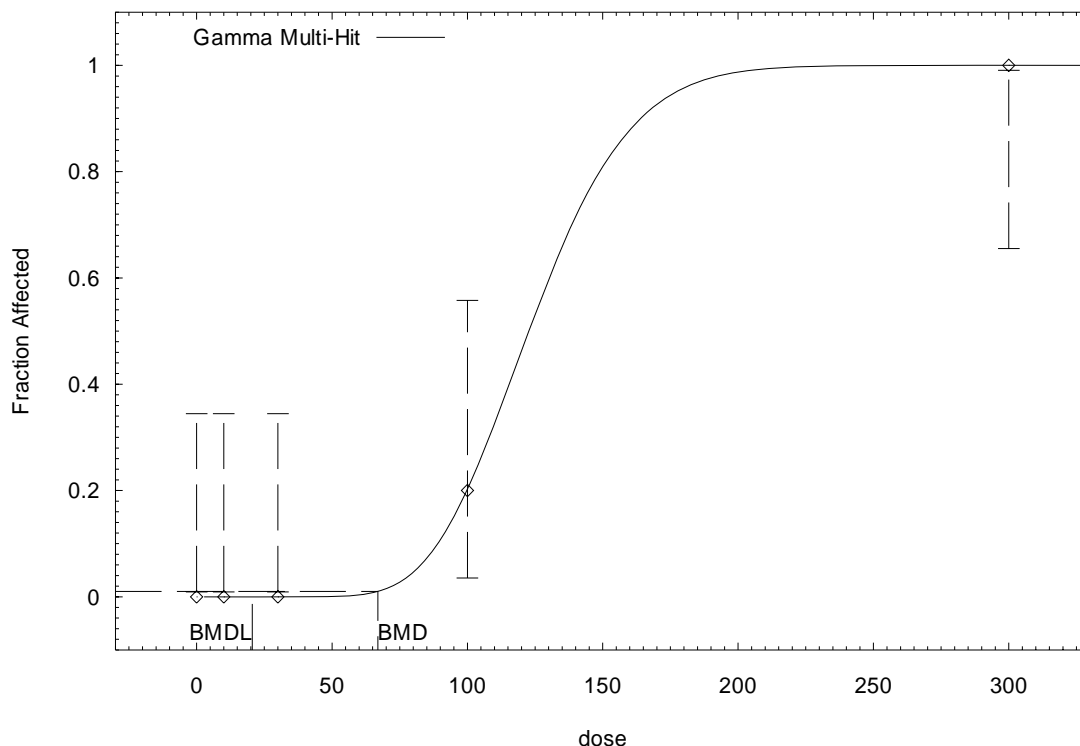
The form and parameters of the gamma model for 5% extra risk for female mouse cytomegaly are as follows:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * \text{CumGamma}[\text{slope} * \text{dose}, \text{power}] \quad (\text{Eq. C-5})$$

where CumGamma(.) is the cumulative Gamma distribution function

- background = 0
- slope = 0.0143677 (se = 0.0164918)
- power = 18

Gamma Multi-Hit Model with 0.95 Confidence Level



14:10 02/02 2006

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

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

$$P[\text{response}] = \text{background} + (1 - \text{background}) * \text{CumGamma}[\text{slope} * \text{dose}, \text{power}] \quad (\text{Eq. C-6})$$

where CumGamma(.) is the cumulative Gamma distribution function

background = 0

slope = 0.143677 (se = 0.0164918)

power = 18

1 **Liver Weight Data**

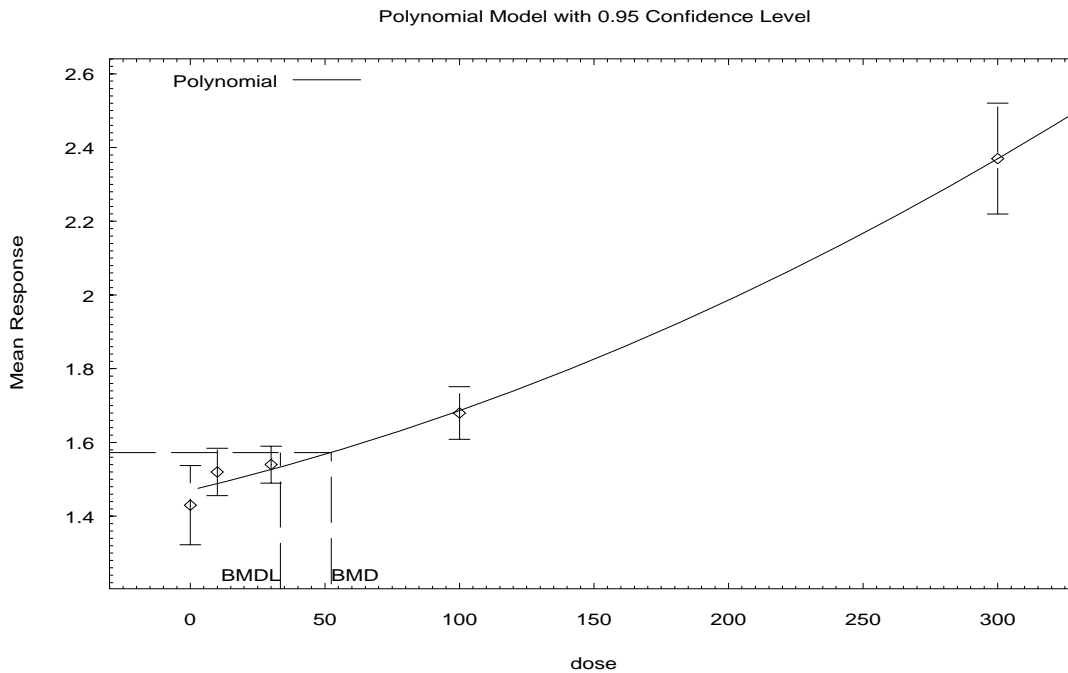
2 Absolute liver weight and liver-to-body weight ratio (relative liver weight) in female
 3 mice were also considered as potential bases of the RfC. All available models in the EPA
 4 BMDS (version 1.3.2) were fit to absolute liver weight data and liver-to-body weight ratio for
 5 female B6C3F1 mice from the 13-week inhalation study (NTP, 1985d). The data that were
 6 modeled are shown in Table 5-12. The model outputs are displayed in Table C-2. Second-
 7 degree polynomial models provided the best fit for the absolute and relative liver weight data for
 8 female mice, as determined by the AIC, and yielded BMCL_{1sd}s of 33.51 ppm and 33.90 ppm,
 9 respectively (Table C-2). See Figures C-7 and C-8 for a plot of observed and predicted values
 10 1sd and 0.5sd, respectively, for absolute liver weight. See Tables C-3 and C-4 for model outputs
 11 for the second-degree polynomial models for 1sd and 0.5sd extra risk for absolute liver weight.
 12 See Figures C-9 and C-10 for a plot of the observed and predicted values for the second-degree
 13 polynomial model for 1sd and 0.5sd extra risk for relative liver weight. Model outputs are
 14 displayed in Tables C-5 and C-6. The BMC_{0.5sd} and BMCL_{0.5sd} are presented in Table 5-14.
 15

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

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

16 ^aStatistical tests indicated that variances were not constant across exposure groups. Model
 17 results are for non-homogeneous variance, with the exception of the linear and third-degree
 18 polynomial models for liver-to-body weight ratio.

19 ^bModeled as a continuous variable using one standard deviation as the BMR.
 20



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

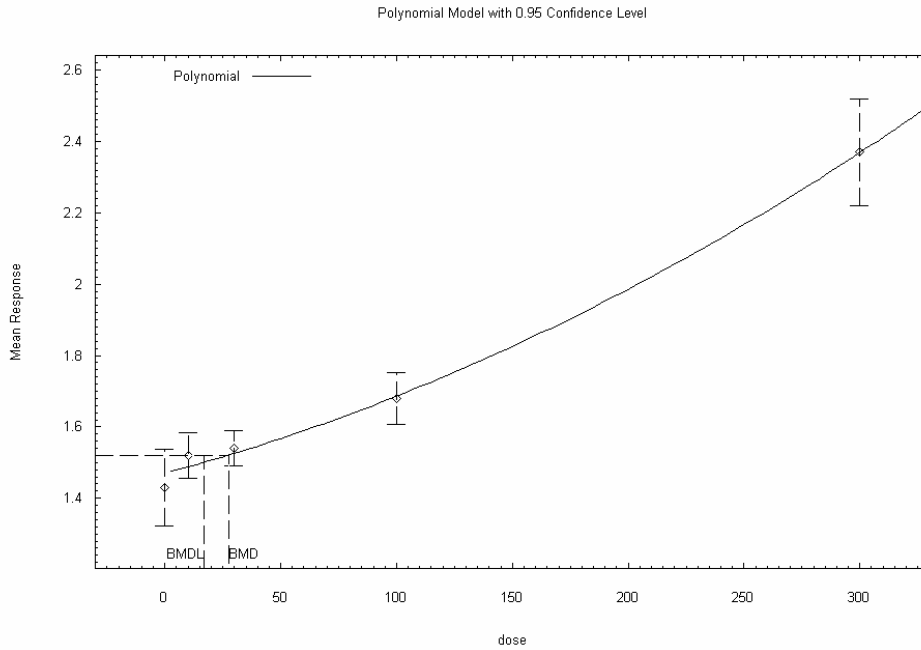
The second-degree polynomial model form of the response function for the female mouse absolute liver weight data is:

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

The variance was modeled as:

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

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

7

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

8

9

The form of the response function is:

10

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

11

12

13

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

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

14

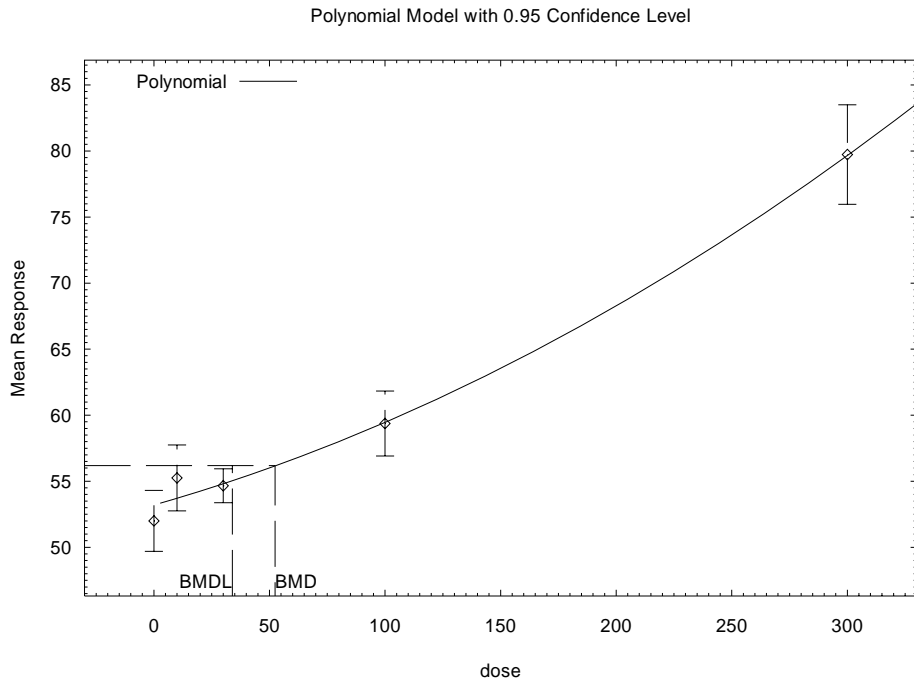
15

1

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

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

2
3
4



16:08 05/25 2006

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

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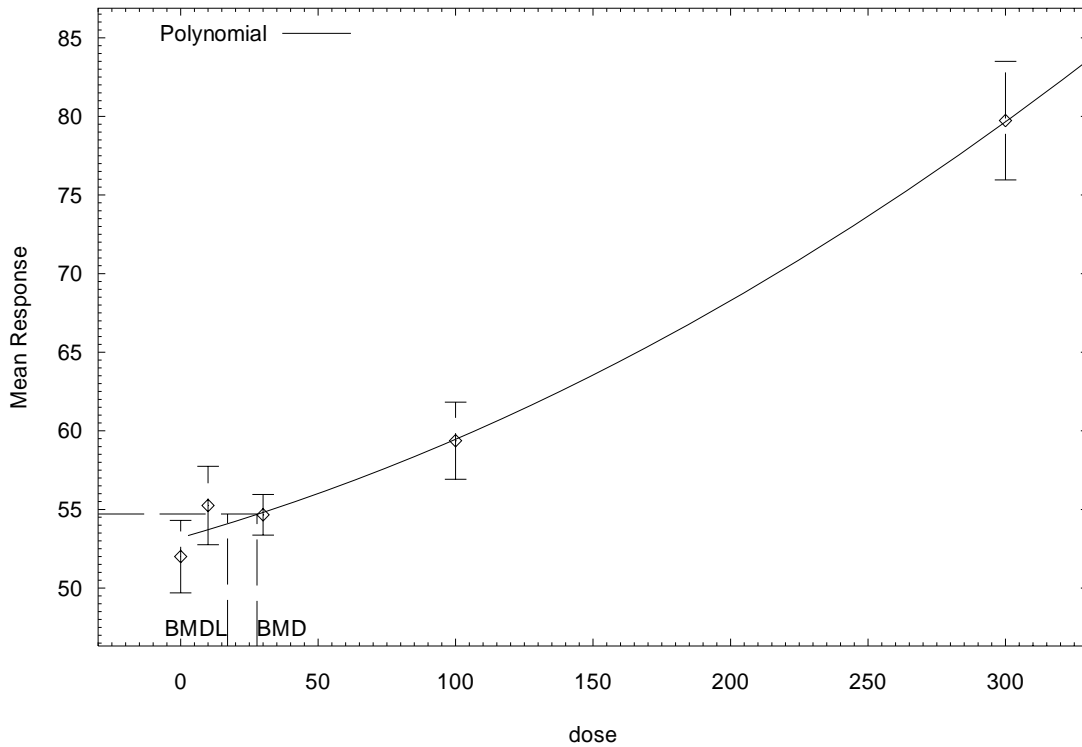
The second degree polynomial model form of the response function for the female mice relative liver weight is:

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

The variance was modeled as:

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

Polynomial Model with 0.95 Confidence Level



10:00 02/06 2006

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

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

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

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

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

13

1

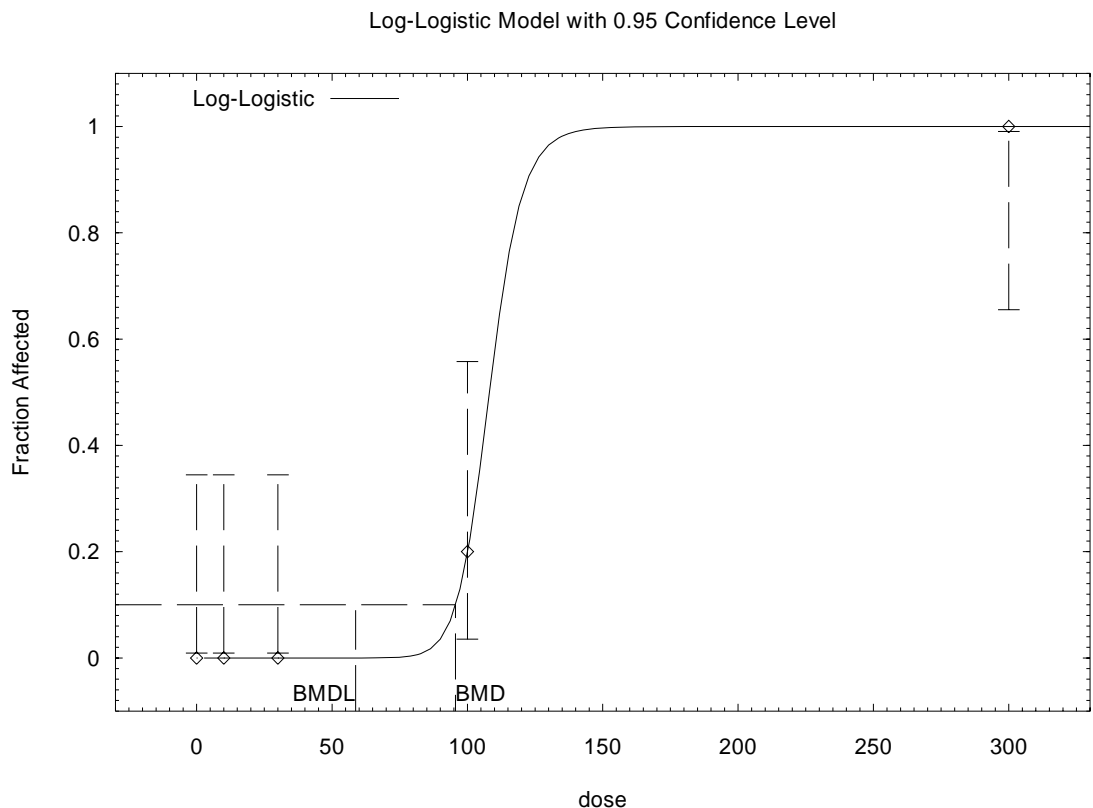
Table C-6. Second-degree polynomial model parameter estimates for 0.5 standard deviation extra risk in relative liver weight for female B6C3F1 mice with variance as a function of dose

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

2

3

1 The average BMCL₁₀ from the log-logistic and gamma models for cytomegaly in female
 2 mice was selected as the point of departure to derive the subchronic and chronic RfC for
 3 bromobenzene. Full modeling results for the log-logistic model appear after Figure C-11 and
 4 full modeling results for the gamma model appear after Figure C-12.



15:30 03/03 2006

6
7
8 **Figure C-11. Full modeling results for 10% extra risk for cytomegaly in the**
 9 **log-logistic model in female B6C3F1 mice treated by inhalation that were**
 10 **used to estimate the RfC**

11
12
13 The form of the probability function is:

14
15
$$P[\text{response}] = \text{background} + (1 - \text{background}) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))]$$
 (Eq. C-11)

16
17 Dependent variable = response

18 Independent variable = dose

19 Slope parameter is restricted as slope ≥ 1

20

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

6
7 User has chosen the log transformed model

8
9 Default Initial Parameter Values
10 background = 0
11 intercept = -8.09038
12 slope = 1.74428

13
14
15 Asymptotic Correlation Matrix of Parameter Estimates

16
17 (***) The model parameter(s) -background -slope have been estimated at a boundary
18 point, or have been specified by the user, and do not appear in the correlation matrix)

19
20 intercept
21
22 intercept 1

23
24
25 Parameter Estimates

Variable	Estimate	Std. Err.
background	0	NA
intercept	-84.2793	0.790565
slope	18	NA

31
32 NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
33 has no standard error.

34
35
36 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

42
43 AIC: 12.008

44

1 Goodness of Fit

2

3

	Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
4						
5	-----					
6	0.0000	0.0000	0.000	0	10	0
7	10.0000	0.0000	0.000	0	10	-1.581e-009
8	30.0000	0.0000	0.000	0	10	-3.112e-005
9	100.0000	0.2000	2.000	2	10	-2.199e-005
10	300.0000	1.0000	10.000	10	10	0.0003213

11

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

13

14

15 Benchmark Dose Computation

16

17 Specified effect = 0.1

18

19 Risk Type = Extra risk

20

21 Confidence level = 0.95

22

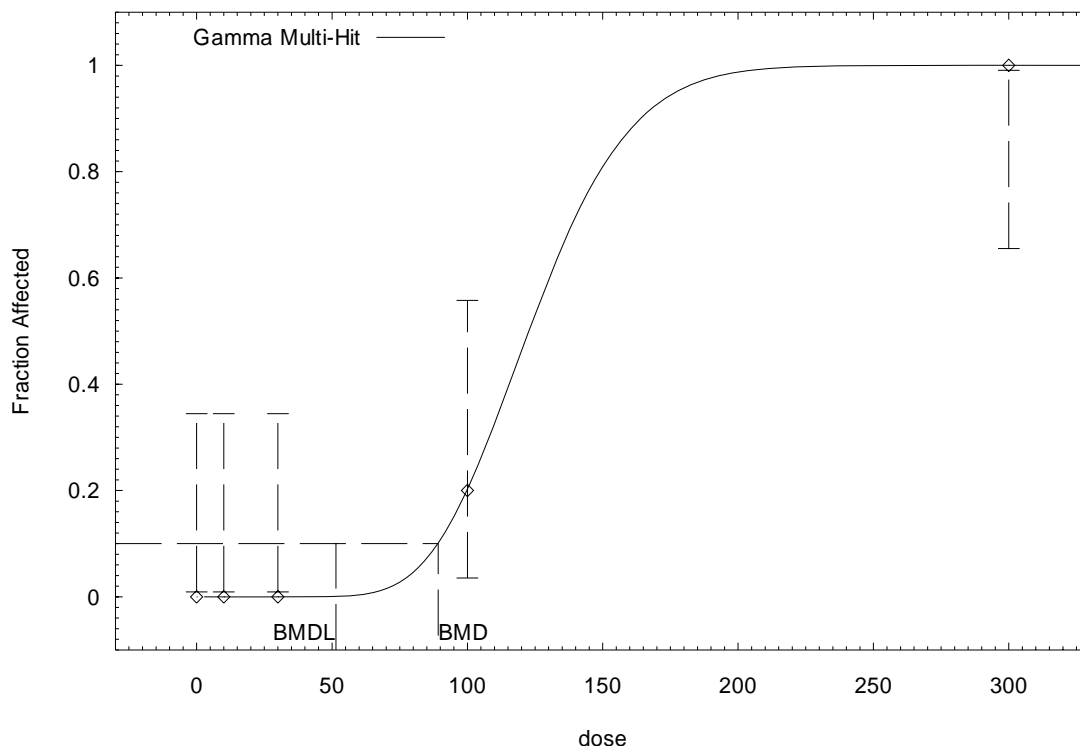
23 BMD = 95.5947

24

25 BMDL = 58.7312

26

Gamma Multi-Hit Model with 0.95 Confidence Level



14:43 03/03 2006

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

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * \text{CumGamma}[\text{slope} * \text{dose}, \text{power}] \quad (\text{Eq. C-12})$$

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

1 slope = 0.00531194
 2 power = 1.3
 3
 4

5 Asymptotic Correlation Matrix of Parameter Estimates

6
 7 (*** The model parameter(s) -Background -Power have been estimated at a boundary
 8 point, or have been specified by the user, and do not appear in the correlation matrix)
 9

10 Slope

11 Slope 1
 12
 13
 14

15 Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA
Slope	0.143677	0.0164918
Power	18	NA

16
 17
 18
 19
 20
 21
 22 NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
 23 has no standard error.
 24
 25

26 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

27
 28
 29
 30
 31
 32
 33 AIC: 12.0082
 34
 35

36 Goodness of Fit

Dose	Est._Prob.	Expected	Scaled		Residual
			Observed	Size	
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		

1
2
3
4
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11
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14

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 89.2392
BMDL = 51.4215