

DRAFT - DO NOT CITE OR QUOTE

EPA/635/R-07/002

[www.epa.gov/iris](http://www.epa.gov/iris)



**TOXICOLOGICAL REVIEW**

**OF**

**BROMOBENZENE**

(CAS No. 108-86-1)

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

*June 2007*

**NOTICE**

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

U.S. Environmental Protection Agency  
Washington, DC

## **DISCLAIMER**

This document is a preliminary draft for review purposes only and does not constitute U.S. Environmental Protection Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

## CONTENTS—TOXICOLOGICAL REVIEW OF BROMOBENZENE (CAS No. 108-86-1)

LIST OF FIGURES .....	vi
LIST OF TABLES .....	vii
LIST OF ABBREVIATIONS AND ACRONYMS .....	x
FOREWORD .....	xi
AUTHORS, CONTRIBUTORS, AND REVIEWERS .....	xii
1. INTRODUCTION .....	1
2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS .....	3
3. TOXICOKINETICS RELEVANT TO ASSESSMENTS .....	6
3.1. ABSORPTION .....	6
3.2. DISTRIBUTION.....	6
3.3. METABOLISM.....	7
3.4. ELIMINATION .....	11
3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS (PBTK).....	12
4. HAZARD IDENTIFICATION.....	13
4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS .....	13
4.2. LESS-THAN-LIFETIME AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION .....	13
4.2.1. Oral Exposure .....	13
4.2.1.1. Subchronic Toxicity .....	13
4.2.1.2. Chronic Toxicity.....	23
4.2.2. Inhalation Exposure .....	24
4.2.2.1. Subchronic Toxicity .....	24
4.2.2.2. Chronic Toxicity.....	31
4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION.....	31
4.3.1. Reproductive Toxicity Studies.....	31
4.3.2. Developmental Toxicity Studies.....	31
4.4. OTHER STUDIES.....	31
4.4.1. Acute Toxicity Studies.....	31
4.4.2. Genotoxicity Studies.....	32
4.4.3. Tumor Promotion Studies .....	35
4.5. MECHANISTIC STUDIES.....	35
4.5.1. Mechanistic Studies of Liver Effects.....	35
4.5.2. Mechanistic Studies of Kidney Effects.....	41
4.5.3. Genomic/Proteomic Responses of the Liver to Bromobenzene .....	42
4.5.4. Similarities Between Bromobenzene and Chlorobenzene.....	43
4.6. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS .....	48
4.6.1. Oral .....	48
4.6.2. Inhalation .....	48

4.6.3. Mode of Action Information.....	49
4.7. EVALUATION OF CARCINOGENICITY.....	51
4.8. SUSCEPTIBLE POPULATIONS .....	51
4.8.1. Possible Childhood Susceptibility .....	51
4.8.2. Possible Gender Differences.....	51
4.8.3. Other .....	52
5. DOSE-RESPONSE ASSESSMENTS .....	53
5.1. ORAL REFERENCE DOSE .....	53
5.1.1. Subchronic Oral RfD .....	53
5.1.1.1. Choice of Principal Study and Critical Effect - with Rationale and Justification .....	53
5.1.1.2. Methods of Analysis - Including Models (PBPK, BMD, etc.).....	54
5.1.1.3. Subchronic RfD Derivation - Including Application of Uncertainty Factors (UFs).....	59
5.1.2. Chronic Oral RfD.....	60
5.1.2.1. Choice of Principal Study and Critical Effect - with Rationale and Justification .....	60
5.1.2.2. Methods of Analysis - Including Models (PBPK, BMD, etc.).....	60
5.1.2.3. Chronic RfD Derivation - Including Application of Uncertainty Factors (UFs).....	60
5.1.3. Previous Oral Assessment.....	62
5.2. INHALATION REFERENCE CONCENTRATION (RfC) .....	62
5.2.1. Subchronic Inhalation RfC .....	62
5.2.1.1. Choice of Principal Study and Critical Effect - with Rationale and Justification.....	62
5.2.1.2. Methods of Analysis - Including Model (PBPK, BMD, etc.) .....	64
5.2.1.3. Subchronic RfC Derivation - Including Application of Uncertainty Factors (UFs).....	68
5.2.2. Chronic Inhalation RfC.....	69
5.2.2.1. Choice of Principal Study and Critical Effect - with Rationale and Justification.....	69
5.2.2.2. Methods of Analysis - Including Model (PBPK, BMD, etc.) .....	70
5.2.2.3. Chronic RfC Derivation - Including Application of Uncertainty Factors (UFs).....	70
5.3. CANCER ASSESSMENT.....	71
6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE.....	72
6.1. HUMAN HAZARD POTENTIAL.....	72
6.2. DOSE RESPONSE .....	73
6.2.1. Noncancer/Oral.....	73
6.2.2. Noncancer/Inhalation.....	73
6.2.3. Cancer/Oral.....	74
6.2.4. Cancer/Inhalation.....	74

7. REFERENCES .....75

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

APPENDIX B: BENCHMARK DOSE CALCULATIONS FOR THE RfD.....B-1

APPENDIX C. BENCHMARK DOSE CALCULATIONS FOR THE RfC .....C-1

## LIST OF FIGURES

2-1	Chemical structure of bromobenzene .....	3
3-1	Proposed metabolic scheme for bromobenzene in mammals .....	8
4-1	Chemical structure of bromobenzene and chlorobenzene .....	43

**LIST OF TABLES**

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) .....15

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.....17

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) .....21

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.....22

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) .....25

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 .....26

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) .....29

4-8 Incidences of male and female B6C3F1 mice with liver and kidney lesions following repeated exposure to bromobenzene vapors for 13 weeks .....30

4-9 Results of bromobenzene genotoxicity testing .....33

4-10 The effect of CYP inhibition on the hepatotoxicity and metabolism of single intraperitoneal doses of bromobenzene .....36

4-11 The influence of various treatments on the metabolism of bromobenzene (BB) and severity of bromobenzene-induced hepatic necrosis in rats administered single intraperitoneal doses of bromobenzene .....39

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 .....46

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 .....	46
5-1	Incidences of male and female Fischer 344/N rats and B6C3F1 mice with liver lesions following administration of bromobenzene by gavage 5 days/week for 90 Days .....	55
5-2	Benchmark doses (BMD <sub>10S</sub> and BMDL <sub>10S</sub> ) from best fitting models predicting combined incidences of Fischer 344/N rats or B6C3F1 mice with liver lesions.....	55
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.....	55
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).....	56
5-5	Benchmark doses (BMD <sub>10</sub> and BMDL <sub>10</sub> ) 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.....	57
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.....	57
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) .....	58
5-8	The power model estimated BMD and BMDLs associated with 10% extra risk for increase SDH serum levels in B6C3F1 female mice exposed to bromobenzene by gavage 5 days/week for 90 days .....	58
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 .....	64
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 .....	66
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 .....	66
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) .....	67



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 .....67

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.....68

## 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	$\gamma$ -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](mailto:hotline.iris@epa.gov) (email address).

## **AUTHORS, CONTRIBUTORS, AND REVIEWERS**

### **CHEMICAL MANAGER**

Carolyn L. Smallwood, Environmental Scientist  
National Center for Environmental Assessment  
U.S. Environmental Protection Agency  
Cincinnati, OH

### **AUTHORS**

Carolyn L. Smallwood, Environmental Scientist  
National Center for Environmental Assessment  
U.S. Environmental Protection Agency  
Cincinnati, OH

David W. Wohlers, Ph.D.  
Peter R. McClure, Ph.D., DABT  
Daniel J. Plewak, B.S.  
Environmental Science Center  
Syracuse Research Corporation  
Syracuse, NY

Under Contract No. 68-C-00-122 with Battelle Memorial Institute and EPA Contract No. 68-C-03-147 to Syracuse Research Corporation

### **REVIEWERS**

This document and the accompanying IRIS Summary have been peer reviewed by EPA scientists and independent scientists external to EPA. Comments from all peer reviewers were evaluated carefully and considered by the Agency during the finalization of this assessment. During the finalization process, the IRIS Program Director achieved common understanding of the assessment among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and EPA's regional offices.

### **INTERNAL EPA REVIEWERS**

Stiven Foster  
National Center for Environmental Assessment  
Washington, DC

John Fox  
National Center for Environmental Assessment  
Washington, DC

Martin Gehlhaus  
National Center for Environmental Assessment  
Washington, DC

Steven S. Kueberuwa  
Health and Ecological Criteria Division  
Office of Science and Technology, Office of Water  
Washington, DC

Allan Marcus  
National Center for Environmental Assessment  
Research Triangle Park, NC

Chandrika Moudgal  
National Center for Environmental Assessment  
Cincinnati, OH

Sederick Rice  
National Center for Environmental Assessment  
Washington, DC

Jeff Swartout  
National Center for Environmental Assessment  
Cincinnati, OH

# 1. INTRODUCTION

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

The RfD and RfC provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m<sup>3</sup>) 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/m<sup>3</sup> 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.

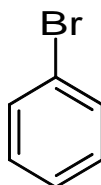
1 EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines*  
2 *for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Guidelines for Carcinogen Risk*  
3 *Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-*  
4 *Life 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 <sub>6</sub> H <sub>5</sub> 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 <sup>2</sup> 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 <sub>ow</sub> = 2.99 (Hansch et al., 1995)
Flash point:	51°C (Budavari, 2001)
Heat of combustion:	-1.98x10 <sup>7</sup> 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 <sub>oc</sub> = 150
Air pollution factors:	1 mg/m <sup>3</sup> = 0.15 ppm, 1 ppm = 6.53 mg/m <sup>3</sup> (Verschueren, 2001)
Henry's Law constant:	2.47x10 <sup>-3</sup> atm m <sup>3</sup> /mol at 25°C (Shiu and Mackay, 1997)
OH reaction rate constant:	7.70x10 <sup>13</sup> cm <sup>3</sup> /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 ( $4.5 \times 10^3$  kg) in 1986,  
2 1990, 1994, 1998, and 2002 (U.S. EPA, 2002). U.S. imports of bromobenzene were  $2.00 \times 10^3$  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  $2.47 \times 10^{-3}$  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).

### 3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

#### 3.1. ABSORPTION

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

#### 3.2. DISTRIBUTION

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

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

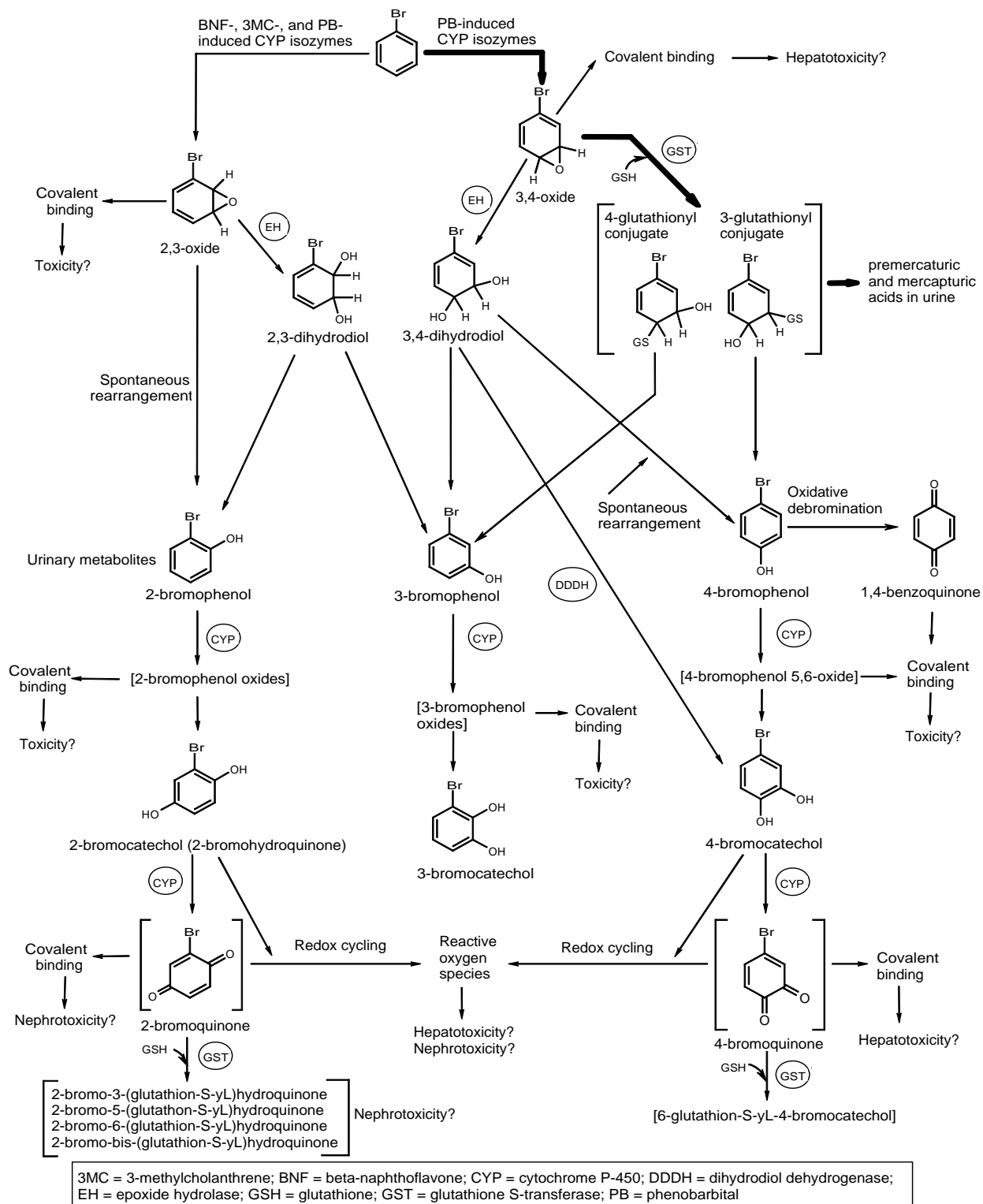
1 In another study, concentrations of bromobenzene in tissues from rats 10 hours after  
2 intraperitoneal injection of 5 mg of bromobenzene were highest in adipose tissue (3.38 µg/g),  
3 followed by liver (0.18 µg/g), seminal fluid (0.15 µg/g), blood (0.12 µg/g), brain (0.08 µg/g), and  
4 pectoral 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 <sup>14</sup>C-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 <sup>14</sup>C-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



1  
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 <sup>14</sup>C-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 <sup>14</sup>C-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  $\gamma$ -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  $^{14}\text{C}$ -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  $^{14}\text{C}$ -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 <sup>14</sup>C-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](mailto: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  $\geq 100$  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 <sup>a</sup>
Body weight (g)	343.0 ± 12.9	330.3 ± 12.2	342.3 ± 18.5	331.3 ± 20.0	293.0 <sup>b</sup> ± 11.9	203.1 <sup>c</sup>
Liver weight (g)	9.16 ± 0.66	Not available	10.64 <sup>b</sup> ± 0.76	11.29 <sup>b</sup> ± 0.69	11.87 <sup>b</sup> ± 0.80	10.50
Difference (%) <sup>d</sup>	--		+16.2	+23.3	+29.6	+14.6
Ratio liver/body weight	26.72 ± 1.88	Not available	31.08 <sup>b</sup> ± 1.18	34.10 <sup>b</sup> ± 0.68	40.56 <sup>b</sup> ± 3.16	51.70 <sup>c</sup>
Difference (%) <sup>d</sup>	--		+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 <sup>b</sup> ± 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 <sup>b</sup> ± 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 <sup>b</sup> ± 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 <sup>a</sup>
Body weight (g)	192.8 ± 9.0	197.1 ± 11.9	193.5 ± 9.1	187.6 ± 8.2	182.3 <sup>b</sup> ± 10.5	167.4 <sup>b</sup> ± 9.8
Liver weight (g)	4.68 ± 0.35	5.23 <sup>b</sup> ± 0.37	5.55 <sup>b</sup> ± 0.36	6.28 <sup>b</sup> ± 0.40	7.85 <sup>b</sup> ± 0.49	9.11 <sup>b</sup> ± 0.57
Difference (%) <sup>d</sup>	--	+11.6	+18.6	+34.2	+67.7	+94.7
Ratio liver/body weight	24.25 ± 1.13	26.55 <sup>b</sup> ± 1.23	28.69 <sup>b</sup> ± 1.20	33.48 <sup>b</sup> ± 1.37	43.11 <sup>b</sup> ± 2.38	54.78 <sup>b</sup> ± 6.64
Difference (%) <sup>d</sup>	--	+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 <sup>b</sup> ± 1.47	3.78 ± 0.98	61.60 ± 143.07	23.00 ± 17.00

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

4 <sup>b</sup>Statistically significantly increased from controls ( $p < 0.05$ ) based on student's two-tailed t-test

5 <sup>c</sup>Outside 3 standard deviations from the control mean

6 <sup>d</sup>Change 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  $\geq 400$  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  $\geq 200$  mg/kg-day in males  
16 and  $\geq 400$  mg/kg-day in females), inflammation (doses  $\geq 200$  mg/kg-day in males), and necrosis  
17 (doses  $\geq 400$  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) <sup>a</sup>											
	0		50		100		200		400		600 <sup>b</sup>	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
<b>Males</b>												
Liver, centrilobular												
Inflammation	2/10	1.0	2/10	1.0	2/10	1.0	7/10 <sup>d</sup>	1.6	9/10 <sup>d</sup>	2.1	7/10 <sup>d</sup>	2.1
Cytomegaly	0/10		0/10		0/10		4/10 <sup>d</sup>	1.5	10/10 <sup>d</sup>	2.0	9/10 <sup>d</sup>	2.4
Necrosis	0/10		0/10		0/10		3/10	1.3	9/10 <sup>d</sup>	2.0	9/10 <sup>d</sup>	2.4
Mineralization	0/10		0/10		0/10		0/10		0/10		2/10	2.5
Combined <sup>c</sup>	2/10		2/10		2/10		7/10 <sup>d</sup>		10/10 <sup>d</sup>		10/10 <sup>d</sup>	
Kidney, tubule												
Necrosis	0/10		0/10		0/10	2.0	0/10		0/10		6/10 <sup>d</sup>	2.2
Degeneration	2/10	1.0	1/10	1.0	2/10		4/10	1.0	1/10	2.0	7/10 <sup>d</sup>	2.6
Casts	0/10		0/10		0/10		1/10	1.0	3/10	2.0	7/10 <sup>d</sup>	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 <sup>d</sup>	1.9	0/10	
<b>Females</b>												
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 <sup>d</sup>	2.4	10/10 <sup>d</sup>	2.6
Necrosis	0/10		0/10		0/10		0/10		7/10 <sup>d</sup>	2.0	9/10 <sup>d</sup>	2.7
Mineralization	0/10		0/10		0/10		0/10		0/10		1/10	3.0
Combined <sup>c</sup>	2/10		2/10		4/10		5/10		10/10 <sup>d</sup>		10/10 <sup>d</sup>	
Kidney, tubule												
Necrosis	0/10		0/10		0/10		0/10		0/10		6/10 <sup>d</sup>	2.3
Degeneration	0/10		0/10		0/10		0/10		1/10	2.0	8/10 <sup>d</sup>	3.0
Casts	0/10		0/10		0/10		0/10		0/10		6/10 <sup>d</sup>	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 <sup>d</sup>	2.1	2/10	2.0

<sup>a</sup>Incidence = number of animals in which lesion was found/number of animals in which organ was examined.

<sup>b</sup>Most male and female rats of the 600 mg/kg-day dose level died during the study, which may have affected incidences of selected lesions.

<sup>c</sup>Incidences of rats with one or more of the liver lesion types (cytomegaly, necrosis, inflammation, mineralization), extracted from individual animal histopathologic results provided to Syracuse Research Corporation by NTP.

<sup>d</sup>Statistically significantly different from control groups according to Fisher's exact test ( $p < 0.05$ ), performed by Syracuse Research Corporation.

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

Source: NTP (1985a)

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  $\geq 200$  mg/kg-day in male  
34 rats and at doses  $\geq 400$  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  $\geq 200$  mg/kg-day, while the liver:body weight ratio was significantly increased at dose  
7 levels  $\geq 100$  mg/kg-day and the liver:brain weight ratio was significantly increased at dose levels  
8  $\geq 400$  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  $\geq 200$  mg/kg-day, relative to sex-  
13 matched controls, but the magnitude only approached a 2-fold increase (a biologically significant  
14 level) at  $\geq 200$  mg/kg-day in males and  $\geq 400$  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  $\geq 200$  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 <sup>a</sup> ± 2.0	30.5 ± 2.5
Liver weight (g)	1.05 ± 0.14	1.13 ± 0.15	1.12 ± 0.12	1.25 <sup>a</sup> ± 0.22	1.27 <sup>a</sup> ± 0.11	1.56 <sup>a</sup> ± 0.16
Difference (%) <sup>b</sup>	--	+7.6	+6.7	+19.1	+21.0	+48.6
Ratio liver/body weight	33.4 ± 2.41	33.9 ± 3.52	36.0 <sup>a</sup> ± 1.91	37.3 <sup>a</sup> ± 4.48	45.3 <sup>a</sup> ± 1.83	51.2 <sup>a</sup> ± 3.48
Difference (%) <sup>b</sup>	--	+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 <sup>a</sup> ± 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 <sup>a</sup> ± 19.3	89 <sup>a</sup> ± 28.3	101 <sup>a</sup> ± 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 <sup>a</sup> ± 1.0	23.4 ± 0.6	23.6 ± 0.8
Liver weight (g)	0.86 ± 0.06	0.96 <sup>a</sup> ± 0.08	1.01 <sup>a</sup> ± 0.08	1.08 <sup>a</sup> ± 0.06	1.12 <sup>a</sup> ± 0.07	1.30 <sup>a</sup> ± 0.06
Difference (%) <sup>b</sup>	--	+11.6	+17.4	+25.6	+30.2	+51.2
Ratio liver/body weight	38.1 ± 1.42	40.2 <sup>a</sup> ± 2.02	42.5 <sup>a</sup> ± 1.62	44.4 <sup>a</sup> ± 2.12	48.0 <sup>a</sup> ± 2.13	55.2 <sup>a</sup> ± 2.56
Difference (%) <sup>b</sup>	--	+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 <sup>a</sup> ± 1.7	23 <sup>a</sup> ± 4.6	43 <sup>a</sup> ± 18.8

<sup>a</sup>Statistically significantly increased from controls ( $p < 0.05$ ) based on Student's two-tailed t-test

<sup>b</sup>Change relative to controls

Source: NTP (1985b)

2  
3  
4  
5

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

<sup>a</sup>Incidence = number of animals in which lesion was found/number of animals in which organ was examined.

<sup>b</sup>Cytomegaly and mineralization were not diagnosed in 5 high-dose male mice that died on treatment day 1

<sup>c</sup>Incidences of mice with one or more of the liver lesion types (cytomegaly, necrosis, inflammation, mineralization), extracted from individual animal histopathologic results provided to Syracuse Research Corporation by NTP.

<sup>d</sup>Statistically significantly different from control groups according to Fisher's exact test ( $p < 0.05$ ), performed by Syracuse Research Corporation.

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

NE = Not examined.

Source: NTP (1985b)

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  $\geq 200$  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/m<sup>3</sup>) 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  $\geq 100$  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  $\geq 10$  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)**

<b>Male rats</b>					
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 <sup>b</sup> ± 1.59	14.33 <sup>c</sup> ± 1.67
Difference (%) <sup>a</sup>		+ 4%	+ 5%	+ 13%	+ 20%
Ratio liver/body weight x 1000	33.37 ± 2.86	37.31 ± 1.96	36.68 ± 2.05	42.11 <sup>c</sup> ± 2.09	46.26 <sup>c</sup> ± 3.86
Difference (%) <sup>a</sup>		+ 10.5%	+ 9%	+ 21%	+ 28%
Right kidney weight	0.98 ± 0.06	1.04 ± 0.05	1.87 ± 0.05	1.07 <sup>b</sup> ± 0.11	1.11 <sup>c</sup> ± 0.09
Ratio right kidney/body weight x 1000	3.09 ± 0.06	3.22 ± 0.17	3.16 ± 0.16	3.43 <sup>c</sup> ± 0.19	3.60 <sup>c</sup> ± 0.11
Difference (%) <sup>a</sup>		+ 4%	+ 2%	+ 10%	+ 14%
<b>Female rats</b>					
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 <sup>c</sup> ± 0.30	8.22 <sup>c</sup> ± 0.63
Difference (%) <sup>a</sup>		+ 7%	+ 4%	+ 12%	+ 23%
Ratio liver/body weight x 1000	34.12 ± 1.83	35.05 ± 1.82	35.68 ± 2.84	38.56 <sup>c</sup> ± 1.62	43.54 <sup>c</sup> ± 2.53
Difference (%) <sup>a</sup>		+ 3%	+ 4%	12%	22%
Right kidney weight	0.62 ± 0.05	0.65 ± 0.03	0.66 ± 0.06	0.66 <sup>b</sup> ± 0.03	0.70 <sup>c</sup> ± 0.05
Ratio kidney/body weight x 1000	3.31 ± 0.21	3.39 ± 0.09	3.62 <sup>b</sup> ± 0.26	3.53 <sup>b</sup> ± 0.18	3.73 <sup>c</sup> ± 0.16
Difference (%) <sup>a</sup>		+ 2%	+ 9%	+ 6%	+ 11%

2 <sup>a</sup>Change relative to controls

3 <sup>b</sup>Statistically significantly different from controls ( $p < 0.05$ ) based on student's two-tailed t-test

4 <sup>c</sup>Outside 3 standard deviations from the control mean

5 Source: NTP (1985d)

1

**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
<b>Males</b>										
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
<b>Females</b>										
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)

6  
 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/m<sup>3</sup>) 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)**

<b>Male mice</b>					
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 (%) <sup>a</sup>		-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 <sup>b</sup> ± 2.42	
Difference (%) <sup>a</sup>		+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 <sup>b</sup> ± 0.75	8.78 ± 0.90	
Difference (%) <sup>a</sup>		+8.7	+9.2	+8.0	
<b>Female mice</b>					
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 <sup>c</sup> ± 1.7
Liver weight (g)	1.43 ± 0.15	1.52 ± 0.09	1.54 <sup>b</sup> ± 0.07	1.68 <sup>c</sup> ± 0.10	2.37 <sup>c</sup> ± 0.21
Difference (%) <sup>a</sup>		+6.3	+7.7	+17.5	+65.7
Ratio liver/body weight x 1000	52.0 ± 3.22	55.25 <sup>b</sup> ± 3.49	54.66 <sup>b</sup> ± 1.80	59.37 <sup>c</sup> ± 3.43	79.73 <sup>c</sup> ± 5.27
Difference (%) <sup>a</sup>		+6.3	+5.1	+14.2	+53.3
Right kidney weight	0.19 ± 0.01	0.20 <sup>c</sup> ± 0.01	0.20 ± 0.02	0.20 <sup>c</sup> ± 0.01	0.23 <sup>c</sup> ± 0.02
Ratio kidney/body weight x 1000	6.80 ± 0.28	7.38 <sup>c</sup> ± 0.25	7.04 ± 0.51	7.14 ± 0.32	7.64 ± 0.45
Difference (%) <sup>a</sup>		+8.5	+3.5	+5.0	+12.4

<sup>a</sup>Change relative to controls

<sup>b</sup>Statistically significantly different from controls ( $p < 0.05$ ) based on Student's two-tailed t-test

<sup>c</sup>Outside 3 standard deviations from the control mean

Source: NTP (1985d)

2  
3  
4  
5  
6

**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 <sup>a</sup>									
	0		10		30		100		300	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
<b>Males</b>										
Liver										
Cytomegaly <sup>b</sup>	0/10		0/10		4/10 <sup>c</sup>	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 <sup>d</sup>	0/10		0/10		2/10	1.5	3/10	2.0	NG	
<b>Females</b>										
Liver										
Cytomegaly	0/10		0/10		0/10		2/10	1.0	10/10 <sup>c</sup>	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 <sup>e</sup>	0/10		0/10		0/10		0/10		2/10	2.0
Kidney <sup>f</sup>										

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

<sup>b</sup> 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.

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

<sup>d</sup>Kidney tubular degeneration could not be distinguished from artifacts of fixation or staining.

<sup>e</sup>Mineralization was not reported in male mice.

<sup>f</sup>No 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 <sup>14</sup>C-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 <sup>14</sup>C-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  $\gamma$ -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  $\text{cm}^2$  of induced glutathione  
17 S-transferase placental form-positive (GST-P<sup>+</sup>) 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  $\text{cm}^2$  of DENA-induced GST-  
20 P<sup>+</sup> 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  $\beta$ -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	$2.8 \pm 0.3^*$	$26 \pm 3$
Bromobenzene (1500 mg/kg-day) + SKF 525A	No specific lesions	$14.4 \pm 0.5$	$149 \pm 8$

14 \*Mean  $\pm$  standard error from 5-7 rats/group; CYP = cytochrome P-450 isozymes; SKF 525 =  
15  $\beta$ -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  $\beta$  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  $\beta$ -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  $^{14}\text{C}$ -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  $^{14}\text{C}$ -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**

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

2 <sup>a</sup>Criteria 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 <sup>b</sup>Half-time of clearance of radioactivity from the whole body of rats administered

6 <sup>14</sup>C-bromobenzene.

7 <sup>c</sup>Significantly 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 <sup>14</sup>C-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 <sup>14</sup>C-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.

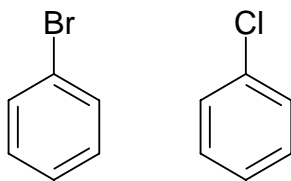
24 Heijne and coworkers (Stierum et al., 2005; Heijne et al., 2004, 2003) used these  
25 techniques to identify changes in gene expression in the rodent liver in response to  
26 bromobenzene. As previously discussed, bromobenzene undergoes CYP-mediated epoxidation  
27 to form the electrophilic 3,4-epoxide, 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  $\gamma$ -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 F<sub>0</sub> and F<sub>1</sub> male rats at exposure levels  $\geq 150$  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 <sup>a</sup>
<b>Males</b>					
Liver					
Necrosis	0/10	0/10	2/10	3/10	7/10 <sup>b</sup>
Degeneration	0/10	0/10	0/10	2/10	1/10
Kidney					
Nephropathy	0/10	0/10	1/10	2/10	2/10
<b>Females</b>					
Liver					
Necrosis	0/10	0/10	1/10	1/10	6/10 <sup>b</sup>
Degeneration	0/10	0/10	0/10	0/10	4/10 <sup>b</sup>
Kidney					
Nephropathy	0/10	0/10	0/10	0/10	7/10 <sup>b</sup>

2 <sup>a</sup>Significantly 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 <sup>b</sup>Statistically 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 <sup>a</sup>	750 <sup>a</sup>
<b>Males</b>						
Liver						
Necrosis	0/10	1/10	1/10	7/10 <sup>b</sup>	10/10 <sup>b</sup>	10/10 <sup>b</sup>
Degeneration	0/10	0/10	0/10	2/10	0/10	0/10
Kidney						
Nephropathy	0/10	NE	0/10	4/10 <sup>b</sup>	9/10 <sup>b</sup>	8/10 <sup>b</sup>
<b>Females</b>						
Liver						
Necrosis	0/10	0/10	0/10	10/10 <sup>b</sup>	8/10 <sup>b</sup>	1/10
Degeneration	0/10	0/10	0/10	0/10	9/10 <sup>b</sup>	4/10 <sup>b</sup>
Kidney						
Nephropathy	0/10	NE	0/10	4/10 <sup>b</sup>	0/10	0/10

8 <sup>a</sup>Significantly 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 <sup>b</sup>Statistically 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  $\geq 200$  mg/kg-day resulted in  
17 significantly increased incidences of histopathologic liver lesions in male and female rats and  
18 male mice ( $\geq 400$  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 ( $\geq 785$  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  $\geq 100$  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  $\geq 30$  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 ( $\geq 785$  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  $\beta$ -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  $^{14}\text{C}$ -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  $\gamma$ -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  $\geq 200$  mg/kg-day in male and female mice and male rats ( $\geq 400$  mg/kg-day in female rats). Significantly increased mean liver weights were observed at doses as low as 50 mg/kg-day in female rats and mice and 100 mg/kg-day in male rats and mice.

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

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

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

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 BMDL<sub>10</sub> (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  $\geq 200$   
35 mg/kg-day, while the liver-to-body weight ratio was significantly increased at dose levels  $\geq 100$   
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<sup>a</sup> 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 <sup>b</sup>	10/10 <sup>b</sup>	10/10 <sup>b</sup>
Female rats	2/10	2/10	4/10	5/10	10/10 <sup>b</sup>	10/10 <sup>b</sup>
Male mice	1/10	0/10	2/10	6/10 <sup>b</sup>	10/10 <sup>b</sup>	10/10 <sup>b</sup>
Female mice	0/10	1/10	2/10	6/10 <sup>b</sup>	9/10 <sup>b</sup>	10/10 <sup>b</sup>

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

<sup>b</sup>Statistically different from control groups according to Fisher's exact test ( $p < 0.05$ ), performed by Syracuse Research Corporation.

Source: NTP (1985a,b)

2

3

4

5

6

7

8

9

10

**Table 5-2. Benchmark doses (BMD<sub>10</sub>s and BMDL<sub>10</sub>s) from best fitting models predicting combined incidences of Fischer 344/N rats or B6C3F1 mice with liver lesions (see Appendix B)**

Data set	Model	BMD <sub>10</sub> s and BMDL <sub>10</sub> s (mg/kg-day)		Fit statistics	
		BMD <sub>10</sub>	BMDL <sub>10</sub>	$\chi^2$ 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 <sub>10</sub>	BMDL <sub>10</sub>	BMD <sub>05</sub>	BMDL <sub>05</sub>	BMD <sub>01</sub>	BMDL <sub>01</sub>
56.1	24.8	36.0	12.7	13.2	2.8

Source: NTP (1985b)

13

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)**

<b>Dose (mg/kg-day)</b>						
	<b>0</b>	<b>50</b>	<b>100</b>	<b>200</b>	<b>400</b>	<b>600</b>
<b>Absolute liver weight (grams)</b>						
<b>Rats</b>						
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	--
<b>Mice</b>						
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
<b>Liver-to-Body Weight Ratio (relative liver weight)</b>						
<b>Rats</b>						
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	--
<b>Mice</b>						
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<sub>1sd</sub> 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 (BMD<sub>10</sub> and BMDL<sub>10</sub>) 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 <sub>1sd</sub> and BMDL <sub>1sd</sub> (mg/kg-day)		Fit-statistics	
		BMD <sub>1sd</sub>	BMDL <sub>1sd</sub>	X <sup>2</sup> p-value	AIC
<b>Absolute liver weight (grams)</b>					
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
<b>Liver-to-body weight ratio (relative liver weight)</b>					
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 <sub>1sd</sub>	BMDL <sub>1sd</sub>	BMD <sub>0.5sd</sub>	BMDL <sub>0.5sd</sub>
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  $\geq 200$  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	
			$\chi^2$ 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<sub>1sd</sub> from the best fitting model for liver weight changes was 25.8 mg/kg-day, which  
 20 was very similar to the lowest BMDL<sub>10</sub> 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.

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

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

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

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

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

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



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  $\text{BMDL}_{10}$  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<sub>D</sub>). 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  $\geq 100$   
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 98<sup>th</sup> percentile (or below the 2<sup>nd</sup>  
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 <sub>10</sub> (ppm)	BMCL <sub>10</sub> (ppm)	$\chi^2$ <i>p</i> -value	AIC
Log-logistic <sup>a</sup>	95.59	<b>58.73</b>	1.00	12.01
Gamma <sup>b</sup>	89.24	<b>51.42</b>	1.00	12.01
Multi-stage <sup>c</sup>	77.09	40.33	0.999	12.17
Weibull <sup>b</sup>	92.34	47.08	1.00	14.01
Log-probit <sup>a</sup>	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 <sup>a</sup>Slope restricted to >13 <sup>b</sup>Restrict power > = 14 <sup>c</sup>Restrict 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 <sub>10</sub>	BMCL <sub>10</sub>	BMC <sub>05</sub>	BMCL <sub>05</sub>	BMC <sub>01</sub>	BMCL <sub>01</sub>
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 <sup>a</sup> ± 0.07	1.68 <sup>a</sup> ± 0.10	2.37 <sup>b</sup> ± 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 <sup>a</sup> ± 2.42	--
Female	52.00 ± 3.22	55.25 <sup>a</sup> ± 3.49	54.66 <sup>a</sup> ± 1.80	59.37 <sup>b</sup> ± 3.43	79.73 <sup>b</sup> ± 5.27

2 <sup>a</sup>Statistically significantly different from controls ( $p < 0.05$ ) based on Student's two-tailed t-test

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

7 <sup>a</sup>Statistical 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 <sup>b</sup>Modeled 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 <sub>1sd</sub>	BMCL <sub>1sd</sub>	BMC <sub>0.5sd</sub>	BMCL <sub>0.5sd</sub>
Absolute liver weight	52.38	33.51	27.65	16.83
Liver-to-body weight ratio	52.42	33.90	27.76	17.08

Source: NTP (1985d)

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

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

Following U.S. EPA (1994b) methodology, the human equivalent concentration (HEC) for an extra respiratory effect produced by a category 3 gas, such as bromobenzene (not highly water soluble or reactive in the respiratory tract, the liver as the critical extrarespiratory target), is calculated by multiplying the duration-adjusted BMCL or NOAEL by the ratio of the blood:gas partition coefficients in animals and humans  $[(H_{b/g})_A / (H_{b/g})_H]$ . Because bromobenzene blood:gas partition coefficients are not available for humans or mice, a default value of 1 is used for this ratio. The BMCL<sub>10</sub> of 55 ppm for hepatocellular cytomegaly in female mice was converted to 353.2 mg/m<sup>3</sup> (55 ppm x MW[157] / 24.45 = 353.2 mg/m<sup>3</sup>), 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  $63 \text{ mg/m}^3$ . The  
3  $\text{BMCL}_{10\text{HEC}}$  of  $63 \text{ mg/m}^3$  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 ( $\text{UF}_A$ ). 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 ( $\text{UF}_H$ ). Although hepatotoxicity was observed only in  
15 female mice, a 300-ppm ( $1926 \text{ mg/m}^3$ ) 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 ( $\text{UF}_D$ ). 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 BMCL<sub>10</sub> 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 BMCL<sub>10</sub>  
15 of 55 ppm for hepatocellular cytomegaly in female mice was converted to a BMCL<sub>10HEC</sub> of 63  
16 mg/m<sup>3</sup> (see Section 5.2.1.3 for details regarding conversion to the HEC). The BMCL<sub>10HEC</sub> of 63  
17 mg/m<sup>3</sup> 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<sub>A</sub>). 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 [ $(H_{b/g})_A / H_{b/g})_H$ ], was applied to derive the BMCL<sub>10HEC</sub> 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<sub>H</sub>). Although hepatotoxicity was observed only in  
29 female mice, a 300-ppm (1926 mg/m<sup>3</sup>) 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<sub>D</sub>). 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 ( $\geq 785$  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/m<sup>3</sup> ( $55$  ppm  $\times$  MW[157] / 24.45 = 353.2 mg/m<sup>3</sup>), 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  $63 \text{ mg/m}^3$ . The  
3 subchronic RfC was derived by dividing the  $\text{BMCL}_{10\text{HEC}}$  of  $63 \text{ mg/m}^3$  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

## 7. REFERENCES

- 1  
2  
3  
4 Aarstad, K; Becker, R; Dahl, J; Dybing, E; Nilsen, OG. (1990) Short term inhalation of  
5 bromobenzene: Methodology and absorption characteristics in mouse, rat, and rabbit. *Pharmacol*  
6 *Toxicol* 67:284-287.
- 7 Aniya, Y; McLenithan, JC; Anders, MW. (1988) Isozyme selective arylation of cytosolic  
8 glutathion S-transferase by [14C]bromobenzene metabolites. *Biochem Pharmacol*  
9 37(2):251-257.
- 10 Atkinson, R. (1989) Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical  
11 with organic compounds. *J Phys Chem Ref Data*. Monograph 1.
- 12 Bambal, RB; Hanzlik, RP. (1995) Bromobenzene 3,4-oxide alkylates histidine and lysine side  
13 chains of rat liver proteins *in vivo*. *Chem Res Toxicol* 8(5):729-735.
- 14 Barber, LB; Thurman, EM; Schroeder, MP. (1988) Long-term fate of organic micropollutants in  
15 sewage-contaminated groundwater. *Environ Sci Technol* 22:205-211.
- 16 Becher, R; Dahl, JE; Aarstad, K; Nilsen, OG; Dybing, E. (1989) Lung and liver damage in mice  
17 after bromobenzene inhalation: effects of enzyme inducers. *Inhal Toxicol* 1:181-196.
- 18 Bidleman, TF. (1988) Atmospheric processes. Wet and dry deposition of organic compounds are  
19 controlled by their vapor-particle partitioning. *Environ Sci Technol* 22(4):361-367.
- 20 Brodie, BB; Reid, WD; Cho, AK; Sipes, G; Krishna, G; Gillette, JR. (1971) Possible mechanism  
21 of liver necrosis caused by aromatic organic compounds. *Proc Natl Acad Sci* 68:160-164.
- 22 Brodzinsky, R; Singh, HB. (1982) Volatile Organic Chemicals in the Atmosphere: An  
23 Assessment of Available Data. Prepared by the Environmental Sciences Research Laboratory,  
24 U.S. Environmental Protection Agency, Research Triangle Park, NC. EPA/600/S3-83-027.
- 25 Brondeau, MT; Bonnet, P; Guenier, JP; de Ceaurriz, J. (1983) Short-term inhalation test for  
26 evaluating industrial hepatotoxicants in rats. *Toxicol Lett* 19:139-146.
- 27 Brondeau, MT; Ban, M; Bonnet, P; Guenier, JP; de Ceaurriz, J. (1986) Concentration-related  
28 changes in blood and tissue parameters of hepatotoxicity and their interdependence in rats  
29 exposed to bromobenzene and 1,2-dichlorobenzene. *Toxicol Lett* 31:159-166.
- 30 Budavari, S. (2001) Bromobenzene. In: O'Neil, MJ; Smith, A; Heckelman, PE; et al., Eds. *The*  
31 *Merck Index*, 13<sup>th</sup> ed. Rahway, NJ: Merck & Co., Inc. pp. 234-235.
- 32 Casini, AF; Pompella, A; Comporti, M. (1984) Glutathione depletion, lipid peroxidation, and  
33 liver necrosis following bromobenzene and iodobenzene intoxication. *Toxicol Pathol*  
34 12:295-299.



- 1 Casini, AF; Pompella, A; Comporti, M. (1985) Liver glutathione depletion induced by  
2 bromobenzene, iodobenzene, and diethylmaleate poisoning and its relation to lipid peroxidation  
3 and necrosis. *Am J Pathol* 118:225-237.
- 4 Casini, AF; Ferrali, M; Pompella, A; Maellaro, E; Comparti, M. (1986) Lipid peroxidation and  
5 cellular damage in extrahepatic tissues of bromobenzene-intoxicated mice. *Am J Pathol*  
6 123(3):520-531.
- 7 Chakrabarti, S; Brodeur, J. (1984) Dose-dependent metabolic excretion of bromobenzene and its  
8 possible relationship to hepatotoxicity in rats. *J Toxicol Environ Health* 14:379-391.
- 9 Chan, K; Jensen, NS; Silber, PM; O'Brien, PJ. (2007) Structure-activity relationships for  
10 halobenzene induced cytotoxicity in rat and human hepatocytes. *Chem Biol Interact*  
11 165(3):165-174.
- 12 Chiou, CT; Freed, VH; Schmedding, DW; Kohnert, RL. (1977) Partition coefficient and  
13 bioaccumulation of selected organic chemicals. *Environ Sci Technol* 11(5):475-478.
- 14 CITI (Chemicals Inspection and Testing Institute). (1992) Biodegradation and bioaccumulation.  
15 Data of existing chemicals based on the CSCL Japan. Japan Chemical Industry Ecological-  
16 Toxicology & Information Center.
- 17 Colacci, A; Arfellini, G; Mazzullo, M; Prodi, G; Grilli, S. (1985) The covalent binding of  
18 bromobenzene with nucleic acids. *Toxicol Pathol* 13:276-282.
- 19 Dahl, JE; Becher, R; Aarstad, K; Nilson, OG; Dybing, E. (1990) Species differences in short  
20 term toxicity from inhalation exposure to bromobenzene. *Arch Toxicol* 64:370-376.
- 21 Forkert, PG. (1985) Bromobenzene causes Clara cell damage in mice. *Can J Physiol Pharmacol*  
22 63:1480-1484.
- 23 Freitag, D; Ballhorn, L; Geyer, H; Korte, F. (1985) Environmental hazard profile of organic  
24 chemicals. An experimental method for the assessment of the behavior of organic chemicals in  
25 the ecosphere by means of simple laboratory tests with <sup>14</sup>C labelled chemicals. *Chemosphere*  
26 14(10):1589-1616.
- 27 Galloway, SM; Armstrong, MJ; Reuben, C; et al. (1987) Chromosome aberrations and sister  
28 chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ Mol*  
29 *Mutagen* 10:1-175.
- 30 Girault, I.; Rougier, N; Chesné, C; et al. (2005) Simultaneous measurement of 23 isoforms from  
31 the human cytochrome P450 families 1-2 by quantitative reverse transcriptase-polymerase chain  
32 reaction. *Drug Metab Dispos* 33:1803-1810.
- 33 Green, MD; Shires, TK; Fischer, LJ. (1984) Hepatotoxicity of acetaminophen in neonatal and  
34 young rats. 1. Age-related changes in susceptibility. *Toxicol Appl Pharmacol* 74:116-124.

- 1 Hansch, C; Leo, A; Hoekman, D. (1995) Exploring QSAR. Hydrophobic, electronic, and steric  
2 constants. In: Heller, SR, Ed. ACS professional reference book. Washington, DC: American  
3 Chemical Society.
- 4 Heijne, WHM; Stierum, RH; Slijper, M; van Bladeren, PJ; van Ommen, B. (2003)  
5 Toxicogenomics of bromobenzene hepatotoxicity: A combined transcriptomics and proteomics  
6 approach. *Biochem Pharmacol* 65:857-875.
- 7 Heijne, WHM; Slitt, AL; van Bladeren, PJ; et al. (2004) Bromobenzene-induced hepatotoxicity  
8 at the transcriptome level. *Toxicol Sci* 79(2):411-422.
- 9 Heijne, WHM; Lamers, RJAN; van Bladeren, PJ; Groten, JP; van Nesselrooij, JHJ; van Ommen,  
10 B. (2005) Profiles of metabolites and gene expression in rats with chemically induced hepatic  
11 necrosis. *Toxicol Pathol* 33:425-433.
- 12 Heikes, DL; Jensen, SR; Fleming-Jones, ME. (1995) Purge and trap extraction with GC-MS  
13 determination of volatile organic compounds in table-ready foods. *J Agric Food Chem*  
14 43:2869-2875.
- 15 Hellman, B. (1993) NIOH and NIOSH basis for an occupational health standard: Chlorobenzene.  
16 *Arbete och Hälsa* 31:1-73 (also published by the U.S. Department of Health and Human Services  
17 under a joint agreement with NIOH and NIOSH. Available at  
18 <http://www.cdc.gov/niosh/pdfs/93-102a.pdf>).
- 19 Herren-Freund, SL; Pereira, MA. (1986) Carcinogenicity of by-products of disinfection in mouse  
20 and rat liver. *Environ Health Perspect* 69:59-65.
- 21 HSDB (Hazardous Substances Data Bank). (2003) Bromobenzene. Last review dated September  
22 19, 1996. National Library of Medicine, National Toxicology Program, Bethesda, MD. Available  
23 at <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>.
- 24 IBT (Industrial Bio-Test Laboratories). (1977) Teratogenic study with monochlorobenzene in  
25 albino rats. Report to Monsanto Company, St. Louis, MO. BTL No. 76-89; IBT No.  
26 8533-09115. May 18, 1977.
- 27 Ito, N; Tsuda, H; Tatematsu, M; et al. (1988) Enhancing effect of various hepatocarcinogens on  
28 induction of preneoplastic glutathione S-transferase placental form positive foci in rats - an  
29 approach for a new medium-term bioassay system. *Carcinogenesis* 9:387-394.
- 30 John, JA; Hayes, WC; Hanley, TR, Jr.; Johnson, KA; Gushow, TS; Rao, KS. (1984) Inhalation  
31 teratology study on monochlorobenzene in rats and rabbits. *Toxicol Appl Pharmacol*  
32 76:365-373.
- 33 Jollow, DJ; Mitchell, JR; Zampaglione, N; Gillette, JR. (1974) Bromobenzene-induced liver  
34 necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the  
35 hepatotoxic metabolite. *Pharmacology* 11:151-169.

- 1 Kato, R; Vassanelli, P; Frontino, G; Chiesara, E. (1964) Variation in the activity of microsomal  
2 drug-metabolizing enzymes in rats in relation to the age. *Biochem Pharmacol* 13:1037-1051.
- 3 Kluwe, WM; Maronpot, RR; Greenwell, A; Harrington, F. (1984) Interactions between  
4 bromobenzene dose, glutathione concentrations, and organ toxicities in single- and multiple-  
5 treatment studies. *Fundam Appl Toxicol* 4:1019-1028.
- 6 Koen, YM; Hanzilk, RP. (2002) Identification of seven proteins in the endoplasmic reticulum as  
7 targets for reactive metabolites of bromobenzene. *Chem Res Toxicol* 15(5):699-706.
- 8 Koen, YM; Williams, TD; Hanzlik, RP. (2000) Identification of three protein targets for reactive  
9 metabolites of bromobenzene in rat liver cytosol. *Chem Res Toxicol* 13(12):1326-1335.
- 10 Krusekopf, S; Roots, I; Hildebrandt, AG; Kleeberg, U. (2003) Time-dependent transcriptional  
11 induction of CYP1A1, CYP1A2 and CYP1B1 mRNAs by H<sup>+</sup>/K<sup>+</sup>-ATPase inhibitors and other  
12 xenobiotics. *Xenobiotica* 33(2):107-118.
- 13 Lau, SS; Monks, TJ. (1988) The contribution of bromobenzene to our current understanding of  
14 chemically-induced toxicities. *Life Sci* 42:1259-1269.
- 15 Lau, SS; Monks, TJ. (1997a) Bromobenzene hepatotoxicity: A paradigm of reactive electrophilic  
16 metabolites binding covalently to tissue macromolecules. Is there light at the end of the tunnel?  
17 In: McCuskey, R, Ed. *Comprehensive Toxicology*, Vol. 9: Hepatic and Gastrointestinal Toxicity.  
18 Austin, TX: Elsevier-Science, University of Texas. pp. 465-473.
- 19 Lau, SS; Monks, TJ. (1997b) Bromobenzene nephrotoxicity: A model of metabolism-dependent  
20 toxicity. In: Goldstein, RS, Ed. *Comprehensive toxicology*, Vol 7: Renal Toxicology. Austin,  
21 TX: Elsevier-Science, University of Texas. pp. 617-632.
- 22 Lau, SS; Zannoni, VG. (1979) Hepatic microsomal epoxidation of bromobenzene to phenols and  
23 its toxicological implication. *Toxicol Appl Pharmacol* 50:309-318.
- 24 Lau, SS; Zannoni, VG. (1981a) Bromobenzene metabolism in the rabbit. Specific forms of  
25 cytochrome P-450 involved in 2,3- and 3,4-epoxidation. *Mol Pharmacol* 20:234-235.
- 26 Lau, SS; Zannoni, VG. (1981b) Bromobenzene epoxidation leading to binding on  
27 macromolecular protein sites. *J Pharmacol Exp Ther* 219(2):563-572.
- 28 Lau, SS; Abrams, GD; Zannoni, VG. (1980) Metabolic activation and detoxification of  
29 bromobenzene leading to cytotoxicity. *J Pharmacol Exp Ther* 214(3):703-714.
- 30 Lau, SS; Monks, TJ; Gillette, JR. (1984a) Identification of 2-bromohydroquinone as a metabolite  
31 of bromobenzene and *o*-bromophenol: implications for bromobenzene-induced nephrotoxicity. *J*  
32 *Pharmacol Exp Ther* 230(2):360-366.
- 33 Lau, SS; Monks, TJ; Greene, KE; Gillette, JR. (1984b) The role of *ortho*-bromophenol in the  
34 nephrotoxicity of bromobenzene in rats. *Toxicol Appl Pharmacol* 72:539-549.

- 1 Lertratanangkoon, K; Horning, MG. (1987) Bromobenzene metabolism in the rat and guinea pig.  
2 Drug Metab Dispos 15(1):1-11.
- 3 Lertratanangkoon, K; Horning, EC; Horning, MG. (1987) Conversion of bromobenzene to 3-  
4 bromophenol: a route to 3- and 4-bromophenol through sulfur-series intermediates derived from  
5 the 3,4-oxide. Drug Metab Dispos 15(5):857-867.
- 6 Lertratanangkoon, K; Horning, EC; Horning, MG. (1993) Pathways of formation of 2-, 3- and  
7 4-bromophenol from bromobenzene. Proposed mechanism for C-S lyase reactions of cysteine  
8 conjugates. Res Commun Chem Pathol Pharmacol 80(3):259-282.
- 9 Lewis, RJ. (1997) Bromobenzene. In: Hawley's Condensed Chemical Dictionary, 13<sup>th</sup> ed. New  
10 York, NY: John Wiley & Sons, Inc. pp. 164.
- 11 Lide, DR. (2000) CRC Handbook of Chemistry and Physics, 81<sup>st</sup> ed. Washington, DC: CRC  
12 Press. pp. 3-30.
- 13 Loudon, GM. (1988) Organic chemistry, 2<sup>nd</sup> ed. Menlo Park, California: Benjamin/Cummings  
14 Publishing Company, Inc. p. 10.
- 15 Lyman, WJ; Reehl, WF; Rosenblatt, DH. (1990) Handbook of chemical property estimation  
16 methods. Environmental behavior of organic compounds. Washington, DC: American Chemical  
17 Society.
- 18 Madhu, C; Klaassen, CD. (1992) Bromobenzene-glutathione excretion into bile reflects toxic  
19 activation of bromobenzene in rats. Toxicol Lett 60(2):224-236.
- 20 McCann, J; Choi, E; Yamaskai, E; Ames, BN. (1975) Detection of carcinogens as mutagens in  
21 the Salmonella/microsome test: assay of 300 chemicals. Proc Nat Acad Sci USA 12:5135-5139.
- 22 Meister, A. (1982) 5-Oxoprolinuria (pyroglutamic aciduria) and other disorders of the  $\gamma$ -glutamyl  
23 cycle. In: Stanbury, JB; Wyngaarder, JB; Frederickson, JL; et al., Eds. Metabolic Basis of  
24 Inherited Diseases, 5<sup>th</sup> ed. New York, NY: McGraw-Hill.
- 25 Merrick, AB; Davies, MH; Schnell, RC. (1986) Effect of sodium selenite upon bromobenzene  
26 toxicity in rats. II. Metabolism. Toxicol Appl Pharmacol 83:279-286.
- 27 Miller, NE; Thomas, D; Billings, RE. (1990) Bromobenzene metabolism *in vivo* and *in vitro*.  
28 The mechanism of 4-bromocatechol formation. Drug Metab Dispos 18(3):304-308.
- 29 Minami, K; Saito, T; Narahara, M; Tomita, H; Kato, H; Sugiyama, H; Katoh, M; Nakajima, M;  
30 Yokoi, T. (2005) Relationship between hepatic gene expression profiles and hepatotoxicity in  
31 five typical hepatotoxicant-administered rats. Toxicol Sci 87(1):296-305.
- 32 Mitchell, JR; Reid, WD; Christie, B; Moskowitz, J; Krishma, G; Brodie, BB. (1971)  
33 Bromobenzene-induced hepatic necrosis: species differences and protection by SKF 525-A. Res  
34 Commun Chem Pathol Pharmacol 2(6):877-888.

- 1 Mohtashamipur, E; Triebel, R; Straeter, H; Norpoth, K. (1987) The bone marrow clastogenicity  
2 of eight halogenated benzenes in male NMRI mice. *Mutagenesis* 2(2):111-113.
- 3 Monks, TJ; Hinson, JA; Gillette, JR. (1982) Bromobenzene and p-bromophenol toxicity and  
4 covalent binding *in vivo*. *Life Sci* 30:841-848.
- 5 Monks, TJ; Lau, SS; Highet, RJ. (1984a) Formation of nontoxic reactive metabolites of  
6 *p*-bromophenol: identification of a new glutathione conjugate. *Drug Metab Dispos*  
7 12(4):432-437.
- 8 Monks, TJ; Lau, SS; Pohl, LR; Gillette, JR. (1984b) The mechanism of formation of *o*-  
9 bromophenol from bromobenzene. *Drug Metab Dispos* 12(2):193-198.
- 10 Monks, TJ; Lau, SS; Highet, RJ; Gillette, JR. (1985) Glutathione conjugates of 2-  
11 bromohydroquinone are nephrotoxic. *Drug Metab Dispos* 13(5):553-559.
- 12 Nair, RS; Barter, JA; Schroeder, RE; et al. (1987) A two-generation reproduction study with  
13 monochlorobenzene vapor in rats. *Fund Appl Toxicol* 9(4):678-686.
- 14 Nakamura, S-I; Oda, Y; Shimada, T; Oki, I; Sugimoto, K. (1987) SOS-inducing activity of  
15 chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: examination  
16 with 151 chemicals. *Mutat Res* 192:239-246.
- 17 National Research Council. (1983) Risk assessment in the federal government: managing the  
18 process. Committee on the Institutional Means for Assessment of Risks to Public Health,  
19 Commission on Life Sciences, NRC. Washington, DC: National Academy Press.
- 20 NTP (National Toxicology Program). (1985a) Subchronic gavage study of bromobenzene in rats.  
21 National Institutes of Health, National Toxicology Program, Research Triangle Park, NC.  
22 [Unpublished study]. April, 1985. (Available by calling EPA's IRIS Hotline at (202)566-1676,  
23 by fax at (202)566-1749 or by email at iris@epa.gov).
- 24 NTP (National Toxicology Program). (1985b) Subchronic gavage study of bromobenzene in  
25 mice. National Institutes of Health, National Toxicology Program, Research Triangle Park, NC.  
26 [Unpublished study]. May, 1985. (Available by calling EPA's IRIS Hotline at (202)566-1676, by  
27 fax at (202)566-1749 or by email at iris@epa.gov).
- 28 NTP (National Toxicology Program). (1985c) Subchronic inhalation study of bromobenzene in  
29 rats. National Institutes of Health, National Toxicology Program, Research Triangle Park, NC.  
30 [Unpublished study]. October, 1985. (Available by calling EPA's IRIS Hotline at (202)566-  
31 1676, by fax at (202)566-1749 or by email at iris@epa.gov).
- 32 NTP (National Toxicology Program). (1985d) Subchronic inhalation study of bromobenzene in  
33 mice. National Institutes of Health, National Toxicology Program, Research Triangle Park, NC.  
34 [Unpublished study]. November, 1985. (Available by calling EPA's IRIS Hotline at (202)566-  
35 1676, by fax at (202)566-1749 or by email at iris@epa.gov).

- 1 NTP (National Toxicology Program). (1985e) NTP Technical report on the toxicology and  
2 carcinogenesis studies of chlorobenzene (CAS No. 108-90-7) in F344/N rats and B6C3F1 mice  
3 (gavage studies). National Institutes of Health, National Toxicology Program, Research Triangle  
4 Park, NC. NTP TR 261. NIH Publication No. 86-2517. NTIS PB86-144714.
- 5 NTP (National Toxicology Program). (1986a) Pathology Working Group summary report on  
6 bromobenzene (C55492) subchronic study (basic and special) F344 rats and B6C3F1 mice.  
7 National Institutes of Health, National Toxicology Program, Research Triangle Park, NC.  
8 (Available by calling EPA's IRIS Hotline at (202)566-1676, by fax at (202)566-1749 or by email  
9 at [iris@epa.gov](mailto:iris@epa.gov)).
- 10 NTP (National Toxicology Program). (1986b) Pathology Working Group (PWG) review of  
11 bromobenzene (C55492) by inhalation in F344 rats and B6C3F1 mice 90-day study. National  
12 Institutes of Health, National Toxicology Program, Research Triangle Park, NC. (Available by  
13 calling EPA's IRIS Hotline at (202)566-1676, by fax at (202)566-1749 or by email at  
14 [iris@epa.gov](mailto:iris@epa.gov)).
- 15 Ogino, Y. (1984a) Studies on the biological toxicity of several hydrocarbon pollutants of the  
16 environment. III. Metabolites and excretion rates of brominated benzenes. *Okayama Igakkai*  
17 *Zasshi* 96(5/6):553-567.
- 18 Ogino, Y. (1984b) Studies on the biological toxicity of several brominated benzene pollutants of  
19 the environment. IV. Biological fate of brominated benzenes in animals. *Okayama Igakkai*  
20 *Zasshi* 96(5/6):569-578.
- 21 Patrick, RS; Kennedy, JS. (1964) S<sup>35</sup>-labelled amino acids in experimental liver disease. *J Pathol*  
22 *Bacteriol* 88:107-114.
- 23 Pienta, RJ; Poiley, JA; Lebherz, WB. (1977) Morphological transformation of early passage  
24 golden Syrian hamster embryo cells derived from cryopreserved primary cultures as a reliable *in*  
25 *vitro* bioassay for identifying diverse carcinogens. *Int J Cancer* 19:642-655.
- 26 Popper, H; Koch-Weser, D; De La Hueraga, J. (1952) Serum and hepatic enzymes in  
27 experimental liver damage. *J Mt Sinai Hosp* 19:256-265.
- 28 Prodi, G; Arfellini, G; Colacci, A; Grilli, S; Mazzullo, M. (1986) Interaction of halocompounds  
29 with nucleic acids. *Toxicol Pathol* 14(4):438-444.
- 30 Ramel, C; Magnusson, J. (1979) Chemical induction of nondisjunction in *Drosophila*. *Environ*  
31 *Health Perspect* 1:59-66.
- 32 Reid, WD. (1973) Mechanism of renal necrosis induced by bromobenzene or chlorobenzene.  
33 *Exp Mol Pathol* 19:197-214.
- 34 Reid, W; Krishna, G. (1973) Centrilobular hepatic necrosis related to covalent binding of  
35 metabolites of halogenated aromatic hydrocarbons. *Exp Mol Pathol* 18:80-99.

- 1 Reid, WD; Christie, B; Krishna, G; Michell, JR; Moskowitz, J; Brokie, BB. (1971)  
2 Bromobenzene metabolism and hepatic necrosis. *Pharmacology* 6:41-55.
- 3 Reid, WD; Ilett, KF; Glick, JM; Krishna, G. (1973) Metabolism and binding of aromatic  
4 hydrocarbons in the lung. Relationships to experimental bronchiolar necrosis. *Am Rev Respir*  
5 *Dis* 107:539-551.
- 6 Riddick, JA; Bunger, WB; Sakano, TK. (1986) Bromobenzene. In: *Techniques of Chemistry.*  
7 *Organic solvents: Physical properties and methods of purification*, 4<sup>th</sup> ed., Vol. 2. New York,  
8 NY: John Wiley & Sons. pp. 534.
- 9 Rombach, EM; Hanzlik, RP. (1997) Detection of benzoquinone adducts to rat liver protein  
10 sulfhydryl groups using specific antibodies. *Chem Res Toxicol* 10(12):1407-1411.
- 11 Rombach, EM; Hanzlik, RP. (1998) Identification of a rat liver microsomal esterase as a target  
12 protein for bromobenzene metabolites. *Chem Res Toxicol* 11(3):178-184.
- 13 Rombach, EM; Hanzlik, RP. (1999) Detection of adducts of bromobenzene 3,4-oxide with rat  
14 liver microsomal protein sulfhydryl groups using specific antibodies. *Chem Res Toxicol*  
15 12(2):159-163.
- 16 Rosenkranz, S; Poirier, LA. (1979) Evaluation of the mutagenicity and DNA-modifying activity  
17 of carcinogens and noncarcinogens in microbial systems. *J Natl Cancer Inst* 62(4):873-892.
- 18 Roth, RA. (1981) Effect of pneumotoxicants on lactate dehydrogenase activity in airways of rats.  
19 *Toxicol Appl Pharmacol* 57:69-78.
- 20 Rush, CF; Newton, JF; Maita, K; Kuo, CH; Hook, JB. (1984) Nephrotoxicity of phenolic  
21 bromobenzene metabolites in the mouse. *Toxicology* 30:259-272.
- 22 Shamilov, TA. (1969) Toxicity characteristics and hazards of bromobenzene. *Gig Tr Prof Zabol*  
23 13(9):56-58.
- 24 Shiu, W-Y; Mackay, D. (1997) Henry's law constants of selected aromatic hydrocarbons,  
25 alcohols, and ketones. *J Chem Eng Data* 42:27-30.
- 26 Simmon, VF. (1979) *In vitro* mutagenicity assays of chemical carcinogens and related  
27 compounds with *Salmonella typhimurium*. *J Natl Cancer Inst* 62(4):893-899.
- 28 Simmon, VF; Rosenkranz, HS; Zeiger, E; Poirier, LA. (1979) Mutagenic activity of chemical  
29 carcinogens and related compounds in the intraperitoneal host-mediated assay. *J Natl Cancer Inst*  
30 62(4):911-918.
- 31 Sipes, IG; Gigon, PL; Krishna, G. (1974) Biliary excretion of metabolites of bromobenzene.  
32 *Biochem Pharmacol* 23:451-455.
- 33 Slaughter, DE; Hanzlik, RP. (1991) Identification of epoxide- and quinone-derived  
34 bromobenzene adducts to protein sulfur nucleophiles. *Chem Res Toxicol* 4(3):349-359.

- 1 Slaughter, DE; Zheng, J; Harriman, S; Hanzlik, RP. (1993) Identification of covalent adducts to  
2 protein sulfur nucleophiles by alkaline permethylation. *Anal Biochem* 208(2):288-295.
- 3 Stierum, R; Heijne, W; Kienhuis, A; van Ommen, B; Groten, J. (2005) Toxicogenomics concepts  
4 and applications to study hepatic effects of food additives and chemicals. *Toxicol Appl*  
5 *Pharmacol* 207(Suppl. 2):179-188.
- 6 Sullivan, TM; Born, GS; Carlson, GP; Kessler, WV. (1983) The pharmacokinetics of inhaled  
7 chlorobenzene in the rat. *Toxicol Appl Pharmacol* 71:194-203.
- 8 Swann, RL; Laskowski, DA; McCall, PJ; Vander Kuy, K; Dishburger, HJ. (1983) A rapid  
9 method for the estimation of the environmental parameters octanol/water partition coefficient,  
10 soil sorption constant, water to air ratio, and water solubility. *Res Rev* 85:18-28.
- 11 Szymańska, JA. (1998) Hepatotoxicity of brominated benzenes: Relationship between chemical  
12 structure and hepatotoxic effects in acute intoxication of mice. *Arch Toxicol* 72(2):97-103.
- 13 Szymańska, JA; Piotrowski, JK. (2000) Hepatotoxicity of monobromobenzene and  
14 hexabromobenzene: Effects of reported dosage in rats. *Chemosphere* 41(10):1689-1696.
- 15 U.S. EPA. (1987) Drinking water health advisory for bromobenzene. External Review Draft.  
16 Prepared by the Environmental Criteria and Assessment Office, Office of Health and  
17 Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, OH for the  
18 Office of Drinking Water, Washington, DC. ECAO-CIN-W001.
- 19 U.S. EPA. (1988) Recommendations for and documentation of biological values for use in risk  
20 assessment. EPA/600/6-87/008. NTIS PB88-179874/AS. February 1988.
- 21 U.S. EPA. (1991) Guidelines for developmental toxicity risk assessment. Federal Register  
22 56(234):63798-63826. Available at <http://www.epa.gov/iris/backgr-d.htm>.
- 23 U.S. EPA. (1994a) Interim policy for particle size and limit concentration issues in inhalation  
24 toxicity: Notice of availability. Federal Register 59(206):53799. Available at  
25 <http://www.epa.gov/iris/backgr-d.htm>.
- 26 U.S. EPA. (1994b) Methods for derivation of inhalation reference concentrations and application  
27 of inhalation dosimetry. EPA/600/8-90/066F. Available at <http://www.epa.gov/iris/backgr-d.htm>.
- 28 U.S. EPA. (1995) Use of the benchmark dose approach in health risk assessment.  
29 EPA/630/R-94/007.
- 30 U.S. EPA. (1996) Guidelines for reproductive toxicity risk assessment. Federal Register  
31 61(212):56274-56322. Available at <http://www.epa.gov/iris/backgr-d.htm>.
- 32 U.S. EPA. (1998a) Guidelines for neurotoxicity risk assessment. Federal Register  
33 63(93):26926-26954. Available at <http://www.epa.gov/iris/backgr-d.htm>.



- 1 U.S. EPA. (1998b) Science policy council handbook: Peer review. Prepared by the Office of  
2 Science Policy, Office of Research and Development, Washington, DC. EPA/100/B-98/001.
- 3 U.S. EPA. (2000a) Science policy council handbook: Peer review, 2<sup>nd</sup> ed. Prepared by the Office  
4 of Science Policy, Office of Research and Development, Washington, DC. EPA/100/B-00/001.  
5 Available at <http://www.epa.gov/iris/backgr-d.htm>.
- 6 U.S. EPA. (2000b) Science policy council handbook: Risk characterization. Prepared by the  
7 Office of Science Policy, Office of Research and Development, Washington, DC.  
8 EPA/100/B-00/002.
- 9 U.S. EPA. (2000c) Benchmark dose technical support document. External Review Draft, Office  
10 of Research and Development, Risk Assessment Forum, Washington, DC. EPA/630/R-00/001.  
11 October. Available at <http://www.epa.gov/iris/backgr-d.htm>.
- 12 U.S. EPA. (2002) A review of the reference dose and reference concentration processes. Risk  
13 Assessment Forum, Washington, DC. EPA/630/P-02/0002F. Available at  
14 <http://www.epa.gov/iris/backgr-d.htm>.
- 15 U.S. EPA. (2003) Analysis of national occurrence of the 1998 Contaminant Candidate List  
16 (CCL) regulatory determination priority contaminants in public water systems. U.S.  
17 Environmental Protection Agency, Office of Water. EPA/815/D-01/002. Available at  
18 [http://www.epa.gov/OGWDW/ccl/pdfs/reg\\_determine1/support\\_cc1\\_nation-occur\\_analysis.pdf](http://www.epa.gov/OGWDW/ccl/pdfs/reg_determine1/support_cc1_nation-occur_analysis.pdf).
- 19 U.S. EPA. (2005a) Guidelines for carcinogen risk assessment. U.S. Environmental Protection  
20 Agency, Washington, DC. EPA/630/P-03/001B. Available at  
21 <http://www.epa.gov/iris/backgr-d.htm>.
- 22 U.S. EPA (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to  
23 carcinogens. Risk Assessment Forum, Washington, DC. EPA/630/R-03/003F. Available at  
24 <http://www.epa.gov/iris/backgr-d.htm>. November 3.
- 25 U.S. EPA. (2005c) Peer review handbook, 3<sup>rd</sup> ed. Review draft. Science Policy Council,  
26 Washington, DC. Available at <http://intranet.epa.gov/ospintra/scipol/prhndbk05.doc>.
- 27 Verschueren, K. (2001) Bromobenzene. In: Handbook of Environmental Data on Organic  
28 Chemicals, Vol 1. New York, NY: John Wiley & Sons. pp. 333.
- 29 Waters, NJ; Waterfield, CJ; Farrant, RD; Holmes, E; Nicholson, JK. (2006) Intergrated  
30 metabonomic analysis of bromobenzene-induced hepatotoxicity: novel induction of  
31 5-oxoprolinosis. J Proteome Res 5(6):1448-1459.
- 32 Westrick, JJ; Mello, JW; Thomas, RF. (1984) The groundwater supply survey. JAWWA  
33 76:52-59.
- 34 Zampaglione, N; Jollow, DJ; Stripp, MB; Mitchell, JR; Hamrick, M; Gillette, JR. (1973) Role of  
35 detoxifying enzymes in bromobenzene-induced liver necrosis. J Pharmacol Exp Ther  
36 187:218-227.

- 1 Zheng, J; Hanzlik, RP. (1992) Dihydroxylated mercapturic acid metabolites of bromobenzene.
- 2 Chem Res Toxicol 5(4):561-567.

1  
2  
3  
4

**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 <sub>10S</sub> and BMDL <sub>10S</sub> (mg/kg-day)		x <sup>2</sup> p-value	AIC
	BMD <sub>10</sub>	BMDL <sub>10</sub>		
Log-logistic <sup>a</sup>	172.07	69.23	1.00	46.24
gamma <sup>b</sup>	134.60	54.59	1.00	46.25
Multi-stage <sup>c</sup>	127.91	27.49	1.00	46.27
Quantal quadratic	65.62	49.47	0.88	47.67
Log-probit <sup>a</sup>	160.78	67.44	1.00	48.24
Weibull <sup>b</sup>	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 <sup>a</sup>Slope restricted to >1

15 <sup>b</sup>Restrict power >=1

16 <sup>c</sup>Restrict 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 <sub>10</sub> s and BMDL <sub>10</sub> s (mg/kg-day)		x <sup>2</sup> p-value	AIC
	BMD <sub>10</sub>	BMDL <sub>10</sub>		
Log-logistic <sup>a</sup>	184.67	66.05	0.85	52.66
gamma <sup>b</sup>	161.04	37.75	0.85	52.69
Quantal quadratic	73.60	54.85	0.86	53.01
Multi-stage <sup>c</sup>	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 <sup>b</sup>	126.69	35.45	0.79	54.40
Log-probit <sup>a</sup>	181.98	59.88	0.71	54.66
Quantal linear	21.40	14.41	0.34	57.45

<sup>a</sup>Slope restricted to >1

<sup>b</sup>Restrict power >=1

<sup>c</sup>Restrict betas >=0; Degree of polynomial = 5

3  
4  
5  
6  
7

**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 <sub>10</sub> s and BMDL <sub>10</sub> s (mg/kg-day)		x <sup>2</sup> p-value	AIC
	BMD <sub>10</sub>	BMDL <sub>10</sub>		
Multi-stage <sup>a</sup>	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 <sup>b</sup>	98.67	53.72	0.74	37.86
Gamma <sup>b</sup>	99.40	57.87	0.71	37.97
Log-probit <sup>c</sup>	100.10	63.56	0.66	38.25
Log-logistic <sup>c</sup>	107.28	64.0	0.62	38.61
Quantal linear	22.64	15.65	0.09	46.35

<sup>a</sup>Restrict power >=1

<sup>b</sup>Restrict betas >=0; Degree of polynomial = 5

<sup>c</sup>Slope restricted to >1

8  
9  
10  
11  
12

1

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

Model	BMD <sub>10</sub> s and BMDL <sub>10</sub> s (mg/kg-day)		x <sup>2</sup> p-value	AIC
	BMD <sub>10</sub>	BMDL <sub>10</sub>		
Weibull <sup>a</sup>	56.08	24.81	0.99	40.84
Gamma <sup>a</sup>	59.27	24.92	0.98	40.98
Quantal quadratic	74.86	59.49	0.87	41.65
Log-probit <sup>b</sup>	63.34	35.33	0.91	41.68
Log-logistic <sup>b</sup>	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 <sup>c</sup>	50.55	20.62	0.95	42.83

2 <sup>a</sup>Restrict power >=1

3 <sup>b</sup>Slope restricted to >1

4 <sup>c</sup>Restrict betas >=0; Degree of polynomial = 5

5

6

7

8

9

10

11

12

13

14

15

16

17

18

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

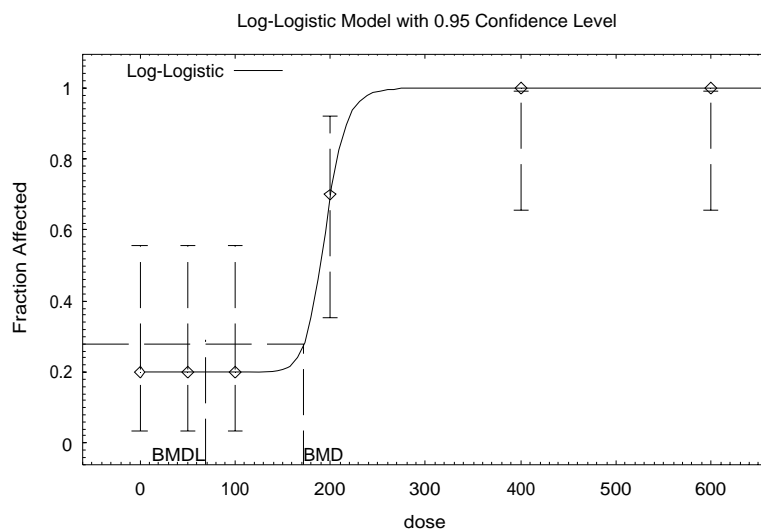
$$P(d) = B_0 + (1 - B_0) / [1 + \exp(-\text{intercept} - \text{slope} * \log(d))] \quad (\text{Eq. B-1})$$

d = exposure dose

B<sub>0</sub> = 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=ED<sub>10</sub>; BMDL=LED<sub>10</sub>**  
 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 BMD<sub>10</sub> 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 (BMDL<sub>10</sub>) 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:

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

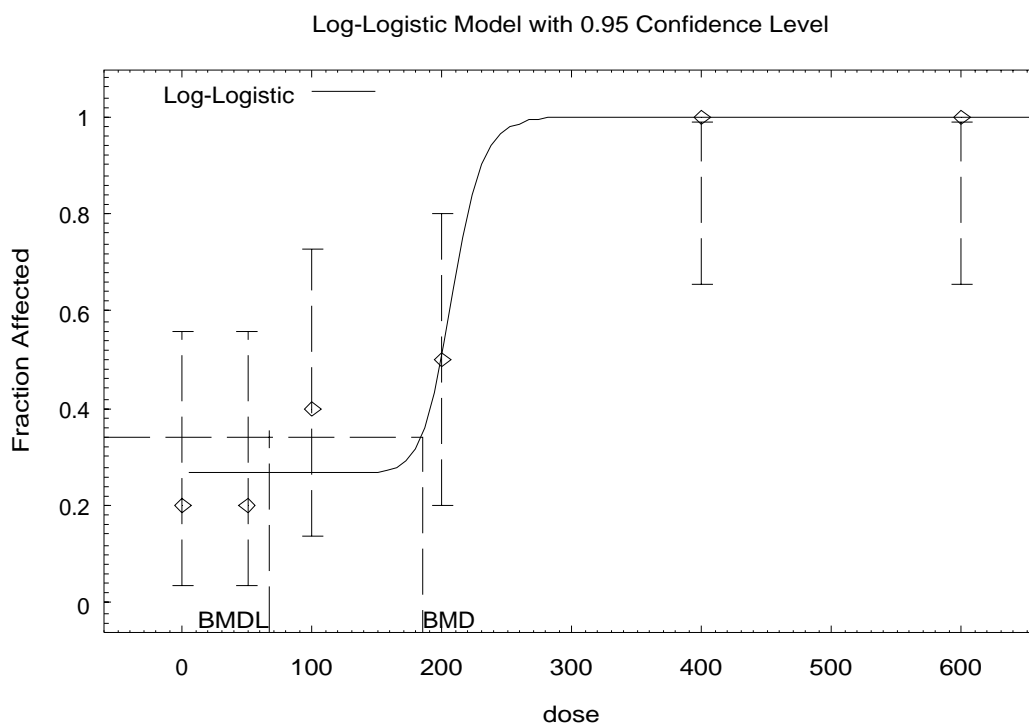
13 d = exposure dose

14 B<sub>0</sub> = 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=ED<sub>10</sub>; BMDL=LED<sub>10</sub>**  
 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 BMD<sub>10</sub> 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 (BMDL<sub>10</sub>) 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  $\beta_2 = 1.63151e+013$

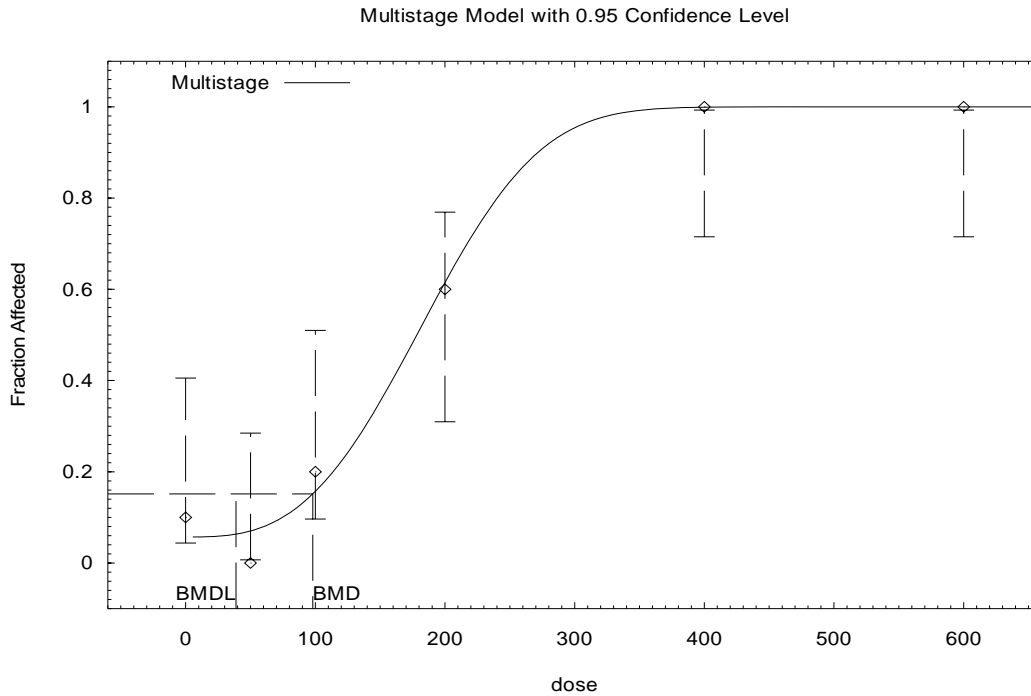
16  $\beta_3 = 0$

17  $\beta_4 = 0.$

18  
 19



1  
2



12:20 02/01 2006

3  
4  
5  
6  
7

**Figure B-3. Observed and predicted incidences of male B6C3F1 mice exhibiting bromobenzene-induced combined liver lesions following following gavage treatment 5 days/week for 90 days. BMD=ED<sub>10</sub>; BMDL=LED<sub>10</sub>**

8  
9  
10  
11  
12  
13  
14  
15  
16

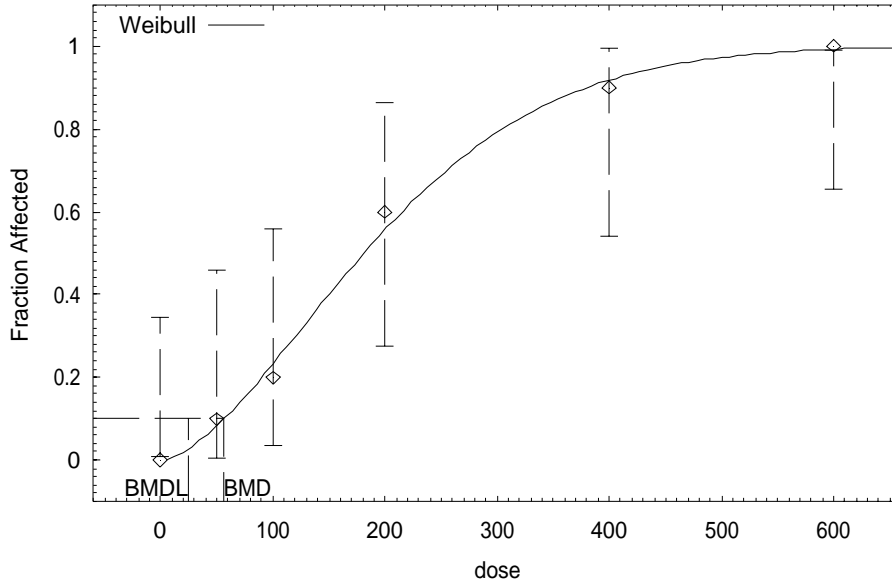
The Weibull model provided the best fit to the female mouse liver lesion data (see Table B-4), and was thus selected to estimate a BMD for liver lesions in female mice from the NTP (1985b) data. The BMD<sub>10</sub> associated with a 10% extra risk for histopathologic liver lesions in female mice was 56.1 mg/kg-day and its lower 95% confidence limit (BMDL<sub>10</sub>) 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:

17  
18  
19  
20  
21  
22  
23

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

- d = exposure dose
- B<sub>0</sub> = 0
- slope = 0.00152103 (se = 0.000322079)
- power = 1.62425 (se = 0.383589)

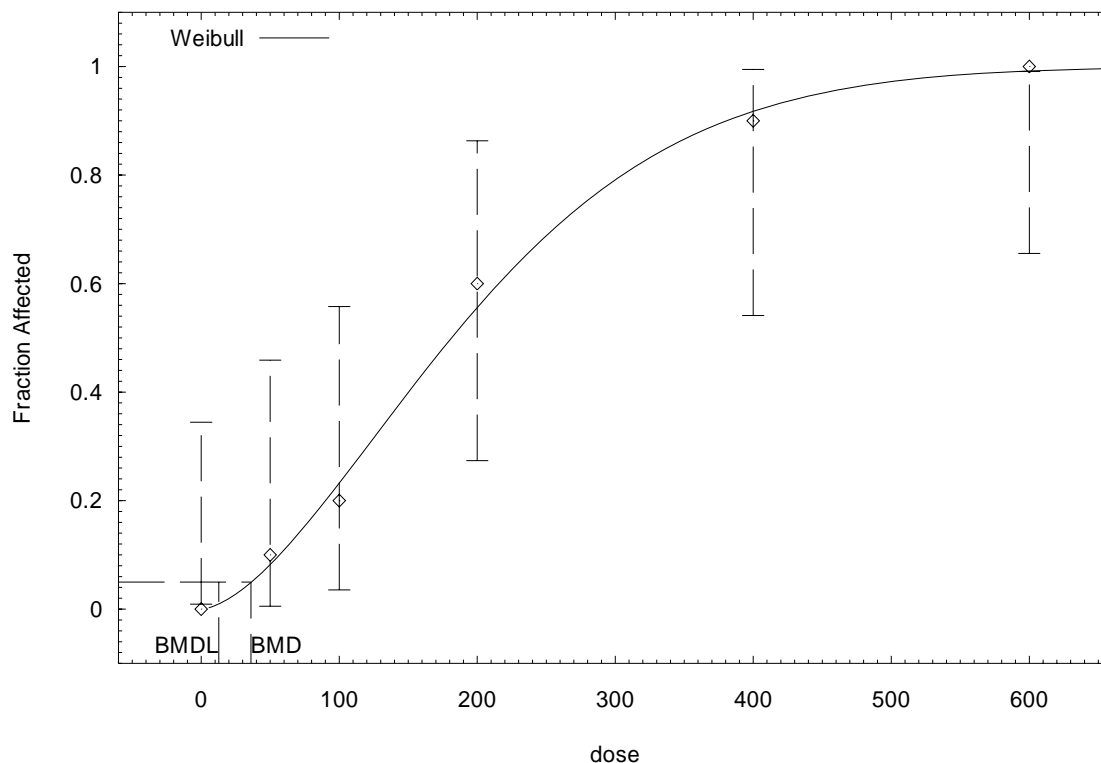
Weibull Model with 0.95 Confidence Level



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=ED<sub>10</sub>; BMDL=LED<sub>10</sub>**

Weibull Model with 0.95 Confidence Level



15:14 02/01 2006

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13

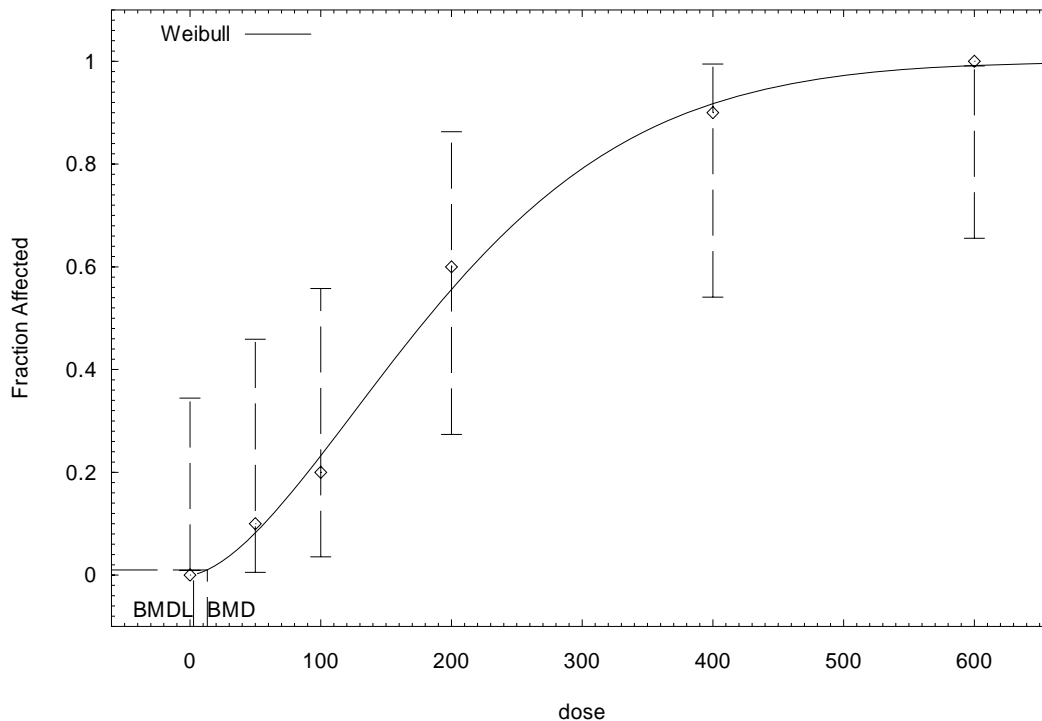
**Figure B-5. Observed and predicted incidences of female B6C3F1 mice exhibiting bromobenzene-induced 5% extra risk for liver lesions following gavage treatment 5 days/week for 90 days**

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

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

background = 0  
slope = 0.000152103 (se = 0.000322079)  
power = 1.62425 (se = 0.383589)

Weibull Model with 0.95 Confidence Level



15:15 02/01 2006

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

12

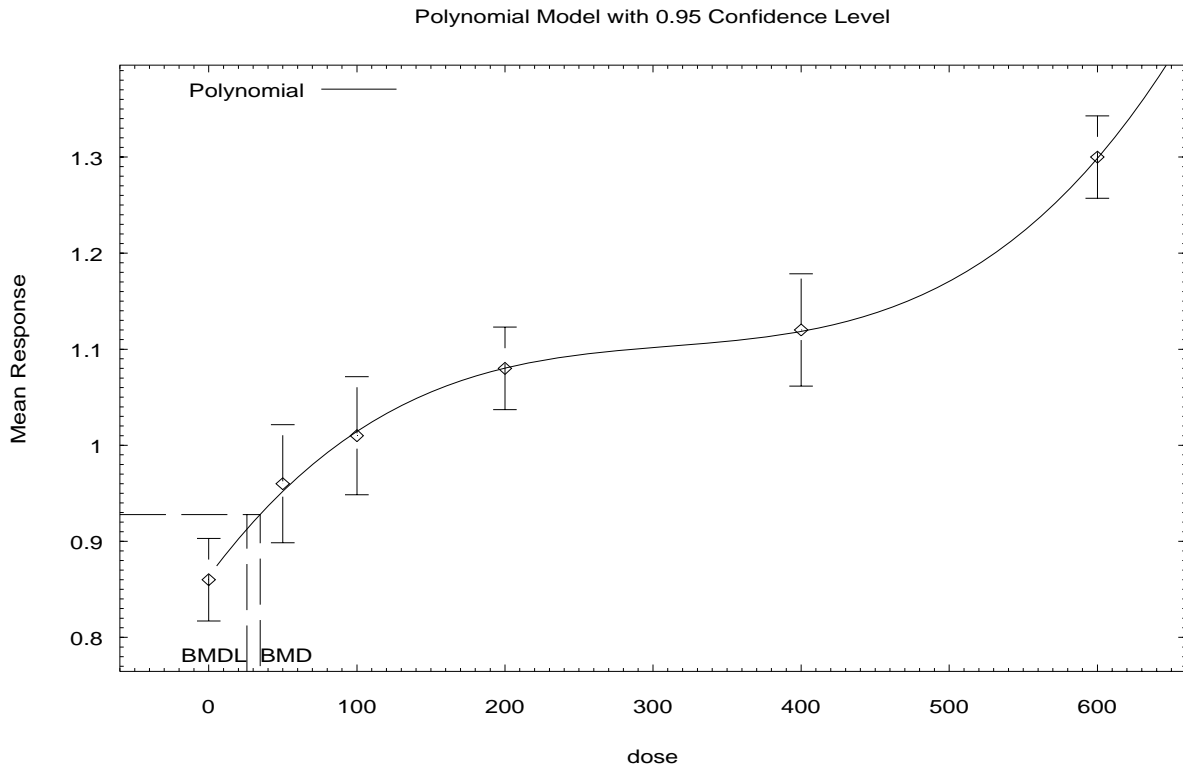
13 All available models in the EPA BMDS (version 1.3.2) were fit to absolute liver weight  
14 and liver-to-body weight data in male and female Fischer 344/N rats and male and female  
15 B6C3F1 mice from the 90-day oral gavage studies (NTP, 1985a,b). The data that were modeled  
16 are shown in Table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

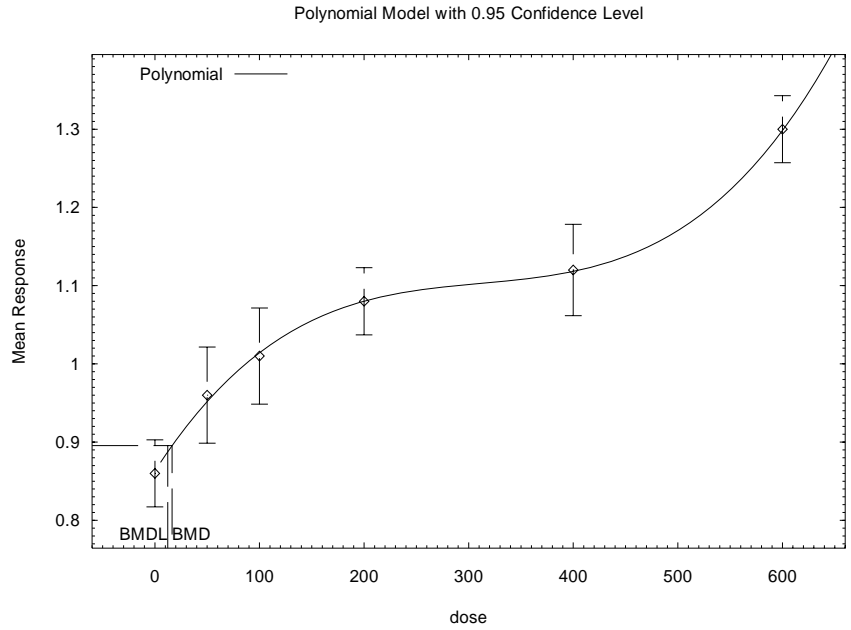


12:19 02/01 2005

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=ED<sub>10</sub>;**  
17 **BMDL=LED<sub>10</sub>**

18

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31



**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.

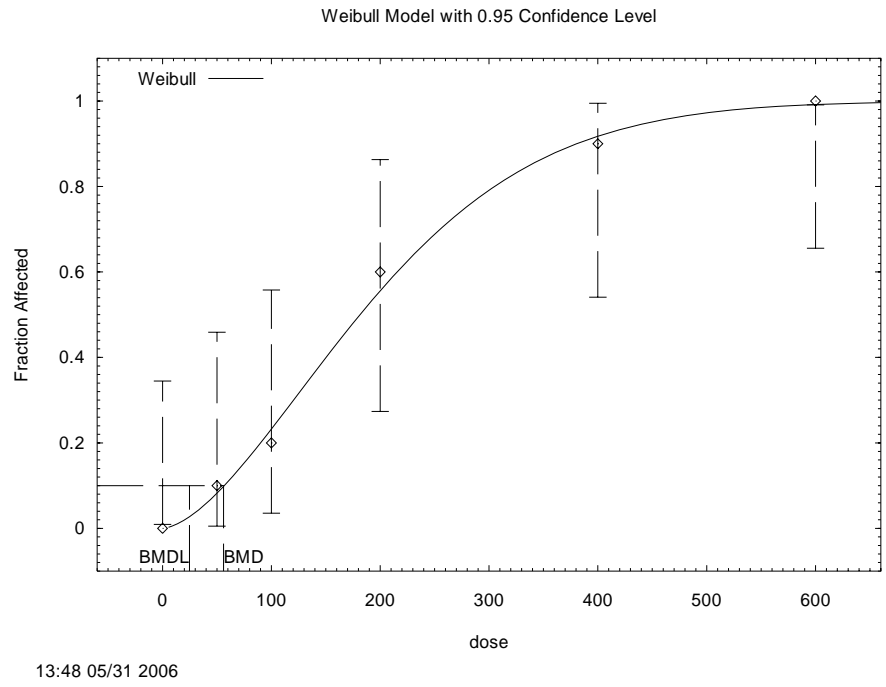
Benchmark Dose Computation

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<sub>1sd</sub> from the best fitting model for liver weight changes was 25.8  
 6 mg/kg-day, which was very similar to the lowest BMDL<sub>10</sub> 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 BMDL<sub>10</sub> 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**

<b>Model</b>	<b>BMC<sub>10</sub> (ppm)</b>	<b>BMCL<sub>10</sub> (ppm)</b>	<b>x<sup>2</sup> p-value</b>	<b>AIC</b>
Log-logistic <sup>a</sup>	95.59	58.73	1.00	12.01
Gamma <sup>b</sup>	89.24	51.42	1.00	12.01
Multi-stage <sup>c</sup>	77.09	40.33	0.999	12.17
Weibull <sup>b</sup>	92.34	47.08	1.00	14.01
Log-probit <sup>a</sup>	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

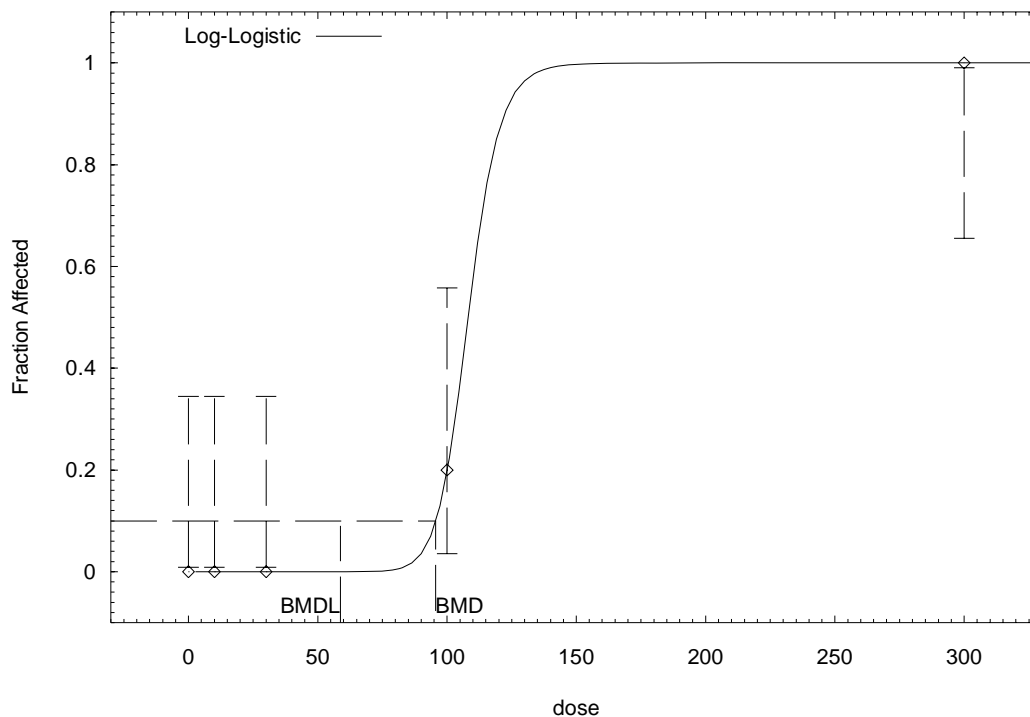
2 <sup>a</sup>Slope restricted to >1

3 <sup>b</sup>Restrict power > = 1

4 <sup>c</sup>Restrict betas > = 0; degree of polynomial = 3 (maximum degree restricted to #dose groups  
5 minus 2)

6

Log-Logistic Model with 0.95 Confidence Level



15:30 03/03 2006

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17

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

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

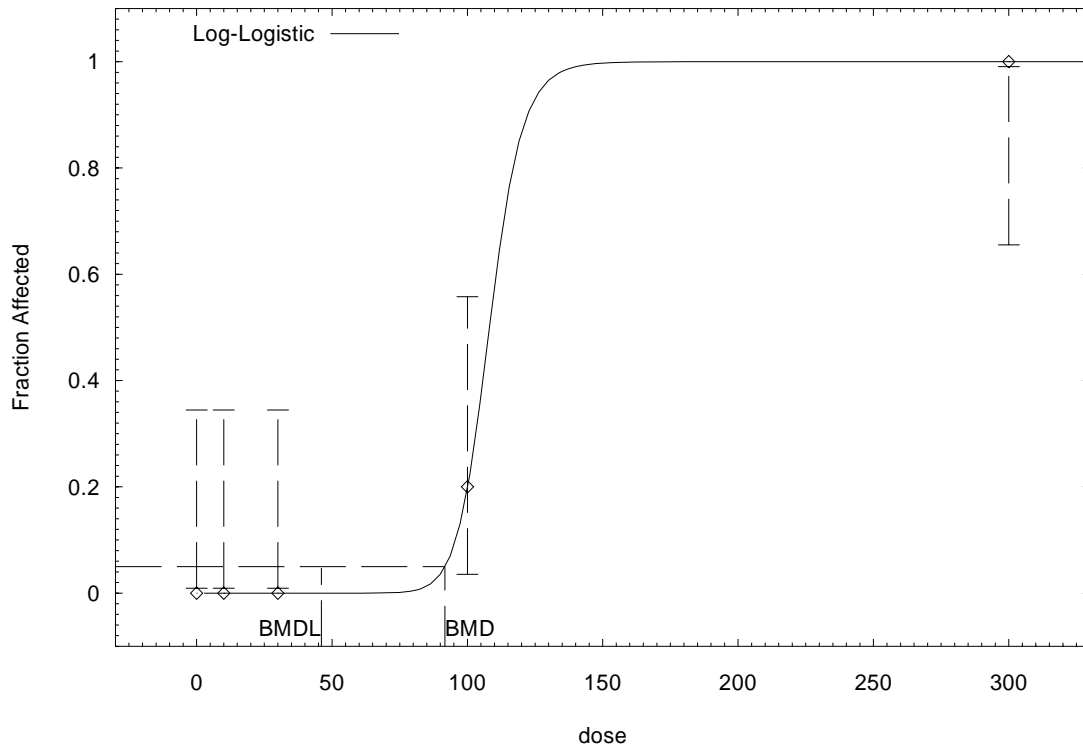
$$P[\text{response}] = \text{background} + (1 - \text{background}) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))] \quad (\text{Eq. C-1})$$

background = 0

intercept = -84.2793 (se = 0.790565)

slope = 18

Log-Logistic Model with 0.95 Confidence Level



14:12 02/02 2006

2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16

**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:

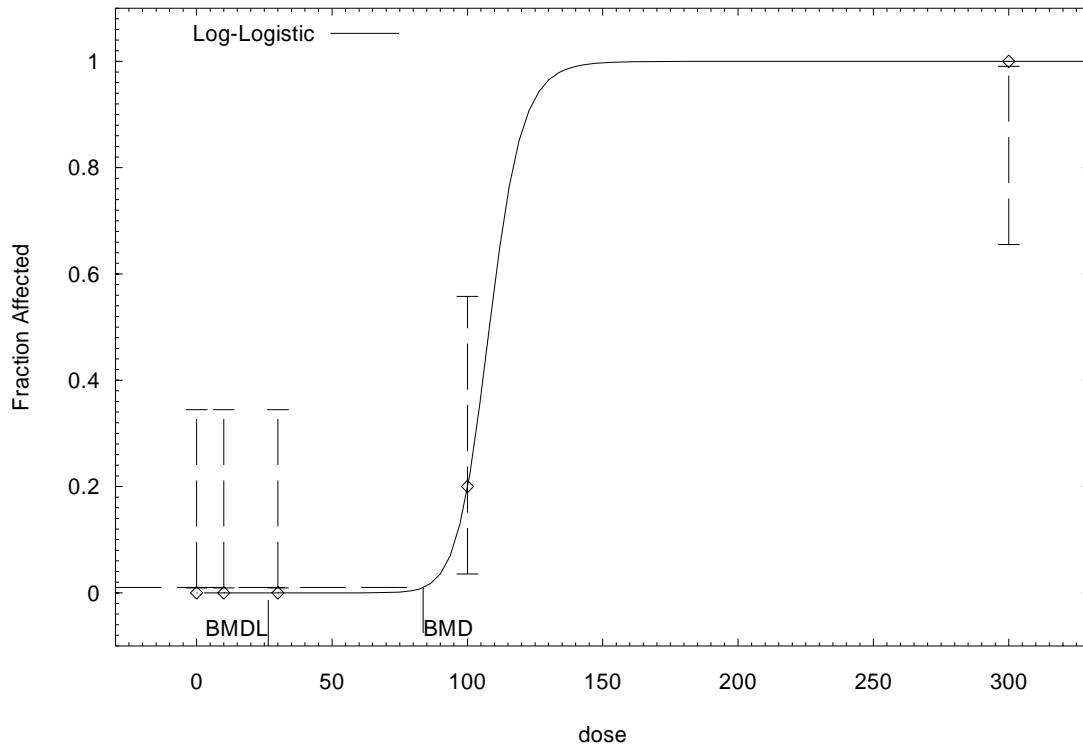
$$P[\text{response}] = \text{background} + (1 - \text{background}) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))] \quad (\text{Eq. C-2})$$

$$\text{background} = 0$$

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

$$\text{slope} = 18$$

Log-Logistic Model with 0.95 Confidence Level



14:13 02/02 2006

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

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

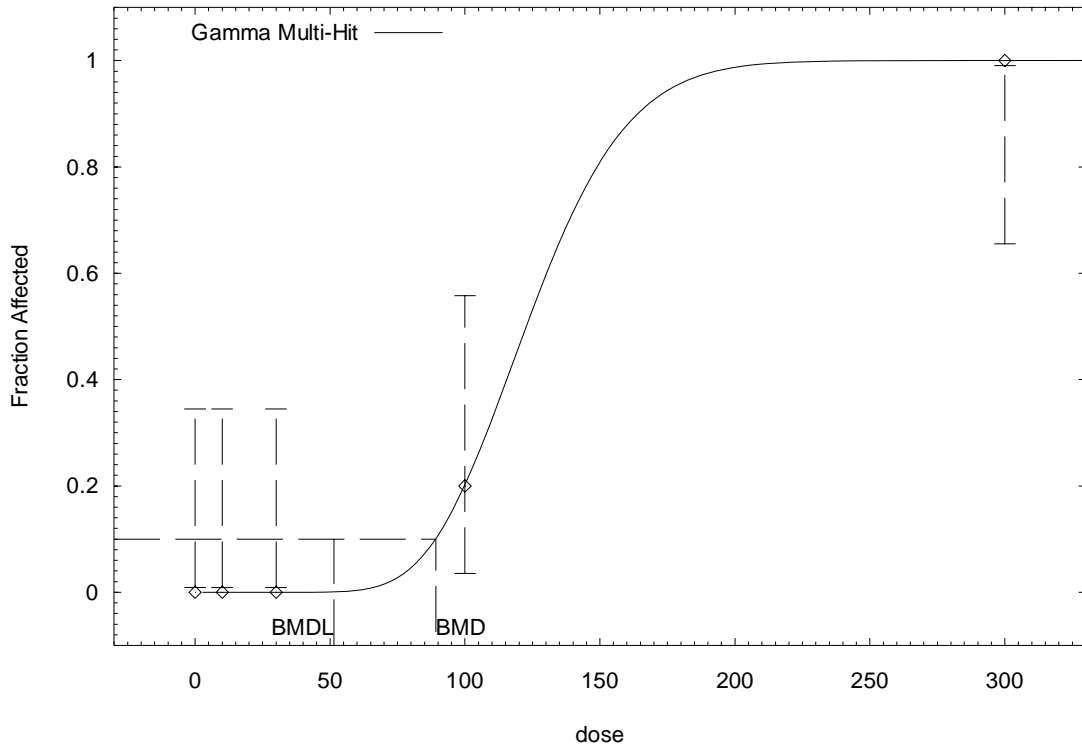
$$P[\text{response}] = \text{background} + (1 - \text{background}) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))] \quad (\text{Eq. C-3})$$

$$\text{background} = 0$$

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

$$\text{slope} = 18$$

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]

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

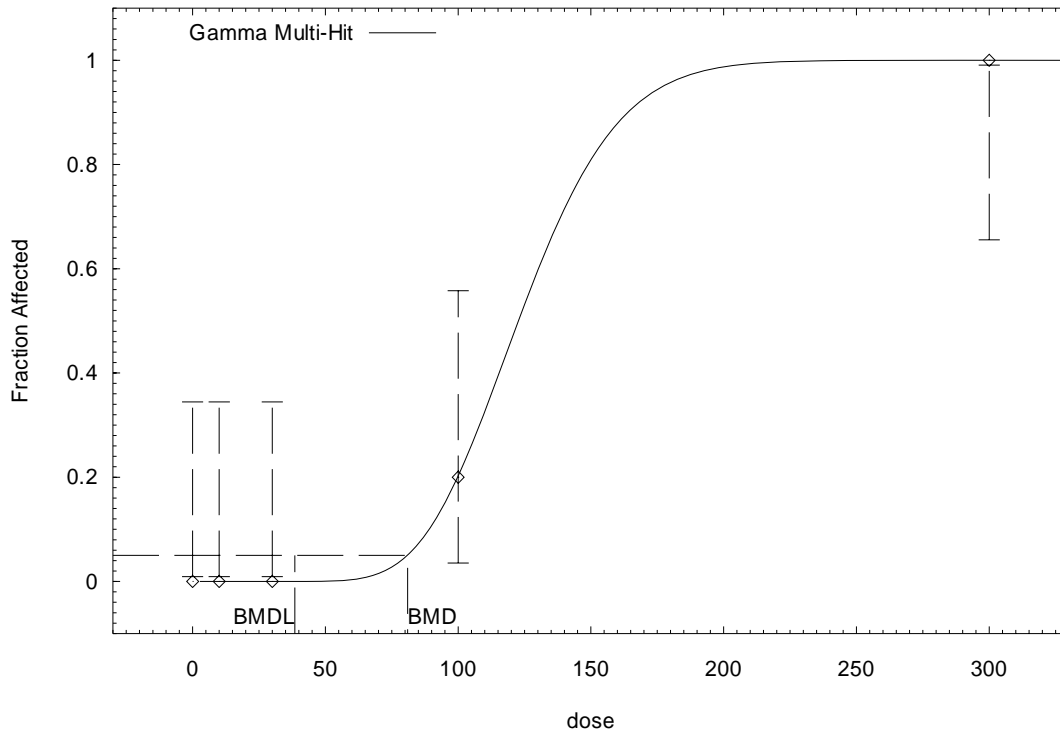
$$14 \quad \text{slope} = 0.143677 \text{ (se} = 0.0164918)$$

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

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:

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

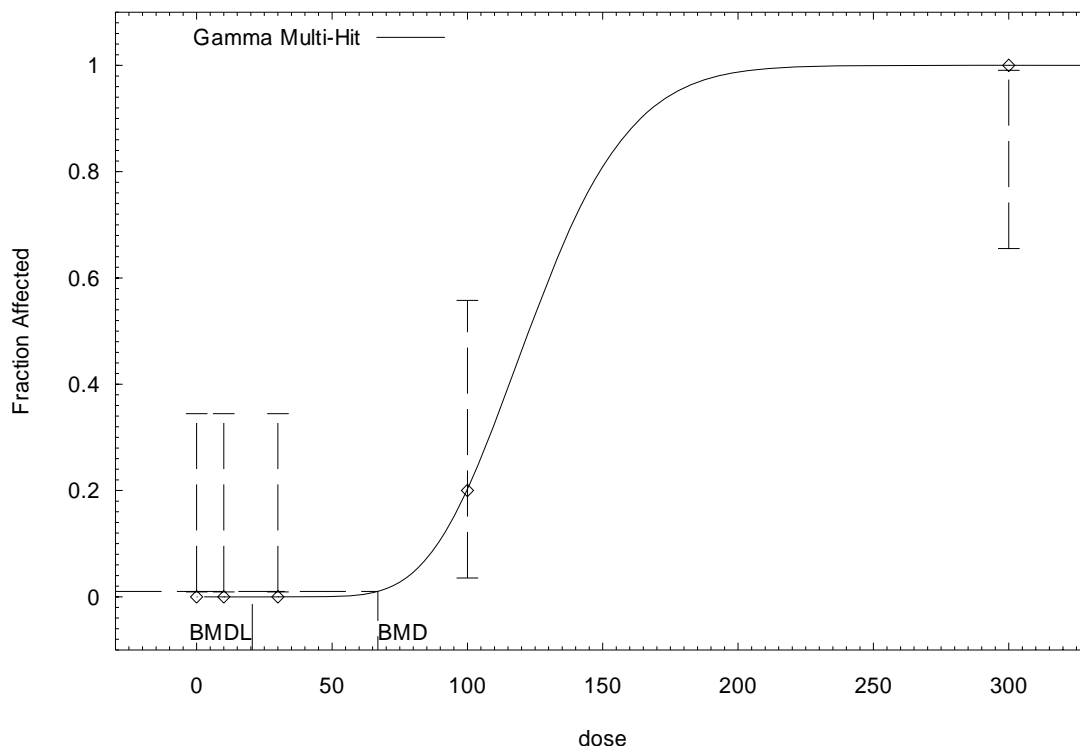
where CumGamma(.) is the cumulative Gamma distribution function

$$\text{background} = 0$$

$$\text{slope} = 0.0143677 \text{ (se} = 0.0164918\text{)}$$

$$\text{power} = 18$$

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**

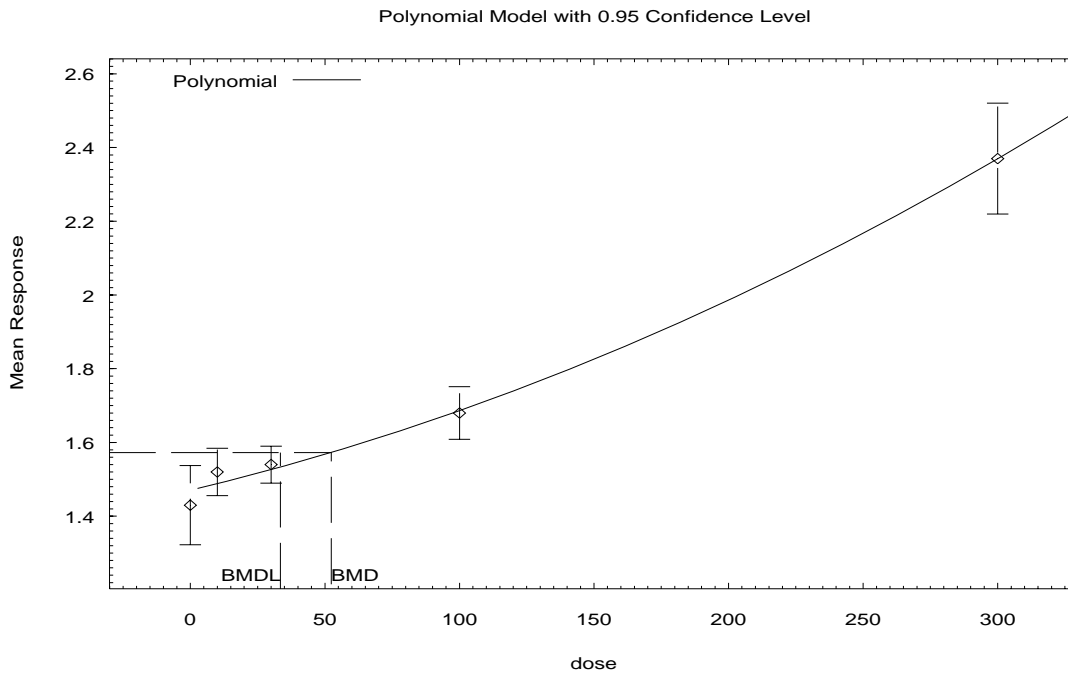
2 Absolute liver weight and liver-to-body weight ratio (relative liver weight) in female  
 3 mice were also considered as potential bases of the RfC. All available models in the EPA  
 4 BMDS (version 1.3.2) were fit to absolute liver weight data and liver-to-body weight ratio for  
 5 female B6C3F1 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<sub>1sd</sub>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<sub>0.5sd</sub> and BMCL<sub>0.5sd</sub> 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 <sup>a</sup>	BMC (ppm)	BMCL <sub>1sd</sub> (ppm)	x <sup>2</sup> p-value	AIC
<b>Absolute liver weight<sup>b</sup></b>				
Linear	35.24	28.39	0.1838	-150.18
<b>Polynomial (2<sup>o</sup>)</b>	<b>52.38</b>	<b>33.51</b>	<b>0.3922</b>	<b>-151.16</b>
Polynomial (3 <sup>o</sup> )	32.67	14.45	0.2891	-149.91
Power	56.82	32.56	0.2901	-150.55
<b>Liver-to-body weight ratio<sup>b</sup></b>				
Linear	41.03	34.52	0.08619	183.82
<b>Polynomial (2<sup>o</sup>)</b>	<b>52.42</b>	<b>33.90</b>	<b>0.09284</b>	<b>182.19</b>
Polynomial (3 <sup>o</sup> )	45.52	18.56	0.09301	184.05
Power	57.55	34.12	0.07211	182.77

16 <sup>a</sup>Statistical 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 <sup>b</sup>Modeled as a continuous variable using one standard deviation as the BMR.



09:10 01/28 2005

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

4  
 5  
 6 The second-degree polynomial model form of the response function for the female mouse  
 7 absolute liver weight data is:

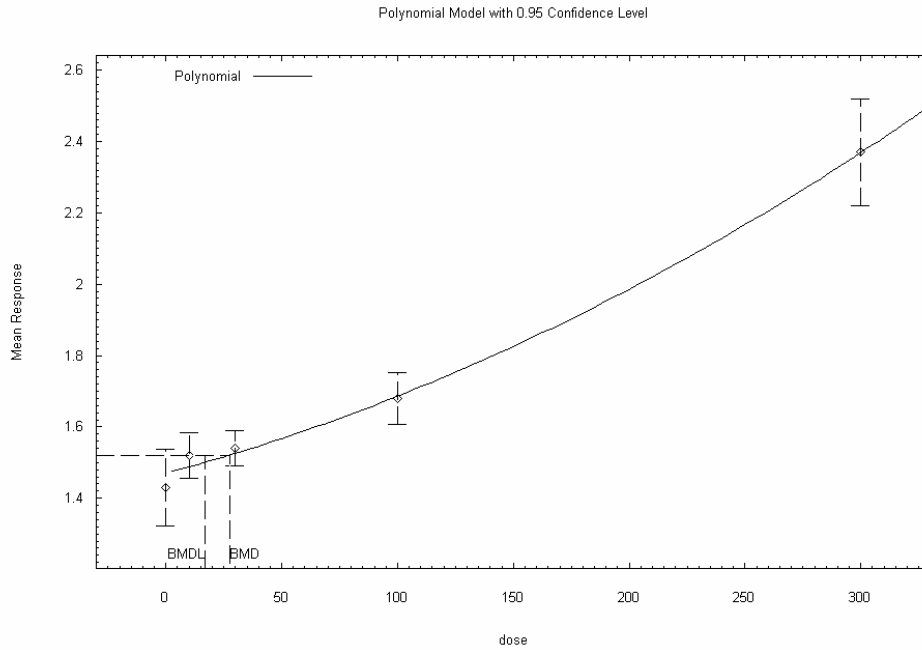
$$8 \quad Y[\text{dose}] = \text{beta}_0 + \text{beta}_1 * \text{dose} + \text{beta}_2 * \text{dose}^2 + \dots \quad (\text{Eq. C-1})$$

9  
 10 The variance was modeled as:

$$11 \quad \text{Var}(i) = \text{alpha} * \text{mean}(i)^\rho \quad (\text{Eq. C-2})$$

12

1



09:55 02/06 2006

2

3

4

5

6

**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

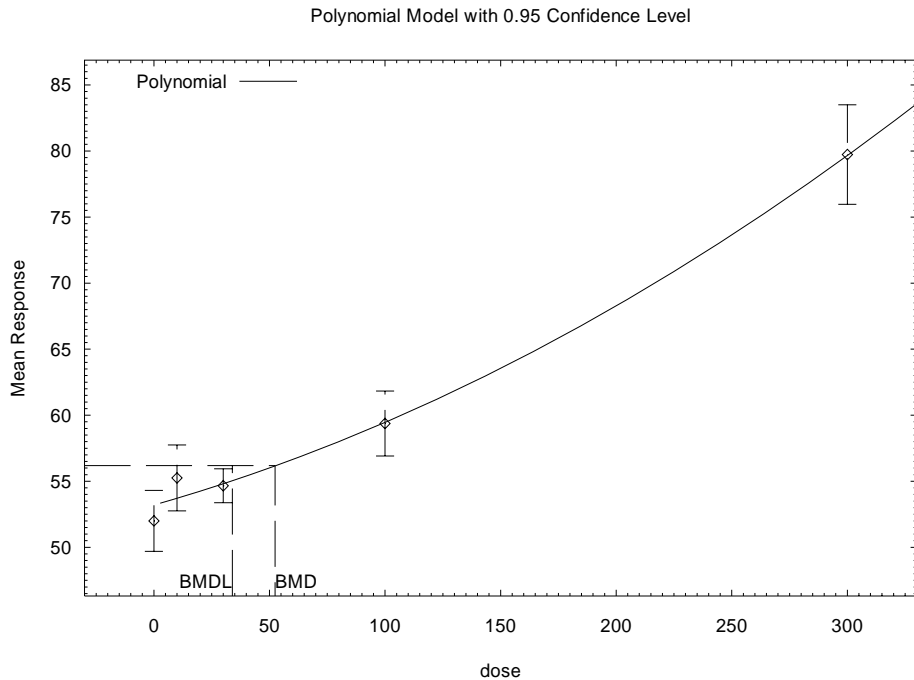
15

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

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

8

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

11

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

13

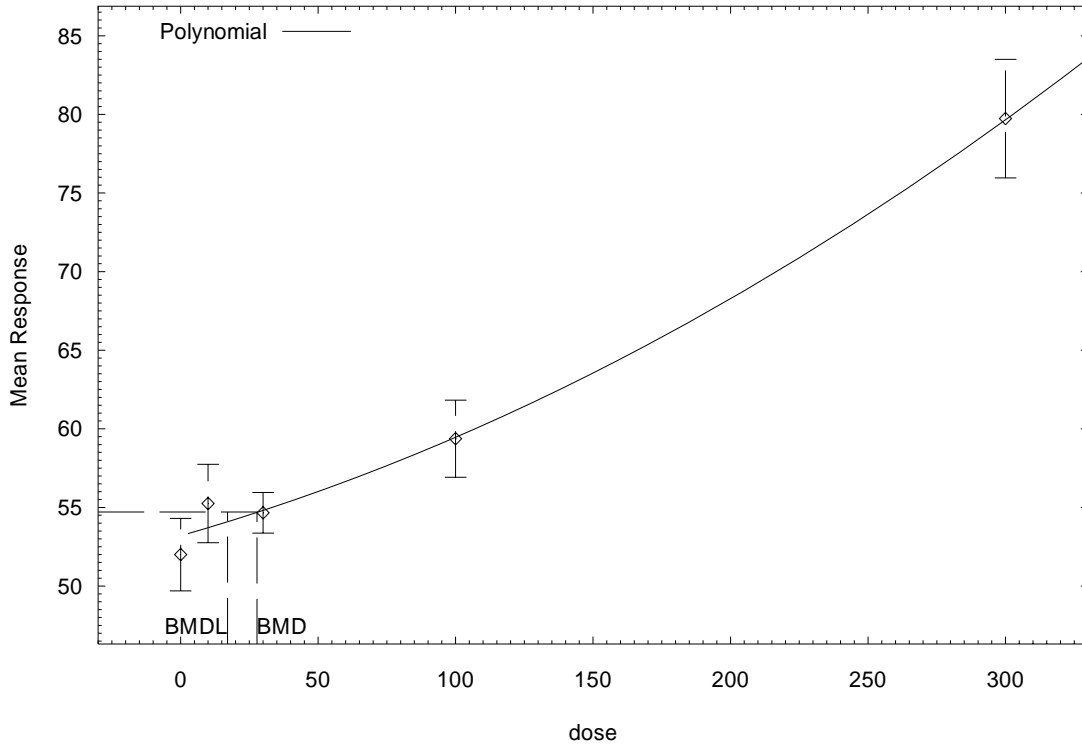
14 The variance was modeled as:

15

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

16

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**

<b>Variable</b>	<b>Estimate</b>	<b>Standard error</b>
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

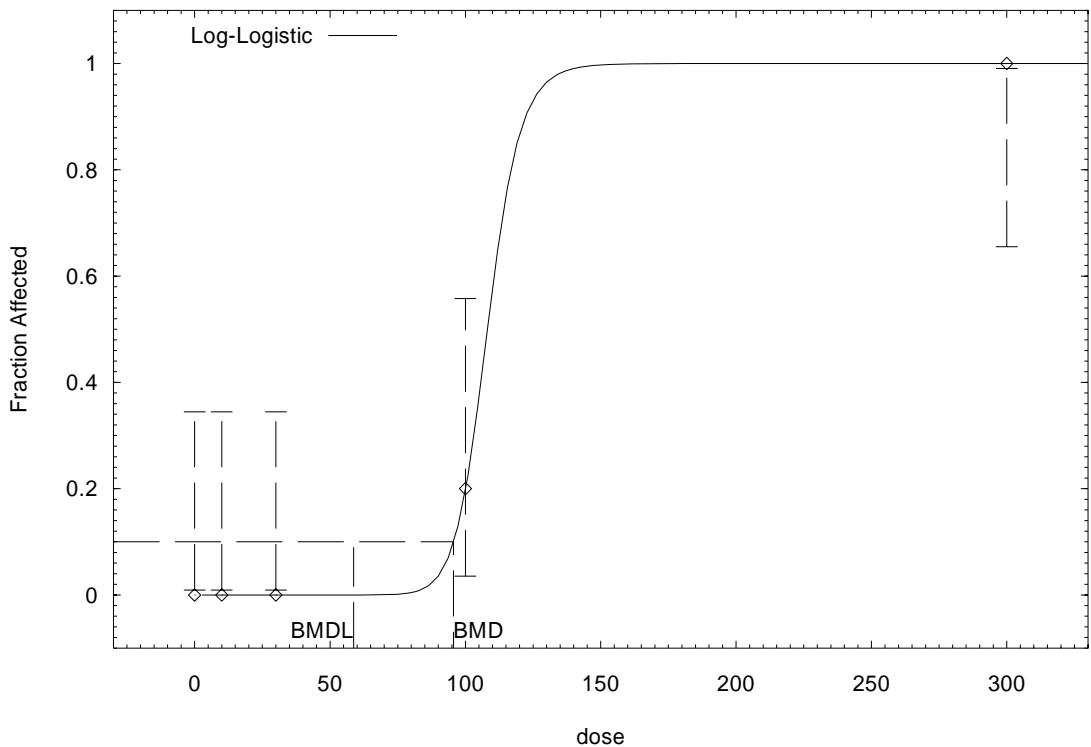
2

3



1 The average BMCL<sub>10</sub> 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.

Log-Logistic Model with 0.95 Confidence Level



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  $\geq 1$

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

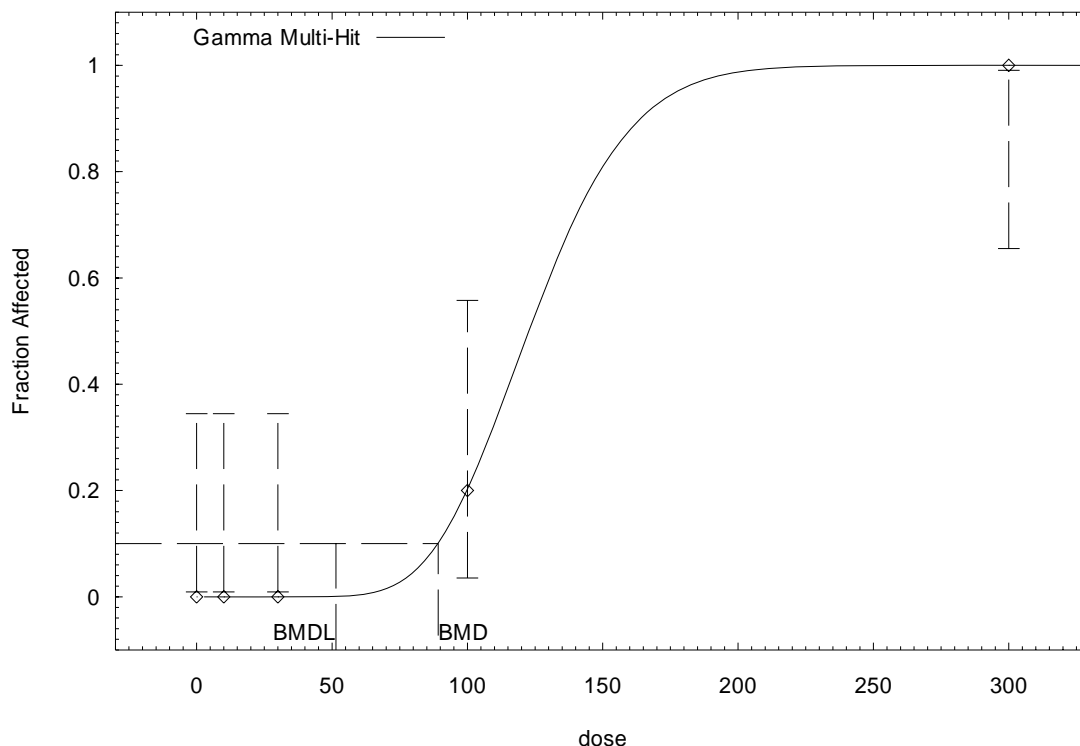
23        BMD                = 95.5947

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  $\geq 1$

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  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14

Benchmark Dose Computation

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