EPA/635/R-03/015
www.epa.gov/iris

TOXICOLOGICAL REVIEW

OF

DICHLOROBENZENES

(CAS Nos. 95-50-1, 541-73-1, 106-46-7)

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

Revised Final Draft
May 2006

NOTICE

This document is an external peer 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 policy on this chemical. It is being circulated for review of its technical accuracy and science policy implications.

U.S. Environmental Protection Agency
Washington, DC

NOTICE

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 for DICHLOROBENZENES
(CAS Nos. 95-50-1, 541-73-1, 106-46-7)

LIST OF TABLES v
LIST OF FIGURES vi
LIST OF ABBREVIATIONS AND ACRONYMS vii
FOREWORD ix
AUTHORS, CONTRIBUTORS, AND REVIEWERS x

1. INTRODUCTION 1

2. CHEMICAL AND PHYSICAL INFORMATION 3

3. TOXICOKINETICS 6
3.1. ABSORPTION 6
3.2. DISTRIBUTION 7
3.3. METABOLISM 11
3.3.1. 1,2-Dichlorobenzene 11
3.3.2. 1,3-Dichlorobenzene 14
3.3.3. 1,4-Dichlorobenzene 15
3.4. ELIMINATION 18
3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS 19

4. HAZARD IDENTIFICATION 23
4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS 23
4.1.1. Oral Exposure 23
4.1.2. Inhalation Exposure 23
4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION 26
4.2.1. Oral Exposure 26
4.2.2. Inhalation Exposure 50
4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION 58
4.3.1. Oral Exposure 58
4.3.2. Inhalation Exposure 62
4.4. OTHER STUDIES 68
4.4.1. Mechanistic Considerations 68
4.4.2. Genotoxicity 73
4.4.3. Short-term Tests of Carcinogenic Potential 79
4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS 80
4.5.1. Oral 80

4.5.2. Inhalation 92
4.5.3. Mode of Action Information 103
4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION 105
4.6.1. 1,2-Dichlorobenzene 105
4.6.2. 1,3-Dichlorobenzene 106
4.6.3. 1,4-Dichlorobenzene 106
4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES 111
4.7.1. Possible Childhood Susceptibility 111
4.7.2. Possible Gender Differences 112

5. DOSE-RESPONSE ASSESSMENTS 113
5.1. ORAL REFERENCE DOSE (RfD) 113
5.1.1. 1,2-Dichlorobenzene 113
5.1.2. 1,3-Dichlorobenzene 116
5.1.3. 1,4-Dichlorobenzene 120
5.2. INHALATION REFERENCE CONCENTRATION (RfC) 125
5.2.1. 1,2-Dichlorobenzene 125
5.2.2. 1,3-Dichlorobenzene 126
5.2.3. 1,4-Dichlorobenzene 127
5.3. CANCER ASSESSMENT 133
5.3.1. 1,2-Dichlorobenzene 133
5.3.2. 1,3-Dichlorobenzene 133
5.3.3. 1,4-Dichlorobenzene 133
5.3.4. Sources of Uncertainty 142

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE 143
6.1. HUMAN HAZARD POTENTIAL 143
6.1.1. 1,2-Dichlorobenzene 143
6.1.2. 1,3-Dichlorobenzene 144
6.1.3. 1,4-Dichlorobenzene 144
6.2. DOSE RESPONSE 146
6.2.1. Noncancer/Oral 146
6.2.2. Noncancer/Inhalation 147
6.2.3. Cancer/Oral and Inhalation 148

7. REFERENCES 150

APPENDIX A A-1

APPENDIX B B-1

LIST OF TABLES

Table 2-1. Chemical identity of dichlorobenzene isomers 4
Table 2-2. Physical and chemical properties of dichlorobenzene isomers 5
Table 3-1. Tissue concentrations of ^{14}C in male Wistar rats at four time points after oral administration of 10 mg/kg ^{14}C-labeled 1,2-dichlorobenzene in corn oil 8
Table 3-2. Tissue concentrations of radioactivity in female CFY/Sprague-Dawley rats during and after exposure to up to 10 consecutive oral 250 mg/kg doses of ^{14}C-labeled 1,4-dichlorobenzene 10
Table 3-3. Parameters in PBPK models for 1,2-dichlorobenzene 21
Table 4-1. Absolute and relative liver weights of female and male beagle dogs exposed to 1,4-dichlorobenzene in gelatin capsules 39
Table 4-2. Summary of liver histopathology incidence in female and male beagle dogs exposed to 1,4-dichlorobenzene in gelatin capsules 40
Table 4-3. Liver lesions in B6C3F$_1$ mice treated with 1,4-dichlorobenzene by gavage for two years 45
Table 4-4. Incidence of neoplastic lesions in mice treated with 1,4-dichlorobenzene via inhalation 57
Table 4-5. Results of selected genotoxicity studies of 1,2-dichlorobenzene 75
Table 4-6. Results of selected genotoxicity studies of 1,3-dichlorobenzene 76
Table 4-7. Results of selected genotoxicity studies of 1,4-dichlorobenzene 78
Table 4-8. Critical effect levels in subchronic, chronic, and developmental oral studies of 1,2-dichlorobenzene 81
Table 4-9. Critical effect levels in subchronic and developmental oral studies of 1,3-dichlorobenzene 84
Table 4-10. Critical effect levels in subchronic, chronic, developmental and reproductive oral studies of 1,4-dichlorobenzene 86
Table 4-11. Critical effect levels in subchronic, developmental and reproductive inhalation studies of 1,2-dichlorobenzene 94
Table 4-12. Critical effect levels in subchronic, chronic, developmental and reproductive inhalation studies of 1,4-dichlorobenzene 100
Table 5-1. Liver and thyroid effects observed in male rats orally exposed to 1,3-dichlorobenzene for 90 days 117
Table 5-2. Summary of BMD analyses for 1,4-dichlorobenzene 131
Table 5-3. Tumor incidence data used for dose-response assessment for 1,4-dichlorobenzene 134
Table 5-4. HEDs corresponding to average daily doses using a body weight scaling factor and time-weighted average body weights for male and female mice 135
Table 5-5. Cancer slope factor based on combined hepatocellular adenoma or carcinoma in male and female B6C3F$_1$ mice (NTP, 1987) 136
Table 5-6. Incidence data for tumors in mice exposed by inhalation 138
Table B-1. Incidence of tubular regeneration in the kidneys of male mice orally exposed to 1,2-dichlorobenzene for 103 weeks B-1
Table B-2. BMD modeling of incidence data for tubular regeneration in the kidneys

of male mice exposed to 1,2-dichlorobenzene for 103 weeks B-2
Table B-3. Incidence of reduced follicular colloidal density of the thyroid in male rats orally exposed to 1,3-dichlorobenzene for 90 days B-5
Table B-4. BMD modeling of incidence data for reduced follicular colloidal density of the thyroid in male rats exposed to 1,3-dichlorobenzene B-6
Table B-5. Summary of incidence of diffuse hepatocellular hypertrophy in male and female beagle dogs exposed to 1,4-dichlorobenzene in gelatin capsules B-9
Table B-6. BMD modeling of incidence data for diffuse hepatocellular hypertrophy in male and female beagle dogs (combined) exposed to 1,4-dichlorobenzene B-10
Table B-7. Incidence of eosinophilic changes in the olfactory epithelium in female rats . . . B-13
Table B-8. BMC analysis for eosinophilic changes in the olfactory epithelium in female rats B-14
Table B-9. Incidence of mineralization of the testes in male mice B-17
Table B-10. BMD modeling of mineralization in the testes of male mice B-18
Table B-11. Tumor incidence data used for dose-response assessment for 1,4-dichlorobenzene B-21
Table B-12. Results of multistage modeling of mouse tumor incidence B-22
Table B-13. Incidence data for tumors in mice exposed by inhalation B-28
Table B-14. Results of multistage modeling of mouse inhalation tumor data B-29

LIST OF FIGURES

Figure 3-1. Metabolism of 1,2-dichlorobenzene. 12
Figure 3-2. Metabolism of 1,3-dichlorobenzene. 14
Figure 3-3. Metabolism of 1,4-dichlorobenzene. 16

LIST OF ABBREVIATIONS AND ACRONYMS

AIC	Akaike Information Criterion
AP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMC	Benchmark concentration
BMCL	The lower 95% bound of the benchmark concentration
BMD	Benchmark dose
BMDL	The lower 95% bound of the benchmark dose
BMDS	Benchmark dose software
BMR	Benchmark response
BrdU	Bromodeoxyuridine
BUN	Blood urea nitrogen
BW	Body weight
CAS	Chemical Abstracts Service
CFR	Cumulative replicating fraction
CHO	Chinese hamster ovary
CI	Confidence interval
CYP	Cytochrome
p,p′-DDE	1,1-Dichloro-2,2 bis(p-chlorophenyl)ethylene
DEN	Diethylnitrosamine
DER	Data Evaluation Record
EPA	Environmental Protection Agency
EROD	7-Ethoxyresorufin O-deethylase
GGT	γ-Glutamyl transpeptidase
GLP	Good laboratory practice
GOFP	Goodness-of-fit *p*-value
GS	Glutamine synthetase
GSH	Glutathione (reduced)
GSSG	Glutathione disulphide (oxidized)
$H_{b/g}$	Blood:gas partition coefficients
HCT	Hematocrit
HEC	Human equivalent concentration
HED	Human equivalent dose
HSDB	Hazardous Substances Data Bank
IARC	International Agency for Research on Cancer
ICI	Imperial Chemical Industries
Ig	Immunoglobulin
i.p.	Intraperitoneal
IRIS	Integrated Risk Information System
JBRC	Japan Bioassay Research Center
Km	Michaelis constant
LD_{50}	Lethal Dose

LDH	Lactate dehydrogenase
LED	The lower 95% bound of the HEC
LOAEL	Lowest-observed-adverse-effect level
MCV	Mean corpuscular volume
MLE	Maximum likelihood estimates
MOA	Mode of action
MTD	Maximum tolerated dose
NAG	β-N-acetylglucosaminidase
NHL	Non-Hodgkin lymphoma
NIOSH	National Institute for Occupational Safety and Health
NOAEL	No-observed-adverse-effect level
NTA	Trisodium nitrilotriacetic acid
NTP	National Toxicology Program
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
PBPK	Physiologically based pharmacokinetic
PROD	7-Pentoxyresorufin O-dependentylase
RBC	Red blood cell
RfC	Reference concentration
RfD	Reference dose
RGDR	Regional gas dose ratio
RTECS	Registry of Toxic Effects of Chemical Substances
SA	Surface area
SEM	Standard error of the mean
SD	Standard deviation
SMR	Standardized mortality ratio
T_3	Triiodothyronine
T_4	Thyroxine
TMP	2,2,4-Trimethylpentane
TSCA	Toxic Substances Control Act
UCL	Upper confidence limit
UF	Uncertainty factor
V_E	minute volume
Vmax	Maximum substrate turnover velocity
WOE	Weight-of-evidence

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

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

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

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Audrey Galizia, Dr.PH
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Edison, NJ

AUTHORS

Audrey Galizia, Dr.PH
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Edison, NJ

Stephen Bosch, B.S.
Environmental Science Center
Syracuse Research Corporation
Syracuse, NY

Marc Odin, M.S., DABT
Environmental Science Center
Syracuse Research Corporation
Syracuse, NY

Mark Osier, Ph.D., DABT
Environmental Science Center
Syracuse Research Corporation
Syracuse, NY

CONTRIBUTORS

Susan Rieth, MPH
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Karen Hogan, MS
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Chandrika Moudgal
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Cincinnati, OH

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

Elin Warn
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency
Washington, DC

Nicole Paquette
Analysis Support Branch, Office of Environmental Information
U.S. Environmental Protection Agency
Washington, DC

Jim Holder

National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Charles Ris
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Femi Adeshina
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Dharm Singh
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

EXTERNAL PEER REVIEWERS

Lynne Haber, Ph.D., Chair
Technology Excellence for Risk Assessment
Cincinnati, OH

Kenny Crump, Ph.D.
Environ Corporation
Ruston, Louisiana

Michael Muller, PhD
ANZAC Research Institute
Sydney, Australia

Harihara M. Mehendale, Ph.D.
The University of Louisiana at Monroe

Glenn Sipes I, Ph.D.
The University of Arizona

Summaries of the external peer reviewers' comments and public comments, and the disposition of their recommendations are provided in Appendix A.

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 dichlorobenzenes. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values, and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects).

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

Development of these hazard identification and dose-response assessments for dichlorobenzene isomers has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk*

Assessment (U.S. EPA, 1998a), *Guidelines for Carcinogen Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2000a, 1998b), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000d), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through October 2005.

2. CHEMICAL AND PHYSICAL INFORMATION

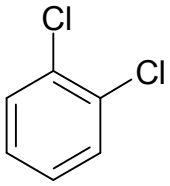
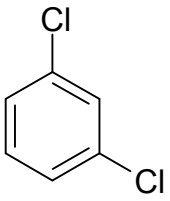
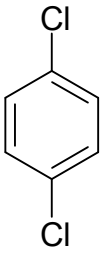
The three dichlorobenzene isomers are 1,2-dichlorobenzene, 1,3-dichlorobenzene, and 1,4-dichlorobenzene (also referred to as ortho-, meta-, and para-dichlorobenzene, respectively). Additional information on their chemical identity is shown in Table 2-1. Physical and chemical properties of the dichlorobenzene isomers are shown in Table 2-2.

Dichlorobenzenes are produced in an isomeric mixture from the reaction of liquid benzene with chlorine gas in the presence of a catalyst at moderate temperature and atmospheric pressure. By altering the reaction conditions and changing the catalyst, the ratio of different chlorinated products can be varied. 1,2-Dichlorobenzene and 1,4-dichlorobenzene are the major dichlorobenzene isomers formed. In a preparation using ferric chloride and sulfur monochloride (S_2Cl_2), a yield of approximately 75% 1,4-dichlorobenzene, 25% 1,2-dichlorobenzene and 0.2% 1,3-dichlorobenzene is obtained (Rossberg et al., 2002; IARC, 1999).

Dichlorobenzenes are used primarily as reactants in chemical synthesis, as process solvents, and as formulation solvents (International Agency for Research on Cancer [IARC], 1999; U.S. EPA, 1981). 1,2-Dichlorobenzene is used in the production of 3,4-dichloroaniline, a base material for herbicides; as a solvent for waxes, gums, resins, tars, rubbers, oils, and asphalts; as an insecticide for termites and locust borers; as a degreasing agent for metals, leather, paper, dry-cleaning, bricks, upholstery, and wool; as an ingredient in metal polishes; in motor oil additive formulations; and in paints (IARC, 1999; U.S. EPA, 1981). 1,3-Dichlorobenzene is used in the production of herbicides, insecticides, pharmaceuticals, and dyes (IARC, 1999; U.S. EPA, 1981). 1,4-Dichlorobenzene is used in air fresheners, as moth repellent in moth balls or crystals, as well as other pesticide applications. 1,4-Dichlorobenzene is also used in the manufacture of 2,5-dichloroaniline and pharmaceuticals, polyphenylene sulfide resins, and in the control of mildew (IARC, 1999; U.S. EPA, 1981).

Production of 1,2-dichlorobenzene in the United States decreased from 24,700 tons in 1975 to 15,800 tons in 1993. Production of 1,4-dichlorobenzene, however, increased from 6800 tons in 1981 to approximately 32,600 tons in 1993. Production of 1,3-dichlorobenzene in the United States during 1983 was less than 500 tons (IARC, 1999). Estimates of U.S. commercial consumption in 1978 indicated negligible consumption of 1,3-dichlorobenzene (<1 kg), about 27,000 kg for 1,2-dichlorobenzene, and about 34,000 kg for 1,4-dichlorobenzene (U.S. EPA, 1981).

Table 2-1. Chemical identity of dichlorobenzene isomers

Characteristic	Dichlorobenzene isomer			Reference
Chemical Name	1,2-Dichlorobenzene	1,3-Dichlorobenzene	1,4-Dichlorobenzene	Lide, 2000
Synonyms	o-Dichlorobenzene; o-Chlorophenyl chloride; PDB; o-Dichlorobenzol; AI3-00053	m-Dichlorobenzene; m-Phenylene dichloride; m-DCB; m-Dichlorobenzol	p-Dichlorobenzene; p-Chlorophenyl chloride; PDB; p-Dichlorobenzol; A13-0050	HSDB, 2005
Trade names	Caswell No 301; Chloroben; Cloroben; Dilatin DB; Dowtherm E	No data	Caswell No 632; Di-chloricide; Evola; Paradi; Paramoth; Paradow; Parazene; Persia-Perazol; Santochlor	HSDB, 2005 Budavari, 1989
Chemical formula	$C_6H_4Cl_2$	$C_6H_4Cl_2$	$C_6H_4Cl_2$	
Chemical structure				Verschueren, 2001
CAS Registry	95-50-1	541-73-1	106-46-7	Budavari et al., 2001
NIOSH RTECS	CZ4500000	CZ4499000	CZ4550000	HSDB, 2005
EPA Hazardous Waste	U070; F002	U071	U072; D027	HSDB, 2005
EPA Pesticide Chemical Code	059401	No data	061501	HSDB, 2005
OHM/TADS	No data	No data	No data	

CAS = Chemical Abstracts Service.
EPA = Environmental Protection Agency.
NIOSH = National Institute for Occupational Safety and Health.
HSDB = Hazardous Substances Data Bank.
OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System.
RTECS = Registry of Toxic Effects of Chemical Substances.

Table 2-2. Physical and chemical properties of dichlorobenzene isomers

Property	1,2-Dichlorobenzene	1,3-Dichlorobenzene	1,4-Dichlorobenzene	Reference
Molecular weight	147.00	147.00	147.00	Lide, 2000
Color	Colorless	Colorless	White	Lewis, 1997
Physical state	Liquid	Liquid	Solid	Verschueren, 2001
Melting point	-16.7 °C	-24.8 °C	52.7 °C	Lide, 2000
Boiling point	180 °C	173 °C	174 °C	Lide, 2000
Density at 20 °C	1.3059 g/mL	1.2884 g/mL	1.2475 g/mL	Lide, 2000
Odor	Pleasant, aromatic	No data	Mothball-like	NIOSH, 1997
Odor threshold: Water Air	 0.01 mg/L 50 ppm	 No data 0.02 ppm	 0.003 mg/L 15-30 ppm	 Verschueren, 2001;Weiss, 1986 Verschueren, 2001
Solubility: Water Organic solvents	 145 mg/L at 25 °C Soluble in alcohol and ether; miscible in acetone	 123 mg/L at 25 °C Soluble in alcohol and ether; miscible in acetone	 79 mg/L at 25 °C Soluble in alcohol; miscible in ether and acetone	 Verschueren, 2001 Budavari et al., 2001 Lide, 2000
Partition coefficients: Log octanol/water Log Koc	 3.43 2.51	 3.53 2.47	 3.44 2.44	 Hansch et al., 1995 Chiou et al., 1993
Vapor pressure at 20 °C	1 mm Hg	2.3 mm Hg	0.6 mm Hg	Verschueren, 2001
Henry's law constant	1.5×10^{-3} atm-m^3/mol	2.83×10^{-3} atm-m^3/mol	2.7×10^{-3} atm-m^3/mol	Staudinger and Roberts, 1996
Autoignition temperature	648.8 °C	No data	No data	Weiss, 1986
Flashpoint	73.9 °C (open cup); 68.3 °C (closed cup)	No data	73.9 °C (open cup); 68.3 °C (closed cup)	Weiss, 1986
Flammability limits	2.2-9.2%	No data	No data	Weiss, 1986
Conversion factor	1 mg/m^3 = 0.166 ppm 1ppm = 6.01 mg/m^3	1 mg/m^3 = 0.166 ppm 1ppm = 6.01 mg/m^3	1 mg/m^3 = 0.166 ppm 1ppm = 6.01 mg/m^3	

3. TOXICOKINETICS

3.1. ABSORPTION

Quantitative data on the extent or rate of absorption of dichlorobenzene isomers in humans following oral or dermal exposure are not available. Data on the absorption of inhaled 1,4-dichlorobenzene by humans were reported by Yoshida et al. (2002a). In seven male volunteers exposed to 2.5 ppm 1,4-dichlorobenzene for 1 hour, mean pulmonary retention was 56 $\pm$ 9% (Yoshida et al., 2002a). Qualitative evidence of absorption in humans comes from reports of the detection of dichlorobenzenes or their metabolites in samples of human breast milk (Mes et al., 1986; Jan, 1983), blood (Hill et al., 1995; Bristol et al., 1982), and urine (Kumagai and Matsunaga, 1997, 1995; Zenser et al., 1997; Hill et al., 1995; Ghittori et al., 1985; Pagnotto and Walkley, 1965). For example, 1,4-dichlorobenzene was detected at concentrations ranging from about 44 to 126 μg/L in urine collected from workers at the end of work shifts (Ghittori et al., 1985). In this study, the mean time-weighted average workplace air concentration of 1,4-dichlorobenzene in the breathing zone was 44.72 mg/m^3 (7.4 ppm). Urinary levels of parent compound or metabolites have been proposed for use as biomarkers of exposure (i.e., markers of absorbed and excreted compound) for workers exposed to 1,2-dichlorobenzene (Kumagai and Matsunaga, 1997, 1995; Zenser et al., 1997) or 1,4-dichlorobenzene (Yoshida et al., 2002b; Ghittori et al., 1985; Pagnotto and Walkley, 1965).

Results from animal studies suggest that 1,2- and 1,4-dichlorobenzene are extensively and rapidly absorbed by the gastrointestinal tract (Bomhard et al., 1998; Hissink et al., 1997a, 1996a, b; Schmidt and Löser, 1977; Azouz et al., 1955). For example, in male Wistar rats given single oral doses of ^{14}C-labeled 1,2-dichlorobenzene, radioactivity in urine collected for up to 175 hours after dosing accounted for about 75, 84, and 75% of the radioactivity for administered doses of 5, 50, and 250 mg/kg body weight, respectively (Hissink et al., 1996a, b). Radioactivity in feces accounted for about 16, 12, and 7% of the respective administered doses. These results indicate that at least 75-84% of the administered dose (assuming that none of fecal radioactivity was absorbed), and up to 82-96% of the dose (assuming that all fecal radioactivity was absorbed and excreted in the bile), was absorbed. Rapid absorption was indicated since peak levels of radioactivity in blood samples occurred at about 6, 10, and 24 hours after administration of 5, 50, and 250 mg/kg doses, respectively (Hissink et al., 1996a, b). In a similarly designed experiment, comparable results were obtained for male Wistar rats given single oral doses of ^{14}C-labeled 1,4-dichlorobenzene (Hissink et al., 1997a). In this study, peak levels of radioactivity in blood

samples appeared to occur at earlier times: about 3, 5, and 8 hours after dosing with 10, 50, and 250 mg/kg, respectively. Radioactivity in urine and feces accounted for about 80% and 4%, respectively, of the administered radioactivity at each dose level (Hissink et al., 1997a). For both of these isomers, radioactivity in exhaled air collected for 24 hours after dose administration accounted for <1% of the administered radioactivity (Hissink et al., 1997a, 1996a, b).

Quantitative oral absorption data for 1,3-dichlorobenzene are not available, but absorption characteristics are likely to be similar to those of the other isomers based on similarities in chemical and physical properties.

Qualitative indication of absorption by the respiratory tract has been reported in several studies of rats exposed to 1,4-dichlorobenzene by inhalation (Umemura et al., 1990, 1989; Hawkins et al., 1980). In female CFY Sprague-Dawley rats exposed to 1000 ppm ^{14}C-labeled 1,4-dichlorobenzene 3 hours/day for up to 10 days, radioactivity was detected in plasma, fat, muscle, lungs, kidneys, and liver after 2, 4, 6, 8, and 10 days of exposure (Hawkins et al., 1980). Likewise, in male F344/DuCrj rats exposed by inhalation to 125 or 500 ppm 1,4-dichlorobenzene for 24 hours, concentrations of 1,4-dichlorobenzene in serum, liver, kidney, and fat rose through the exposure period, reached maximal values 3-6 hours after exposure cessation, and declined thereafter (Umemura et al., 1989). The reported results in these rat studies, however, are inadequate to determine the fraction of inhaled compound that was absorbed.

No data were located regarding the extent and rate of absorption of dichlorobenzene isomers in animals following dermal exposure.

3.2. DISTRIBUTION

Information on the distribution of dichlorobenzene isomers in humans is not available, but results from studies of rats orally exposed to ^{14}C-labeled 1,2- or 1,4-dichlorobenzene indicate the following distributional events after absorption from the gastrointestinal tract: 1) translocation of parent compound to the liver where considerable metabolism occurs; 2) biliary excretion and intestinal reabsorption of metabolites (i.e., enterohepatic circulation); 3) eventual translocation of most metabolites to the kidney for elimination via the urine; 4) temporary storage of parent compound in fat when metabolism is saturated; and 5) minor distribution of parent compound or metabolites to tissues other than fat, kidney, and liver.

No information is available on the distribution of 1,3-dichlorobenzene in animals exposed by any route.

Consistent with events numbered 1, 3, and 5 above are the observations that, 6 hours after dosing rats with 10 mg/kg ^{14}C-labeled 1,2-dichlorobenzene, the highest tissue concentrations of

radioactivity were found in the urinary bladder, kidney, liver, and perirenal fat, and lower concentrations were found in the remaining tissues (Hissink et al., 1996a; see Table 3-1). Radioactivity was rapidly eliminated from all tissues following cessation of exposure. First-order elimination half-times for the various tissues ranged from 8.7 to 19.3 hours (Table 3-1), indicating that no significant storage of parent compound or metabolites occurs in any specific tissue at low doses.

Table 3-1. Tissue concentrations of ^{14}C in male Wistar rats at four time points after oral administration of 10 mg/kg ^{14}C-labeled 1,2-dichlorobenzene in corn oil

Tissue	6 hours	15 hours	30 hours	75 hours	Elimination half-time (assuming 1st order kinetics)
	nmol/g Tissue				Hours
Urinary bladder	183	17	7	0.3	8.7
Kidney	133	16	4	2	13.1
Liver	33	9	3	1	17.0
Perirenal fat	33	14	2	0.2	9.4
Small intestine	29	11	4	0.4	11.6
Plasma	22	9	2	0.4	12.5
Skin	19	3	1	0.4	15.1
Caecum	16	17	3	0.3	11.1
Pancreas	10	3	1	0.2	14.5
Red blood cells	9	3	2	0.6	18.8
Spleen	8	2	0.6	0.2	15.2
Lung	7	3	1	0.3	16.0
Colon	8	12	1	0.2	12.0
Stomach	7	2	1	0.2	14.3
Femur	5	1	0.6	0.1	15.1
Skeletal muscle	5	1	0.5	0.1	9.4
Heart	5	3	0.7	0.2	15.1
Testis	4	2	1	0.2	17.2
Brain	1	0.7	0.3	0.1	19.3
	% of administered dose				
Residual carcass	13%	4%	1%	0.3%	Not determined
Gastrointestinal contents	13%	15%	2%	0.1%	Not determined

Source: Hissink et al., 1996a.

Some storage of parent material or metabolites may occur after exposure to high doses (event number 4 above), as indicated by the lower percentage of radioactivity recovered in urine and feces within 175 hours of administration of a high (250 mg/kg) dose of ^{14}C-labeled 1,2-dichlorobenzene (82%) compared with a low (10 mg/kg) dose (96%) in rats (Hissink et al., 1996a). Unfortunately, tissue distribution data like that in Table 3-1 are not available for other dose levels of 1,2-dichlorobenzene. Such data would confirm the hypothesis that the parent compound is temporarily stored in fat tissue. The Hissink et al. (1996a) study, however, provides indirect evidence that metabolism of 1,2-dichlorobenzene is saturated after a high dose, and that temporary storage of the nonmetabolized parent compound in fat may have occurred. Blood concentrations of parent compound showed a dramatic (>10-fold) drop within 1-2 hours of administration of a 10-mg/kg dose, but showed plateaus following administration of 50-mg/kg (for 3-4 hours) or 250-mg/kg (for 8-10 hours) doses before precipitously dropping thereafter. With the two lower doses, concentrations of total radioactivity in blood showed plateaus between about 3 and 10 hours before declining thereafter. In contrast, after administration of the 250-mg/kg dose, radioactivity concentrations in blood continued to rise for 24 hours before declining thereafter.

More direct support for the temporary storage of parent compound in fat comes from a study in which female CFY/Sprague-Dawley rats were given up to 10 consecutive daily oral doses of 250 mg/kg ^{14}C-labeled 1,4-dichlorobenzene in sunflower oil (Hawkins et al., 1980). Concentrations of radioactivity were determined in several tissues from two animals sacrificed at each of several intervals during the exposure period, and from one animal sacrificed at each of several intervals up to 192 hours after exposure (Table 3-2). The highest tissue concentrations of radioactivity were attained in fatty tissue, followed in decreasing order by concentrations in kidneys, liver, lungs, plasma, and muscle (Table 3-2). Illustrating the temporary nature of the storage of parent compound or metabolites at this fairly high dose level, radioactivity was essentially completely eliminated from all tissues within 120-196 hours of the administration of the last dose (Table 3-2).

Table 3-2. Tissue concentrations of radioactivity in female CFY/Sprague-Dawley rats during and after exposure to up to 10 consecutive oral 250 mg/kg doses of ^{14}C-labeled 1,4-dichlorobenzene

	Fat[a]	Kidney[a]	Liver[a]	Plasma[a]	Lung[a]	Muscle[a]
Number of doses						
2	218	27	11	13	7	5
4	369	29	18	14	13	6
6	170	23	14	12	10	<0.2
8	131	18	15	9	11	8
10	257	16	9	8	9	4
Hours after last dose						
0.5	401	74	117	38	58	12
2	630	81	75	46	347	no sample
4	1423	149	90	48	106	no sample
8	1385	123	101	43	75	23
24	559	31	31	18	13	11
48	56	3	7	2	3	0.2
96	8	2	2	<0.2	2	<0.2
120	<0.2	<0.2	<0.2	<0.2	4	<0.2
192	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2

[a] Concentrations are expressed as ppm and are based on two rats per sacrifice interval during the exposure period and one rat per sacrifice interval after the last dose.

Source: Hawkins et al., 1980

With inhalation exposure, distribution of absorbed dichlorobenzene isomers is expected to be similar to oral exposure distribution, except that a first-pass metabolic effect is not expected. In rats exposed by inhalation to ^{14}C-labeled 1,4-dichlorobenzene (1000 ppm, 4 hours/day for up to 10 days), the patterns for tissue concentrations of radioactivity were very similar to those shown in Table 3-2 for orally exposed rats, except that fat concentrations were higher at most sacrifice intervals, compared to orally exposed rats (Hawkins et al., 1980). The latter observation is consistent with a first-pass metabolic effect following oral exposure that limits the temporary storage of absorbed parent compound in fat, but does not occur with inhalation exposure. Further support for this distribution pattern following inhalation exposure comes from an observation in male F344 DuCrj rats exposed to 500 ppm 1,4-dichlorobenzene for 24 hours, where the highest peak tissue concentrations of parent compound occurred in fat (2.5-3 mg/g) (Umemura et al., 1989). Lower peak concentrations were found in liver (~0.27 mg/g), kidney (~0.26 mg/g), and serum (~0.025 mg/mL) (Umemura et al., 1989). 1,4-Dichlorobenzene concentrations in these tissues declined to very low levels within 24 hours after exposure. This

observation supports the notion that storage of dichlorobenzene isomers in fatty tissues is temporary (i.e., the parent compounds are rapidly eliminated).

3.3. METABOLISM

Data indicate that the dichlorobenzenes are extensively metabolized, as evidenced by low or non-detectable levels of parent compound in the urine or feces in available studies. Proposed general metabolic schemes for each of the dichlorobenzene isomers are presented in Figures 3-1 to 3-3. Metabolism is believed to occur primarily in the liver, and does not appear to depend on the route of administration (Hissink et al., 1997a).

3.3.1. 1,2-Dichlorobenzene

The proposed metabolic pathway for 1,2-dichlorobenzene is shown in Figure 3-1. The initial step in 1,2-dichlorobenzene metabolism is cytochrome (CYP) P450-catalyzed oxidation of the aromatic ring, resulting in formation of an intermediate epoxide (Nedelcheva et al., 1998; Hissink et al., 1996b, c; Bogaards et al., 1995). This epoxide can either react directly with cellular proteins, be conjugated to glutathione (GSH) or glucuronic acid, or be hydrolyzed to form 2,3-dichlorophenol or 3,4-dichlorophenol (Hissink et al., 1996c). The dichlorophenol metabolites can be conjugated with GSH, glucuronic acid or sulfate, or further oxidized to catechols, hydroquinones or benzoquinones (Hissink et al., 1996c; den Besten et al., 1992). Considerable levels of secondary metabolites and only small amounts of dichlorophenols have been detected in the urine of exposed animals, indicating that the secondary metabolism is extensive (Hissink et al., 1996b; Hawkins et al., 1980).

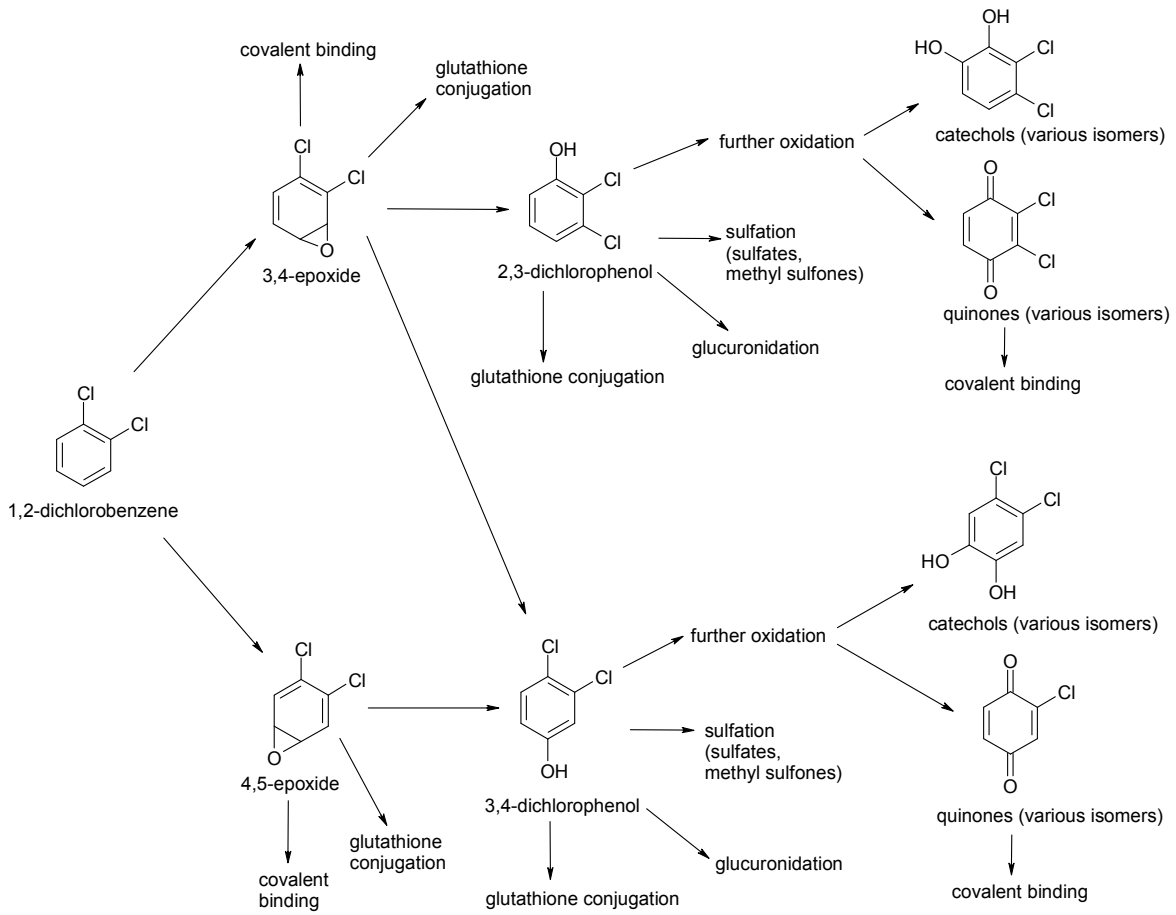


Figure 3-1. Metabolism of 1,2-dichlorobenzene.

CYP P4502E1 is the main CYP P450 isozyme involved in the oxidation of 1,2-dichlorobenzene by human liver microsomes (Nedelcheva et al., 1998; Hissink et al., 1996c; Bogaards et al., 1995). CYP1A1 and CYP1A2 in human microsomes also have shown some activity toward the formation of 1,2-dichlorophenol metabolites, but 2B6, 2C9, 2D6, 2A6, and 3A4 were inactive. In rats and mice, the main isozymes involved in the metabolism of 1,2-dichlorobenzene appear to be CYP2B1/2, with CYP2E1 and CYP3A4 also playing a role (Nedelcheva et al., 1998 ; Lake et al., 1997; Hissink et al., 1996c).

Reports concerning the extent of glucuronidation of 1,2-dichlorobenzene vary widely, with one study reporting virtually no glucuronidation in rats (Hissink et al., 1996b) and another reporting that 48% of the urinary metabolites of 1,2-dichlorobenzene following exposure in

rabbits were glucuronide conjugates (Azouz et al., 1954). It is not known whether this considerable variation results from different study conditions, interspecies variation, or other factors. Sulfation also appears to be a considerable secondary metabolic pathway, accounting for 21-30% of a single oral dose of 1,2-dichlorobenzene in rats and rabbits (Hissink et al., 1996b; Azouz et al., 1954).

In vitro studies have also identified conjugation to GSH, with subsequent metabolism to n-acetyl cysteine and mercapturic acid metabolites, as a potential metabolic pathway. However, the in vivo relevance of this pathway appears to vary considerably from study to study; the source of this variation has not been definitively demonstrated, but is possibly due to interspecies and interstrain differences in metabolism. For 1,2-dichlorobenzene, conjugation to GSH following a single administration accounted for approximately 60% of the dose in rats (Hissink et al., 1996b). In rabbits, mercapturic acid accounted for less than 10% of the urinary metabolites (Azouz et al., 1954).

A minor pathway of possible toxicological significance involves the formation of methyl sulfone metabolites. Following oxidation by CYP P450 in the liver, and possibly following sulfation, the metabolites are secreted into the bile. Within the gut, dichloromethylsulfones are formed as a result of metabolism by intestinal flora, and are then reabsorbed and transported back to the liver. While these compounds represent a proportionally small percentage of the total metabolites, they are extremely potent inducers of CYP P450 enzymes (Kato and Kimura, 1997; Larsen et al., 1990; Kato et al., 1988a, b, 1986; Kimura et al., 1985), with even small levels of methyl sulfones resulting in considerable hepatic enzyme induction.

Fisher et al. (1990) reported that in rat liver slices the majority (>70%) of 1,2-dichlorobenzene was found as GSH or cysteine conjugates, with only small amounts of glucuronide or sulfate conjugates detected. In human liver slices, the conjugation pattern was different, with approximately equal distribution between glucuronide and GSH conjugates, and only minor amounts of the sulfate. Azouz et al. (1955) identified urinary metabolites in rabbits exposed to a single dose of 1,2-dichlorobenzene. 2,3- and 3,4-Dichlorophenol were detected, as were considerable levels of glucuronide and sulfate conjugates; the presence of dihydroquinone metabolites was not reported. Kumagai and Matsunaga (1997) reported that in occupationally exposed humans urinary metabolites of 1,2-dichlorobenzene consisted of 3,4- and 4,5-dichlorocatechol and 2,3- and 3,4-dichlorophenol; there was a linear correlation between exposure concentration and the levels of these four metabolites in the urine. As with the studies in rabbits, the presence of dihydroquinone metabolites of 1,2-dichlorobenzene was not reported.

3.3.2. 1,3-Dichlorobenzene

The proposed pathway for 1,3-dichlorobenzene metabolism is shown in Figure 3-2. While comparatively few studies have characterized the metabolism of this dichlorobenzene isomer, it is believed to follow similar metabolic pathways as 1,2- and 1,4-dichlorobenzene, beginning with metabolism by CYP P450 enzymes to an epoxide, which is then further metabolized to a phenol or to a variety of conjugates.

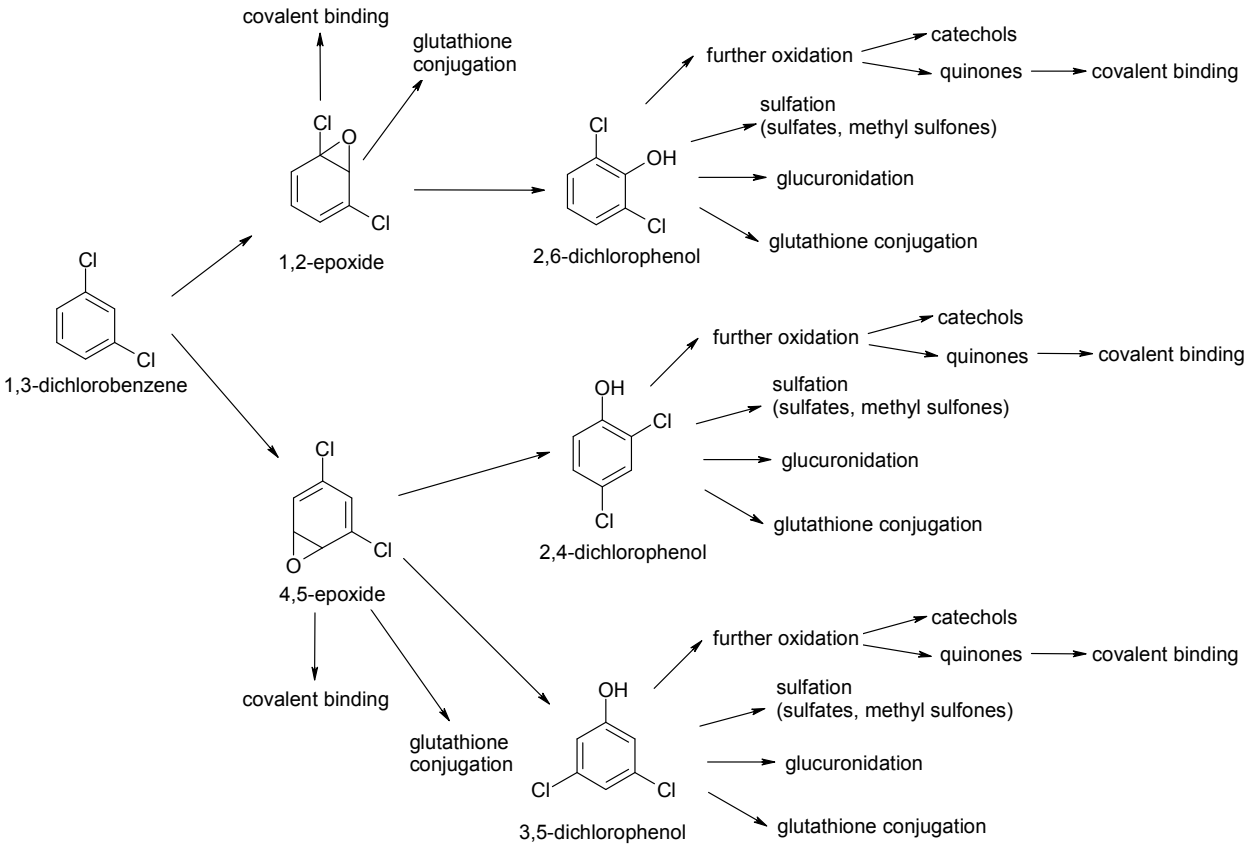


Figure 3-2. Metabolism of 1,3-dichlorobenzene.

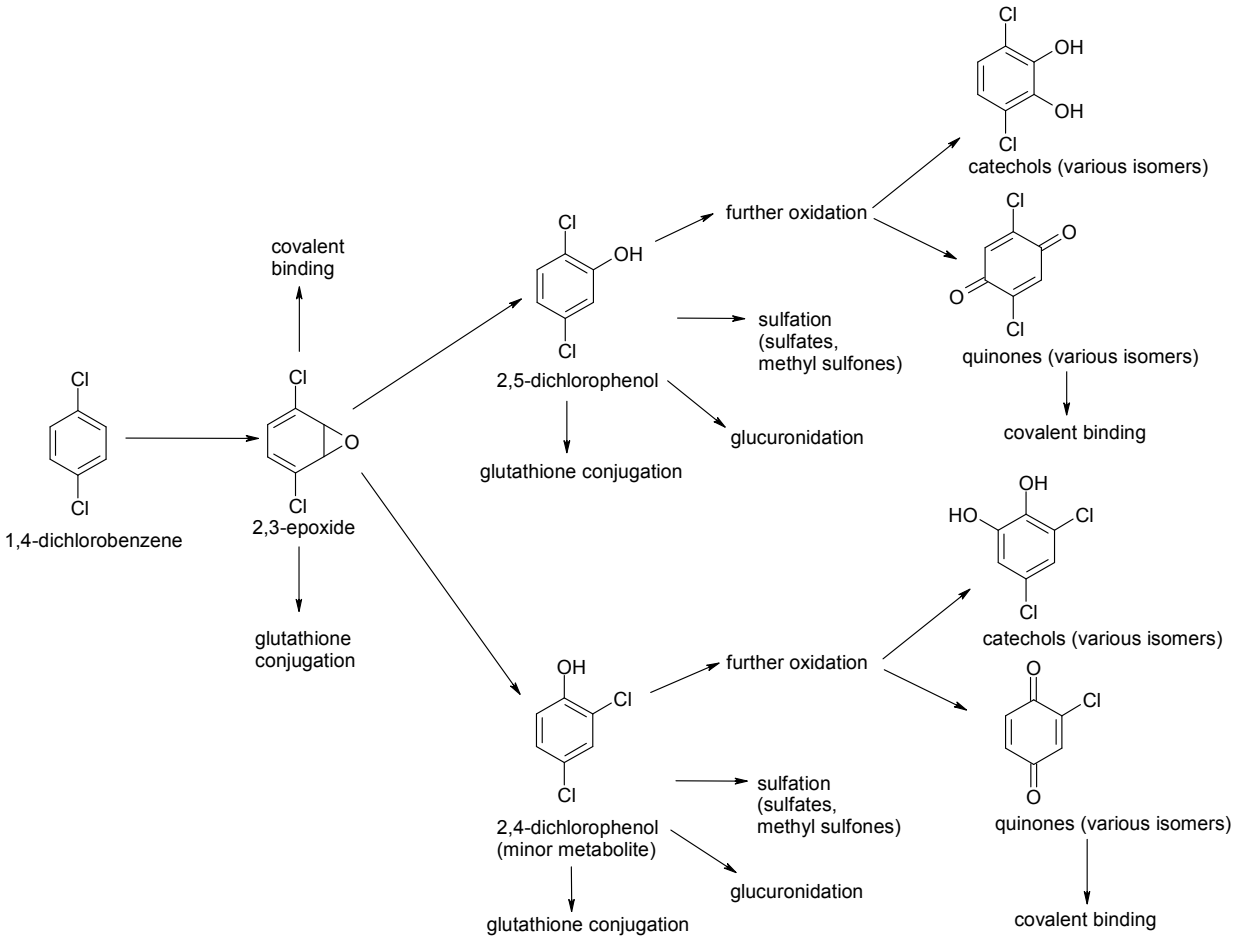
Fisher et al. (1990) reported that in rat liver slices, the majority (~70%) of 1,3-dichlorobenzene was found conjugated to GSH, or as a cysteine conjugate, with only small amounts of the glucuronide or sulfate detected. In human liver slices, the pattern was different, with approximately equal distribution (~40% each) of glucuronide and GSH conjugates, and ~20% of the metabolites as the sulfate. Following in vivo exposure of rats to 1,3-dichlorobenzene, the major sulfur-containing metabolites in the urine were 2,4- and 3,5-dichlorophenyl methyl sulfoxides and 3,5- and 2,4-dichlorophenyl methyl sulfones (Kimura

et al., 1984). Kimura et al. (1992) identified 18 different biliary metabolites in rats exposed to a single dose of 1,3-dichlorobenzene; these were all heavily conjugated dichlorophenyl metabolites, with evidence of both mono- and diol formation, but no conjugated quinone derivatives were detected.

3.3.3. 1,4-Dichlorobenzene

The proposed pathway for 1,4-dichlorobenzene metabolism is shown in Figure 3-3. The first step in the metabolism is CYP P450-catalyzed oxidation of the aromatic ring, generating an epoxide (Nedelcheva et al., 1998; Hissink et al., 1996b; Bogaards et al., 1995; den Besten et al., 1992). In humans, metabolism proceeds predominantly via the 2,3-epoxide (shown in Figure 3-3); in rats and mice, metabolism proceeds via the 1,2- and 2,3- epoxides (Muller, 2002). The epoxide can react directly with cellular proteins, can react directly or via enzymatic catalysis with GSH to form a GSH conjugate, or can be hydrolyzed to 2,5-dichlorophenol (and minor amounts of 2,4-dichlorophenol) (Bogaards et al., 1995). The dichlorophenols can be further oxidized to dichlorocatechols and dichlorohydroquinones, or conjugated with GSH, glucuronic acid or sulfate (Bogaards et al., 1995; den Besten et al., 1992). Considerable levels of secondary metabolites and only small amounts of dichlorophenols have been detected in the urine of exposed animals, indicating that the secondary metabolism is extensive (Hissink et al., 1996b; Hawkins et al., 1980).

CYP P4502E1 is the main P450 isozyme involved in the metabolism of 1,4-dichlorobenzene by human liver microsomes (Nedelcheva et al., 1998; Hissink et al., 1997b, 1996b; Bogaards et al., 1995). CYP1A1 and 1A2 also showed activity toward the formation of 1,4-dichlorophenol metabolites in human microsomes, but 3A4 and 2D6 had low or nondetectable activity. CYP2B1/2, as well as CYP2E1 and CYP3A1, appear to be involved in the metabolism of 1,4-dichlorobenzene in rat and mouse microsomes (Hissink et al., 1997b; Lake et al., 1997), but information on other species (e.g., dogs) is not available.



Note: In humans, metabolism proceeds predominantly via the 2,3-epoxide (shown in this figure). In rats and mice, metabolism proceeds via the 1,2- and 2,3-epxoide (Muller, 2002).

Figure 3-3. Metabolism of 1,4-dichlorobenzene.

The toxicity of 1,4-dichlorobenzene is largely attributable to covalent binding of the epoxide to cellular proteins, although dichlorobenzoquinones may also be important reactive metabolites (Hissink et al., 1997b, 1996b; den Besten et al., 1992) (see Sections 4.4.1.2.1.3 and 4.5.3.3). In contrast to the evidence for covalent binding to proteins, metabolites of 1,4-dichlorobenzene showed only minimal covalent binding to DNA (Nedelcheva et al., 1998; den Besten et al., 1992). In addition, genotoxicity studies did not indicate that 1,4-dichlorobenzene is DNA-reactive (see Section 4.4.2.3). When 1,4-dichlorobenzene was added to liver microsomes from rats treated with P450 inducers, epoxide formation resulted in

considerable covalent binding to proteins (den Besten et al., 1992). Levels of identified metabolites were dichlorohydroquinones > dichlorophenols > dichlorocatechols. Increasing the dose did not change the formation of 2,5-dichlorohydroquinone, but decreased the formation of dichlorophenol and increased covalent binding to microsomal protein.

Conjugation with glucuronic acid is believed to be of considerable importance for the 1,4-isomer. Studies in animals demonstrated that 22–36% of 1,4-dichlorobenzene was eliminated in the urine as the glucuronide conjugate (Hissink et al., 1997a, 1996b; Hawkins et al., 1980; Azouz et al., 1954). Sulfation appears to be the predominant Phase II metabolic pathway, accounting for 27–65% of a single oral dose of 1,4-dichlorobenzene (Hissink et al., 1997a, 1996b; Hawkins et al., 1980; Azouz et al., 1954). GSH conjugation appears to be of minimal importance for 1,4-dichlorobenzene, with only small, if any, detectable levels of mercapturic acid metabolites identified in the urine of exposed animals (Hissink et al., 1997a, 1996b; Azouz et al., 1954). Fisher et al. (1990) reported that in rat liver slices the majority (>60%) of 1,4-dichlorobenzene was found conjugated to GSH, or as a cysteine conjugate, with small amounts of the sulfate detected (~10% of total metabolites). In human liver slices, the pattern was different, with GSH still being the predominant metabolite (~55%), but with an approximately equal distribution of glucuronide and sulfate conjugates (22–24%).

A minor pathway of possible toxicological significance involves the formation of methyl sulfone metabolites. Following oxidation by CYP P450 in the liver, and possibly following sulfation, the metabolites are secreted into the bile. Within the gut, metabolism by intestinal flora leads to formation of dichloromethylsulfones that are then re-absorbed and transported back to the liver. While these represent but a small percentage of the total metabolites, they are extremely potent inducers of CYP P450 enzymes (Kato and Kimura, 1997; Larsen et al., 1990; Kato et al., 1988a, b, 1986; Kimura et al., 1985), causing considerable hepatic enzyme induction.

Following a single oral exposure of 1,4-dichlorobenzene to male Wistar rats, the main sulfur-containing metabolites found in the urine are 2,5-dichlorophenyl methyl sulfoxide and 2,5-dichlorophenyl methyl sulfone. Levels of 2,5-dichlorophenyl methyl sulfone in the blood were higher and more persistent following a single oral dose of 1,4-dichlorobenzene (Kimura et al., 1979). Hissink et al. (1997a) exposed male Wistar rats to 0, 10, 50, or 250 mg/kg 1,4-dichlorobenzene. The major metabolite in bile was the glucuronide of 2,5-dichlorophenol. Approximately 90% of the dichlorobenzene was metabolized to 2,5-dichlorophenol, which was detected in the urine as sulfate (50–60%), glucuronide (20–30%), and the free form (5–10%). The remaining metabolites consisted of N-acetyl-cysteine-S-dihydro-hydroxy-1,4-dichlorobenzene and N-acetyl-cysteine-S-

1,4-dichlorobenzene. No evidence for the formation of hydroquinones was seen, even under conditions of induced oxidative metabolism.

Lake et al. (1997) reported that treatment of male F344 rats (0–300 mg/kg-day) and male B6C3F$_1$ mice (0–600 mg/kg-day) with 1,4-dichlorobenzene for up to 13 weeks resulted in a sustained increase in hepatic CYP P450 levels in both species, but the increase in rats was considerably greater than in mice. Studies have shown that following in vivo exposure, mice, but not rats, showed covalent binding of 1,4-dichlorobenzene to the DNA of liver, kidney, lung, and stomach (Lattanzi et al., 1989). In vitro binding to calf thymus DNA was detected following incubation of 1,4-dichlorobenzene with microsomes from liver or lung from both rats and mice, although the binding with mouse lung microsomes was considerably greater than with rat lung microsomes (Lattanzi et al., 1989).

Nedelcheva et al. (1998) compared the in vitro metabolism of 1,4-dichlorobenzene by human microsomes to that seen in animals and reported that metabolic rates in humans were lower than those in rats or mice. Additional data on species-specific metabolic pathways of 1,4-dichlorobenzene would be useful in determining which animal species, if any, is the most appropriate model for 1,4-dichlorobenzene toxicity and/or carcinogenicity for humans.

3.4. ELIMINATION

In a study of seven adult male volunteers exposed to 2.5 ppm 1,4-dichlorobenzene for 1 hour, Yoshida et al. (2002a) found that mean serum concentrations of the compound decreased by about 70% within 1 hour after the end of exposure. Very little of the absorbed 1,4-dichlorobenzene was exhaled. The mean excretion of 1,4-dichlorobenzene in the urine (as 2,5-dichlorophenol) was 7.7% by 12–16 hours after exposure; excretion beyond 16 hours postexposure was not measured. Information on the elimination of dichlorobenzenes following oral or dermal exposure is not available.

Results from rat studies with 1,2-dichlorobenzene and 1,4-dichlorobenzene indicate that following absorption by the gastrointestinal or respiratory tract, parent compounds are subject to rapid metabolism, and elimination of metabolites principally takes place via urine. Excretion via feces or exhaled breath plays a minor role. Neither parent compounds nor metabolites persist in fat or other tissues (see Tables 3-1 and 3-2).

The rapid elimination of parent compound and metabolites is supported by the report that <0.1% of administered radioactivity was found in the organs, fat, or blood of male or female F344 rats 72 hours after oral administration of 900 mg/kg ^{14}C-labeled 1,4-dichlorobenzene in

corn oil (Klos and Dekant, 1994). In this study, 92–93% of recovered radioactivity was collected within 72 hours in urine, and 6–8% in feces (Klos and Dekant, 1994).

Results from studies with bile duct-cannulated rats have demonstrated the importance of enterohepatic circulation for 1,2- and 1,4-dichlorobenzene following oral exposure. In two bile duct-cannulated Wistar rats given oral doses of 10 mg/kg ^{14}C-labeled 1,2-dichlorobenzene, 60% of total radioactivity was collected in bile within about 30 hours of dosing, whereas in non-cannulated rats, 75–84% orally administered radioactivity from ^{14}C-labeled 1,2-dichlorobenzene was excreted in the urine (Hissink et al., 1996a). In bile duct-cannulated rats orally given 250 mg/kg ^{14}C-labeled 1,4-dichlorobenzene, 10–30% of the radioactivity collected within 24 hours of dosing was in the bile, 40–50% in the urine, and <5% in the feces (Hissink et al., 1997a).

Levels of parent compound or metabolites in urine have been proposed as biomarkers of exposure for people exposed to 1,2-dichlorobenzene or 1,4-dichlorobenzene in the workplace (Kumagai and Matsunaga, 1997, 1995; Zenser et al., 1997; Ghittori et al., 1985; Pagnotto and Walkley, 1965). Concentrations of several metabolites of 1,2-dichlorobenzene (3,4-dichlorocatechol, 4,5-dichlorocatechol, 2,3-dichlorophenol, and 3,4-dichlorophenol) in urine collected at the end of a work shift from 10 male workers were significantly correlated with 8-hour time-weighted-average air concentrations based on personal air monitoring (Kumagai and Matsunaga, 1997). Correlations have also been reported between urinary levels of 1,4-dichlorobenzene (Ghittori et al., 1985) or 2,5-dichlorophenol (Pagnotto and Walkley, 1965) and workplace air concentrations of 1,4-dichlorobenzene. However, ACGIH (2002) currently does not recommend biological exposure indices for workplace exposure to dichlorobenzene isomers.

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

A physiologically based pharmacokinetic (PBPK) model has been developed for 1,2-dichlorobenzene in rats and humans (Hissink et al., 1997c). PBPK models have not been developed for 1,3-dichlorobenzene or 1,4-dichlorobenzene.

The PBPK models for 1,2-dichlorobenzene developed by Hissink et al. (1997c) have four compartments connected by blood flow: 1) rapidly perfused tissues including lung, kidneys and spleen; 2) slowly perfused tissues comprising muscle and skin; 3) fat; and 4) liver, the only compartment in which metabolism is assumed to take place. The models were developed for oral exposure; no respiratory or dermal portals of entry are included. The models assume that uptake from the gastrointestinal tract proceeds as a dose-dependent first-order kinetic process depositing

1,2-dichlorobenzene directly in the liver. For each of the nonmetabolizing compartments, differential equations describe the influx and efflux of 1,2-dichlorobenzene. Equations for the liver also account for 1,2-dichlorobenzene metabolism and reduced GSH synthesis, turnover, and consumption.

Physiological parameters, partition coefficients, biochemical parameters, and absorption rate constants used in the models are shown in Table 3-3. Absorption rate constants were estimated by fitting the parameters to data for rats exposed to 5, 50, or 250 mg/kg 1,2-dichlorobenzene (Table 3-3).

Metabolism in the model was described as the initial, P450-mediated, saturable formation of an epoxide, followed by metabolism via three competing pathways that were assumed to independently follow pseudo first-order kinetics (i.e., to be non-saturable): 1) conversion into dichlorophenol; 2) covalent binding of reactive metabolites to cellular proteins in the presence of GSH and glutathione S-transferase; and 3) conjugation of the epoxide with GSH. The Michaelis-Menten constants Vmax and Km for the saturable CYP-P450 oxidation of 1,2-dichlorobenzene were initially estimated from in vitro experiments with rat and human liver microsomes (Table 3-3). Scaling for use in the models assumed 45 and 77 mg microsomal protein per gram liver for rats and humans, respectively. However, in order to obtain adequate fits to rat data for blood concentrations of parent material or total amount of metabolites, a "best-fit" Vmax value of 17 μmol/hour was used, along with the in vitro Km of 4.8 μM (Table 3-3). This "best-fit" value was about fourfold higher than the rat in vitro Vmax scaled to units of μmol/hour (4.3 μmol/hour; see Table 3-3). Based on the rat data analysis, a factor of four was used to derive a "best-fit" Vmax value of 10,840 μmol/hour from the human in vitro Vmax (2742 μmol/hour; see Table 3-3). The ratio of rate constants for the three epoxide-transforming pathways in rats (5:30:65) was estimated based on the relative amounts of in vitro covalent binding (5%), in vitro and in vivo dichlorophenol formation (25% and 30%), and in vitro and in vivo GSH conjugation (70% and 60%). For the rat model, the first order rate constant for covalent binding was arbitrarily set at 50 $hour^{-1}$; the resulting rates for dichlorophenol formation and GSH conjugation were 300 $hour^{-1}$ and 650 $hour^{-1}$, respectively (Table 3-3). In vitro data obtained with human microsomes similarly formed the basis of the rate constants for these pathways: 5 $hour^{-1}$ for covalent binding; 360 $hour^{-1}$ for dichlorophenol formation; and 650 $hour^{-1}$ for GSH conjugation (Table 3-3). A GSH turnover rate of 0.14 $hour^{-1}$, determined in another study with rats (Potter and Tran, 1993), was used in both the rat and human models (see Table 3-3).

Table 3-3. Parameters in PBPK models for 1,2-dichlorobenzene

Parameter	Rat	Human
Physiologic parameters [a]		
Body weight (kg)	0.258	70
Percentages of body weight		3.14
Liver	4	23.1
Fat	7	2.66
Rapidly perfused	5	62.1
Slowly perfused	75	
Flows (L/hour)		
[QC or QP = 15 L/hour (body weight)$^{0.74}$]		348.0
Cardiac output (QC)	5.50	348.0
Alveolar ventilation (QP)	5.50	
Percentages of cardiac output		25
Liver	25	9
Fat	9	51
Rapidly perfused	51	15
Slowly perfused	15	
Partition coefficients [b]		
Blood:air	423	423
Liver:blood	2.7	2.7
Fat:blood	66.4	66.4
Rapidly perfused:blood	2.7	2.7
Slowly perfused: blood	1.3	1.3
Biochemical parameters		
Oxidative metabolism		
Vmax (nmol/min-mg) (in vitro derived)	0.142 (4.3 µmol/hour)	0.27 (2742 µmol/hour)
Km (µM) (in vitro derived)	4.8	7.5
Vmax (µmol/hour) ("best-fit" values)	17	10840
GSH conjugation of epoxide (hour^{-1})	650	650
Formation of dichlorophenol (hour^{-1})	300	360
Formation of reactive metabolites (hour^{-1})	50	5
GSH turnover rate (hour^{-1})	0.14	0.14
Absorption rate constants [c]		
Ka (hour^{-1})		
5 mg/kg	0.5	–
50 mg/kg	0.18	–
250 mg/kg	0.06	0.06

[a] As per Gargas et al., 1986.
[b] Calculated according to Droz et al. (1989) using water:air, oil:air, and blood:air partition coefficients.
[c] Estimated by fitting parameters to data for rats at indicated dose levels).

Source: Hissink et al., 1997c.

The rat model was used to predict hepatic concentrations of covalently bound metabolites following an oral dose of 250 mg/kg 1,2-dichlorobenzene that was expected to be toxic to the liver (Hissink et al., 1997c). The hepatic concentration in rats, 24 hours after dosing, was 1459 μM. Versions of the human model using different Vmax values predicted that this administered dose level produced much lower hepatic concentrations of covalently bound metabolites in humans. Increasing the human in vitro-derived Vmax value by a factor of 10 did not increase predicted human hepatic concentrations to a value above about 240 μM 24 hours after dosing. Thus, the models predicted that equivalent doses in rats and humans would produce hepatic concentrations of covalently bound metabolites that were at least sixfold higher in rats than in humans.

The models were also used to predict hepatic concentrations of GSH, expressed as percentage of an assumed baseline concentration of 6.5 mM following an oral dose of 250 mg/kg 1,2-dichlorobenzene (Hissink et al., 1997c). The rat model predicted that maximum depletion of GSH (to about 30% of normal) occurred by 15 hours after dosing. In contrast, the human model (using a Vmax value of 10,840 μmol/hour; see Table 3-3) predicted that maximum depletion of GSH (essentially complete) occurred at 10 hours after dosing. Thus, the models predicted that humans may be more susceptible than rats to 1,2-dichlorobenzene-induced depletion of hepatic GSH levels. Hissink et al. (1997c) noted that if depletion of GSH were the only factor involved in acute 1,2-dichlorobenzene hepatotoxicity, the models predicted that humans should be more susceptible than rats at the same dose level. On the other hand, if covalent binding of reactive metabolites were the critical factor, humans should be less susceptible to acute 1,2-dichlorobenzene-induced hepatotoxicity than rats. However, at present, the majority of parameters of the human model were based on direct scaling from the rodent data, rather than having been calibrated based on data from humans. As such, application of the human model should be done with great caution, if at all, as its predictive ability in humans has not been established.

4. HAZARD IDENTIFICATION

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

4.1.1. Oral Exposure

Information on the toxicity of ingested dichlorobenzene isomers in humans is limited to case reports of 1,4-dichlorobenzene exposure. A three-year-old boy developed health effects that included acute hemolytic anemia, methemoglobinemia, and jaundice after playing with moth crystals containing 1,4-dichlorobenzene (Hallowell, 1959). Traces of 2,5-dichloroquinol (2,5-dichlorohydroquinone) and two other phenols were identified in urine collected six days later, but 2,5-dichlorophenol (the major metabolite of p-dichlorobenzene) was not detected. Although ingestion of the chemical presumably occurred, it is likely that inhalation and dermal exposure were also involved. Hematological effects also occurred in a woman who consumed toilet air freshener (composed mainly of p-dichlorobenzene) at a rate of one or two blocks per week throughout pregnancy until about 38 weeks of gestation (Campbell and Davidson, 1970). The woman developed severe microcytic, hypochromic anemia from which she recovered following cessation of exposure; neonatal examination of the child showed no abnormalities.

4.1.2. Inhalation Exposure

4.1.2.1. *1,2-Dichlorobenzene*

Periodic industrial hygiene surveys and medical examinations were conducted in a plant where men were occupationally exposed to 1,2-dichlorobenzene during unspecified handling operations (Hollingsworth et al., 1958). The workers were exposed to an average concentration of 15 ppm (range 1-44 ppm) for unreported durations. No eye or nasal irritation or effects on clinical indices (red blood cell [RBC] count, total and differential white blood cell counts, hemoglobin, hematocrit [HCT], mean corpuscular volume [MCV], blood urea nitrogen [BUN], sedimentation rate, or urinalysis) were attributable to exposure. Additional information on the medical examinations was not provided. Hollingsworth et al. (1958) noted that during repeated vapor inhalation experiments in animals, researchers in his laboratory detected 1,2-dichlorobenzene odor at a concentration of 50 ppm without experiencing eye or nasal irritation. An earlier source (Elkins, 1950) reported that occupational exposure to 100 ppm of 1,2-dichlorobenzene caused irritation of the eyes and respiratory passages. The frequent difficulty of finding accurate exposure information and such concerns as confounders skewing the data is

exemplified here. Unreported durations of exposure and the lack of necessary medical information from the medical histories of the workers make meaningful associations between exposure and adverse health effects, in response to that exposure, questionable.

A retrospective cohort mortality study was conducted among 14,457 male and female workers who were exposed to trichloroethylene and a large number of other organic solvents and chemicals, including 1,2-dichlorobenzene, during the cleaning and repairing of small parts at an aircraft maintenance facility in Utah (Spirtas et al., 1991). The study group consisted of civilian employees who worked for at least 1 year between January 1952 and December 1956, and were followed until December 1982, at which time 9860 of the subjects were still living, and 3832 of the subjects were deceased. Determination of standardized mortality ratios (SMRs) showed that mortality in the entire cohort was slightly reduced for all causes of death (SMR = 92 [95% confidence interval (CI): 90–95], $p<0.01$) and all malignant neoplasms (SMR = 90 [95% CI: 83–97], $p<0.05$) in comparison with expected numbers for the Utah population. The only causes of death assessed for exposure to 1,2-dichlorobenzene (size of subgroup not reported) were multiple myeloma and non-Hodgkin lymphoma (NHL). Mortality from neither of these cancers was significantly increased based on very few observed deaths (no deaths from multiple myeloma in either sex, one death from NHL in men (SMR = 70 [95% CI: 2–388], $p>0.05$), and one death from NHL in women (SMR = 1008 [95% CI: 25–5616], $p>0.05$).

Five cases of blood disorders (two cases of chronic lymphoid leukemia, two cases of acute myeloblastic leukemia, and one case of a myeloproliferative syndrome) were described in people who were exposed to 1,2-dichlorobenzene as a solvent for other chemicals or in chlorinated benzene mixtures (Girard et al., 1969; IARC, 1982). None of these cases had evidence of exposure to unsubstituted benzene. One of the case reports suggested an association between chronic lymphoid leukemia and long-term (10 years) occupational exposure to a solvent mixture containing 80, 2, and 15% of 1,2-, 1,3-, and 1,4-dichlorobenzene, respectively, that was used to clean electrical parts (IARC, 1982).

4.1.2.2. *1,3-Dichlorobenzene*

No relevant information was located regarding the toxicity of inhaled 1,3-dichlorobenzene in humans.

4.1.2.3. *1,4-Dichlorobenzene*

Periodic industrial hygiene and health surveys of 58 men who had been occupationally exposed, intermittently or continually, to 1,4-dichlorobenzene for an average of 4.75 years

(range, 8 months to 25 years) indicated that exposure to 1,4-dichlorobenzene vapor caused eye and nasal irritation (Hollingsworth et al., 1956). These surveys showed that the odor was faint at 15-30 ppm and strong at 30-60 ppm, and that painful irritation of the eyes and nose was usually experienced at 50-80 ppm, although the irritation threshold was higher (80-160 ppm) in workers acclimated to exposure. Concentrations above 160 ppm caused severe irritation and were considered intolerable to people not adapted to it. Odor and irritation were considered to be fairly good warning signs for excessive exposure to 1,4-dichlorobenzene, but the industrial experience indicated that it is possible for people to become sufficiently used to tolerate high concentrations of the vapor (Hollingsworth et al., 1956). Examinations of the workers conducted at various times (not specified) showed no cataracts or any other lens changes or effects on clinical indices (RBC count, total and differential white blood cell counts, hemoglobin, HCT, MCV, BUN, sedimentation rate, or urinalysis) attributable to 1,4-dichlorobenzene exposure. No additional relevant information was provided on the design and results of the health surveys.

Case studies of people who inhaled 1,4-dichlorobenzene provide indications that the liver and nervous system are targets of toxicity in humans, but this conclusion is limited by lack of adequate quantitative exposure information and/or verification that 1,4-dichlorobenzene was the only factor associated with the effects. Available information includes the cases of a man and his wife who were exposed to mothball vapor that "saturated" their home for 3-4 months and died of hepatic failure (acute liver atrophy) within a year of the initial exposure (Cotter, 1953). The man additionally experienced neurological symptoms that included numbness, clumsiness, and slurred speech. Liver damage (yellow atrophy and cirrhosis) was also diagnosed in a woman who demonstrated 1,4-dichlorobenzene products in a department store for more than a year, as well as in an adult man who was occupationally exposed to 1,4-dichlorobenzene in a fur storage plant for approximately 2 years (Cotter, 1953). Neurotoxicity was indicated in a woman who was exposed for 6 years via liberal treatment of her bedroom, bedding, and clothing with 1,4-dichlorobenzene as an insect repellant (Miyai et al., 1988). This person experienced neurological symptoms (severe ataxia, speech difficulties, limb weakness, hyporeflexia) and abnormal brainstem auditory-evoked potentials (marked delays of specific brainwave patterns) that gradually improved following cessation of exposure. Similar reversible neurological symptoms developed in a woman who intentionally inhaled 1,4-dichlorobenzene vapor from deodorizer blocks for several months and had verified exposure (her urine had a characteristic aromatic odor and contained the p-dichlorobenzene metabolite, 2,5-dichlorophenol) (Reygagne et al., 1992).

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Exposure

4.2.1.1. *1,2-Dichlorobenzene*

Groups of 10 young adult white female rats (strain not specified) were administered 1,2-dichlorobenzene in an olive oil-gum arabic emulsion by gavage in doses of 18.8, 188, or 376 mg/kg, 5 days/week for 138 doses in 192 days (adjusted: 13.5, 135, or 270 mg/kg-day) (Hollingsworth et al., 1958). A group of 20 vehicle-exposed females was used as controls. Body weight, absolute organ weights (liver, kidneys, spleen, and heart), hematology, bone marrow values and histology were evaluated. Unspecified numbers of deaths from respiratory infection occurred that were reported to be well-distributed among the groups. No exposure-related effects were observed at 13.5 mg/kg-day, and there were no body weight, hematological, or bone marrow changes at higher doses. Statistically significant ($p \leq 0.02$) increases in absolute liver and kidney weights (37–47% and 22–30% higher than control values, respectively) occurred at ≥135 mg/kg-day. Additional effects were found at 270 mg/kg-day that included slight to moderate cloudy swelling of the liver and significantly decreased spleen weight. No additional relevant information (e.g., incidences of liver lesions) was reported. The increases in liver and kidney weights in the absence of histopathologic or other corroborating evidence of tissue damage are considered to be adaptive, rather than adverse, changes. Therefore, a NOAEL of 135 and LOAEL of 270 mg/kg-day are identified from this study on the basis of liver pathology.

Groups of 10 male and 10 female Sprague-Dawley rats were treated with 1,2-dichlorobenzene in corn oil by gavage in doses of 0, 25, 100, or 400 mg/kg-day for 90 consecutive days (Robinson et al., 1991). Endpoints evaluated during the study included clinical signs, body weight, and food consumption. Evaluations at the end of the exposure period included hematology (8 indices), serum chemistry (12 indices including alkaline phosphatase [AP], aspartate aminotransferase [AST], alanine aminotransferase [ALT], lactate dehydrogenase [LDH] and BUN), urinalysis (6 indices), ophthalmic condition, and selected organ weights (brain, liver, spleen, lungs, thymus, kidneys, adrenal glands, heart, and testes or ovaries). Histologic examinations were performed on selected tissues (liver, kidneys, spleen, adrenal glands, thymus, brain, heart, lungs, and testes or ovaries) in all high-dose rats and one-half of the control group. No clinical signs or effects on survival were observed. Body weight gain was not affected in female rats, but significantly decreased in the males at 400 mg/kg-day (final body weights were 12.8% lower than controls). Food consumption was increased in female rats at 400 mg/kg-day during weeks 11–13. Statistically significant changes in organ weights included

dose-related increases in absolute and relative liver weights in both sexes at ≥100 mg/kg-day, increases in absolute and relative kidney weights in both sexes at 400 mg/kg-day (absolute kidney weight was also increased in females at 100 mg/kg-day), and decreases in absolute (both sexes) and relative (males only) spleen weights at 400 mg/kg-day.

No compound-related alterations in urinalysis or hematological parameters were observed (Robinson et al., 1991). Clinical chemistry changes included increased serum ALT in males at ≥100 mg/kg-day, increased BUN in males at 400 mg/kg-day, and increased total bilirubin in both sexes at 400 mg/kg-day. The increases in serum ALT were statistically significant, but did not increase with dose, and serum levels of other liver-associated enzymes were not increased (AST, LDH, and AP). Histopathologic alterations were observed only in the liver. Statistically significant increases in the incidences of centrilobular degeneration, centrilobular hypertrophy, and single cell necrosis (males only) were observed in both sexes at 400 mg/kg-day. The degeneration, hypertrophy, and necrosis in the high-dose rats occurred in 10/10, 9/10, and 7/10 males and 8/10, 10/10, and 5/10 females, respectively; none of these lesions were present in control animals of either sex. As indicated above, histologic examinations were not performed in the low- and middle-dose groups, and were limited to one half of the control group. Changes in serum ALT and liver weight at 100 mg/kg-day were not considered evidence of hepatotoxicity because the increase in serum ALT was not supported by dose-related changes in other serum enzymes that are indicators of liver damage. Because histopathologic data were not collected at this dose level, the toxicological significance of increased liver weight without an increase in associated enzymes is uncertain. The 400 mg/kg-day dose is an effect level based on hepatic degeneration, hypertrophy and necrosis. A NOAEL and LOAEL were not identified because the 25 and 100 mg/kg-day rats were not examined for histopathology.

Subchronic studies in F344/N rats were performed to determine doses to be used in a chronic rat bioassay (NTP, 1985). Groups of 10 male and 10 female rats were administered 1,2-dichlorobenzene (>99% pure) in corn oil by gavage at doses of 0, 30, 60, 125, 250, or 500 mg/kg, 5 days/week for 13 weeks (adjusted: 0, 21.4, 42.9, 89.3, 179, or 357 mg/kg-day). Evaluations included clinical signs, body weight and food consumption, hematology, clinical chemistry, urine volume, urine uroporphyrins and coproporphyrins, liver porphyrins, organ weights, and necropsies in all groups of animals. Complete histologic examinations were performed on all control and high-dose animals; histology exams in lower dose groups were limited to liver, kidneys and thymus at 89.3 and 179 mg/kg-day. Final body weights were within 7% of control values in all groups of both sexes except for the 357 mg/kg-day male rats, which were 19% less than controls. Early deaths that were presumed by the researchers to be due to

gavage error occurred in two females at 357 mg/kg-day and in one male each from the 0, 21.4, and 89.3 mg/kg-day groups.

Effects mainly occurred in the liver, as shown by histopathologic changes, including centrilobular degeneration or necrosis of individual hepatocytes in most of the rats (8/10 males and 7/8 surviving females, as well as the two females that died early) at 357 mg/kg-day (NTP, 1985). Liver pathology (necrosis of individual hepatocytes) was also significantly increased at 179 mg/kg-day ($p<0.05$, Fisher's Exact test conducted for this assessment; 4/9 males and 5/10 females) relative to controls. Milder degenerative liver lesions were noted in a few animals (1/10 males and 3/10 females) at 89.3 mg/kg-day; the incidence of these lesions was not significantly increased at this dose. No liver lesions were reported in male or female controls.

Relative liver weights were significantly increased at ≥89.3 mg/kg-day in both sexes, while slight decreases in serum triglycerides (357 mg/kg-day; males, 179 mg/kg-day; females) and serum protein (179–357 mg/kg-day; males, 21.4–357 mg/kg-day; females) were observed which may reflect hepatic effects of the chemical at these doses. Serum cholesterol was significantly ($p<0.05$) increased in males at ≥21.4 mg/kg-day (50.0, 17.6, 26.5, 70.6, and 109% higher than controls in the low to high dose groups, not significant at 42.9 mg/kg-day) and females at ≥89.3 mg/kg-day (12.2, 12.2, 32.6, 26.5, and 51.0%). Serum total protein was significantly increased in females at ≥21.4 mg/kg-day (7.8, 4.7, 6.3, 6.3, and 17.2%) and males at ≥179 mg/kg-day (-1.4, 1.4, 0, 7.1, and 7.1%). BUN was not increased in any dose group of either sex, although 24-hour urine volume was 57% higher than controls in 357 mg/kg-day males.

Additional effects observed at 357 mg/kg-day included renal tubular degeneration (6/10 males), lymphoid depletion in the thymus (4/10 males), and some slight hematologic changes (e.g., minimal decreases in hemoglobin, HCT, erythrocyte counts, and MCV in both sexes). Urinary concentrations of uroporphyrin and coproporphyrin were 3–5 times higher than controls in the 357 mg/kg-day males and females, but this increase was not considered indicative of porphyria because total porphyrin concentration in the liver was not altered at any dose level and no pigmentation indicative of porphyria was observed by ultraviolet light at necropsy. At 89.3 mg/kg-day, there was a significant increase in relative liver weight along with degenerative liver lesions (1/10 males and 3/10 females). The 89.3 mg/kg-day is a LOAEL on the basis of significant increase in relative liver weight and the appearance of degenerative liver lesions (1/10 males and 3/10 females). A NOAEL was not identified in this study due to the lack of histopathology data at the two lower doses (21.4 mg/kg-day and 42.9 mg/kg-day).

In the chronic NTP (1985) rat study, groups of 50 male and 50 female F344/N rats were gavaged with 1,2-dichlorobenzene (>99% pure) in corn oil in doses of 0, 60, or 120 mg/kg,

5 days/week for 103 weeks (adjusted: 0, 42.9, or 85.7 mg/kg-day). Evaluations included clinical signs, body weight, necropsy, and histology on all animals. At 1 year, survival in males was 98–100% in the control and low-dose groups, and 88% in the high-dose group, while in females it was 95–100% in all groups. At termination, survival in the 0, 42.9, and 85.7 mg/kg-day groups was 84, 72, and 38% in males and 62, 66, and 64% in females, respectively. Survival to termination in the high-dose male rats was significantly reduced compared with controls (19/50 vs. 42/50, $p<0.001$), but the difference appeared to be mainly due to causes incidental to treatment. There were 20 incidental deaths in the high-dose group compared to 4 in controls; according to NTP, of the 20 deaths, 3 were accidental, 5 were probably due to gavage error, and 12 may have been caused by aspiration. Due to the probable gavage-related deaths in the high-dose male rats, the lower survival of this group does not necessarily mean that the maximum tolerated dose (MTD) was either reached or exceeded. Mean body weight was slightly reduced (~5% less than controls) in males throughout the study at 85.7 mg/kg-day; the only effect in females was a small increase compared to controls after week 32 in both dose groups (final body weights were 11-12% increased at 42.9 and 85.7 mg/kg-day). There were no compound-related increased incidences of nonneoplastic lesions in the liver, kidneys, or any other tissues. This indicates that the high dose of 85.7 mg/kg-day is the NOAEL in the chronic rat study.

There were no 1,2-dichlorobenzene-related increases in tumor incidence in the rats (NTP, 1985). Although the incidence of adrenal gland pheochromocytomas was statistically significantly increased ($p<0.05$) in low-dose males by the life table test (mortality adjusted incidence of 20.9, 40.5, and 21.7% in the control, low-dose and high-dose groups, respectively), the increase in low-dose males was not significant by the incidental tumor test (considered by NTP to be the more appropriate mortality-adjusted test for analysis of nonfatal types of tumors, such as adrenal pheochromocytomas) or by Fisher's Exact test (without mortality adjustment), nor was there a significant trend in the Cochran-Armitage test. No increase in pheochromocytomas was seen in high-dose males. The increased incidence of pheochromocytomas in the low-dose male rats was discounted by NTP (1985) because there was no dose-response trend or high-dose effect, no increased incidence in females, no observation of malignant pheochromocytomas, and questionable toxicological significance of the life table test results (pheochromocytomas were not considered by the researchers to be a life-threatening condition). Incidences of interstitial-cell tumors of the testis were elevated in control and treated groups (47/50, 49/50, 41/50), and occurred with a significant positive trend when analyzed by the life-table test. However, the increase detected by the life-table test was discounted by NTP because this tumor is not considered to be life threatening, and no significant results were

obtained by the incidental tumor test, which is the more appropriate test for non-fatal tumors. The Cochran-Armitage test showed a significant negative trend for the interstitial cell tumors.

Subchronic studies in B6C3F_1 mice were performed to determine doses to be used in a chronic mouse bioassay (NTP, 1985). Groups of 10 male and 10 female mice were administered 1,2-dichlorobenzene (>99% pure) in corn oil by gavage in doses of 0, 30, 60, 125, 250, or 500 mg/kg, 5 days/week for 13 weeks (adjusted: 0, 21.4, 42.9, 89.3, 179, or 357 mg/kg-day). Evaluations included clinical signs, body weight and food consumption, hematology, clinical chemistry, urine uroporphyrins and coproporphyrins, liver porphyrins, organ weights, and necropsies in all groups of animals. Complete histologic examinations were performed on all control and high-dose animals; histology exams in lower dose groups were limited to the liver, spleen, thymus, heart, and muscle at 179 mg/kg-day, and only the liver at 89.3 mg/kg-day.

Mortality occurred in 4/10 males and 3/10 females at 357 mg/kg-day, as well as in one male at 179 mg/kg-day. Final body weights were within 6% of control values in all groups of both sexes except for the 357 mg/kg-day males and females, which were 11 and 19% less than controls, respectively. Effects observed in the liver included histopathologic changes at 357 mg/kg-day (centrilobular necrosis, necrosis of individual hepatocytes, and/or hepatocellular degeneration in 9/10 males and 9/10 females) and 179 mg/kg-day (necrosis of individual hepatocytes, hepatocellular degeneration and/or pigment deposition in 4/10 males). No compound-related liver lesions were observed in females at 179 mg/kg-day, mice of either sex at 89.3 mg/kg-day, or controls. Relative liver weights were significantly increased at 357 mg/kg-day in both sexes, but there were no exposure-related changes in serum levels of ALT, AP, or γ-glutamyl transpeptidase (GGT) in either sex at any dose (no other clinical chemistry indices were examined in the mice). Additional effects, observed only at 357 mg/kg-day, included mineralization of the myocardial fibers of the heart and skeletal muscle (3/10 males and 8/10 females), and lymphoid depletion in the thymus (2/10 males and 2/10 females) and spleen (4/10 males and 2/10 females). There were no hematological changes considered to be biologically significant. The urinary concentration of coproporphyrin was 3–5 times higher than controls in the 357 mg/kg-day females. The increase in urinary coproporphyrin was considered to be moderate, but not indicative of porphyria, because total porphyrin concentration in the liver was only increased twofold in 357 mg/kg-day females, not altered in males at any dose level, and not accompanied by pigmentation indicative of porphyria observed by ultraviolet light at necropsy. The hepatic histopathology findings in mice (NTP, 1985) indicate that the NOAEL and LOAEL were 89.3 and 179 mg/kg-day, respectively.

In the chronic NTP (1985) mouse study, groups of 50 male and 50 female B6C3F_1 mice were gavaged with 1,2-dichlorobenzene (>99% pure) in corn oil at doses of 0, 60, or 120 mg/kg, 5 days/week for 103 weeks (adjusted: 0, 42.9, or 85.7 mg/kg-day). Evaluations included clinical signs, body weight, and necropsy and histology on all animals. No clinical signs were reported, and mean body weight and survival were comparable in control and dosed mice throughout the study, indicating that it was unclear whether an MTD was achieved. The only exposure-related nonneoplastic lesion was a significantly increased incidence (p<0.05, Fisher's Exact test performed for this study evaluation) of renal tubular regeneration in male mice at 85.7 mg/kg-day; incidences in the control, low- and high-dose male groups were 8/48, 12/50, and 17/49, respectively. Though no regeneration or necrotic lesions were found in the kidneys of the female mice and the kidney was not identified as a target organ at higher doses in the subchronic study in mice, an increase in renal tubular regeneration indicates that at some earlier point in the study, tubular degeneration must have occurred which is consistent with an apoptotic response in the kidneys. In addition, tubular degeneration and regeneration could be considered as precursor events to chronic nephritis in the dosed animals. This indicates that highest dose of 85.7 mg/kg-day in the chronic study in mice is a minimal LOAEL for kidney effects (male mice) and the lowest dose of 42.9 mg/kg-day is a NOAEL.

There were no clear compound-related increased incidences of neoplasms in the mice (NTP, 1985). Incidences of malignant histiocytic lymphomas showed a significant positive dose-related trend in male mice (0/50, 1/50, 4/50) and female mice (0/49, 0/50, 3/49), but NTP considered numbers of animals with all types of lymphomas to be a more appropriate basis for comparison. Because malignant lymphocytic lymphomas occurred in male mice (7/50, 0/50, 0/50) with a significant negative dose-related trend, and the combined incidence of all types of lymphomas was not significantly different from that in controls for the male mice (8/50, 2/50, 4/50) or female mice (11/49, 11/50, 13/49) by any of the statistical tests, the increase in histiocytic lymphomas was discounted by NTP. Alveolar/bronchiolar carcinomas were significantly increased in high dose male mice (4/50, 2/50, 10/50). The incidences showed a significant positive increasing trend by the Cochran-Armitage test, but not by the life-table or incidental tumor test. The increase in alveolar/bronchiolar carcinomas was discounted by NTP because the more appropriate combined incidence of male mice with alveolar/bronchiolar adenomas or carcinomas (8/50, 8/50, 13/50) was not significantly greater than controls in any of the tests.

4.2.1.2. *1,3-Dichlorobenzene*

Groups of 10 male and 10 female Sprague Dawley rats were administered daily gavage doses of 0, 9, 37, 147, or 588 mg/kg of 1,3-dichlorobenzene in corn oil for 90 consecutive days (McCauley et al., 1995). Endpoints evaluated during the study included clinical signs and mortality (observed daily), body weight (measured weekly), and food and water consumption (measured weekly). At necropsy, blood was collected for hematology and serum chemistry analyses (erythrocytes, leukocytes, hemoglobin, HCT, MCV, glucose, BUN, creatinine, AP, AST, ALT, cholesterol, LDH, and calcium levels); selected organs (brain, liver, spleen, lungs, thymus, kidneys, adrenal glands, heart, and gonads) were weighed, and comprehensive gross tissue examinations were conducted. Histologic examinations were performed on all tissues that were examined grossly in all high-dose rats and one-half of control rats, as well as on liver, thyroid, and pituitary glands from all animals treated with 9, 37, or 147 mg/kg-day. Inflammatory and degenerative lesions were graded on a relative scale from one to four depending on the severity (minimal, mild, moderate, or marked).

There were no compound-related deaths or overt clinical signs, although other effects occurred at all dose levels (McCauley et al., 1995). Body weight gain was reduced in both sexes at 588 mg/kg-day; final body weights were 24 and 10% lower than controls in males and females, respectively. The weight loss was progressive throughout the exposure period, and occurred despite increased food and water consumption in the affected groups. Average daily food consumption was not significantly altered; however, food intake normalized to body weight was significantly increased (10–13%) in male and female rats in the 588 mg/kg-day group. Water consumption was increased (18%) in the 588 mg/kg-day group, and water consumption normalized for body weight was increased (18–23%) in the male rats at 147 and 588 mg/kg-day and female rats at 588 mg/kg-day. Relative testes and brain weights were significantly increased in males at 588 mg/kg-day, likely reflecting the decreased body weight at this dose.

The researchers did not report the results of their statistical evaluation of the pathology data. Therefore, analysis of incidences of lesions was conducted as part of the evaluation of this study using Fisher's Exact test and a criterion of significance of $p \leq 0.05$. Histologic examinations showed statistically significant increased incidences of reduced colloidal density in thyroid follicles that exceeded normal variability in male rats at ≥9 mg/kg-day and female rats at ≥37 mg/kg-day (incidences in the control to high dose groups were 2/10, 8/10, 10/10, 8/9, and 8/8 in males and 1/10, 5/10, 8/10, 8/10, and 8/9 in females) (McCauley et al., 1995). The authors did not explain why <10 animals were examined in the two high-dose groups. Depletion of colloid density in the thyroid was characterized by decreased follicular size with scant colloid and

follicles lined by cells that were cuboidal to columnar. The severity of the colloid density depletion generally ranged from mild to moderate, increased with dose level, and was greater in males than females. For example, in the 147 and 588 mg/kg-day male groups, the severity was classified as moderate, as compared to mild for the females. Incidences of male rats with thyroid colloidal density depletion of moderate or marked severity were significantly increased at ≥147 mg/kg-day (0/10, 0/10, 2/10, 5/9, and 6/8).

There were significantly increased incidences of cytoplasmic vacuolization in the pars distalis of the pituitary of male rats at ≥147 mg/kg-day (2/10, 6/10, 6/10, 10/10, and 7/7); the incidences in the 9 and 37 mg/kg-day groups were marginally increased (p=0.085). The vacuoles were variably sized, irregularly shaped, often poorly defined, and the severity of the lesions (number of cells containing vacuoles) ranged from minimal to mild and generally increased with increasing dose level. Incidences of male rats with pituitary cytoplasmic vacuolization of moderate or marked severity were significantly increased at 588 mg/kg-day (1/10, 0/10, 2/10, 3/9, and 7/7). Though vacuolization was significantly increased and varied in severity from the low to the high dose, it is unlikely to be an adverse event. In addition, there was no evidence in the study (McCauley et al., 1995) of significant perturbations in circulating levels of sex hormones and so the pituitary cellular changes were highly unlikely to be castration cells, as described in the study, but rather a general response to some stimulus (i.e., decreased T4) to stimulate production of TSH, and therefore an adaptive response. No compound-related pituitary lesions were observed in female rats. Serum cholesterol, which was significantly (p≤0.05) increased in males at ≥9 mg/kg-day and females at ≥37 mg/kg-day in a dose-related manner, is consistent with hypothyroxinemia. The investigators suggested that this serum chemistry change might reflect a disruption of hormonal feedback mechanisms, or target organ effects on the hypothalamus, and/or other endocrine organs.

Pathological changes in the liver were found at 1,3-dichlorobenzene doses higher than 9 mg/kg-day (McCauley et al., 1995). These changes occurred in both sexes at 147 and 588 mg/kg-day, including significant increases (p≤0.05) in relative liver weight (51 and 85% increases in males and 32 and 74% increases in females, compared to controls). Absolute organ weights were not reported. Liver effects were characterized by inflammation, hepatocellular alterations (spherical, brightly eosinophilic homogeneous inclusions), and hepatocellular necrosis. Liver effects that were significantly increased (p≤0.05) included hepatocellular cytoplasmic alterations of minimal to mild severity in males at ≥147 mg/kg-day (incidences in the control to high dose groups were 1/10, 2/10, 1/10, 6/10, and 6/9), and females at 588 mg/kg-day (0/10, 2/10, 0/10, 1/10, and 7/9), and necrotic hepatocyte foci of minimal severity in

both sexes at 588 mg/kg-day (1/10, 2/10, 1/10, 2/10, and 5/9 in males and 0/10, 0/10, 0/10, 3/10, and 5/9 in females). The 588 mg/kg-day dose groups of both sexes were the only ones that displayed an incidence level high enough to be considered treatment-related. Other statistically significant liver-associated effects included significantly increased serum AST levels (90–100% higher than controls) in males at ≥9 mg/kg-day and females at ≥37 mg/kg-day. Serum cholesterol levels were significantly increased in males at ≥9 mg/kg-day and females at ≥37 mg/kg-day, but this change could be thyroid-related, as indicated above. Serum LDH levels were reduced in males at ≥9 mg/kg-day and BUN levels were reduced in both sexes at 588 mg/kg-day, but the biological significance of decreases in these indices is unclear. Relative kidney weight was increased in males at ≥147 mg/kg-day and females at 588 mg/kg-day, but there were no renal histopathologic changes in any of the exposed animals. Other effects included hematological alterations consisting of significant increases in leukocyte levels in males at 147 mg/kg-day and females at 588 mg/kg-day, and erythrocyte levels in males at 588 mg/kg-day.

The McCauley et al. (1995) study found that 1,3-dichlorobenzene caused effects in rats at all tested dose levels, indicating that the LOAEL was 9 mg/kg-day and a NOAEL was not identifiable. The most sensitive target discerned on the basis of histopathology was the thyroid. Incidences of lesions in the pituitary and liver were increased at higher dose levels of ≥147 mg/kg-day, although serum levels of the liver-associated enzyme AST were increased at ≥9 mg/kg-day.

No information regarding the chronic toxicity and carcinogenicity of 1,3-dichlorobenzene in humans or animals were located in the literature searched.

4.2.1.3. *1,4-Dichlorobenzene*

Hepatic porphyria induction was investigated in groups of 5 female rats (strain not reported) that were administered 0, 50, 100, or 200 mg/kg 1,4-dichlorobenzene in corn oil by daily gavage for 30, 60, 90, or 120 days (Carlson, 1977). Study endpoints included absolute liver weight, liver porphyrin content, and urinary excretion of porphyrins, porphobilinogen and δ-aminolevulinic acid; body weight, liver histology, and activity of δ-aminolevulinic acid synthase were not evaluated. Absolute liver weights were significantly ($p \leq 0.05$) increased in the 200 mg/kg-day group at days 30 and 60 (approximately 18 and 25% higher than controls, respectively), but not after 90 or 120 days of exposure. The only additional significant increase in liver weight was in the 50 mg/kg-day group after 120 days. Small (10–24%), but statistically significant increases ($p<0.05$) in liver porphyrin levels occurred at 60 days in the 200 mg/kg-day group and after 120 days at ≥50 mg/kg-day. The toxicological significance of the increased

absolute liver weight is unclear due to the small magnitude and transient nature of the effect, and the lack of information on change relative to body weight (body weight was not measured). The increases in liver porphyrins were considered to be slight and not toxicologically significant, particularly because urinary excretion of δ-aminolevulinic acid and porphobilinogen were not increased. The available information therefore indicates that there was a low potential for porphyria and that there were no clear adverse effect levels for the hepatic endpoints examined in this study.

1,4-Dichlorobenzene in olive oil solution was administered to groups of two young adult white male rats (strain not specified) by gavage at doses of 10, 100, or 500 mg/kg, 5 days/week (adjusted: 7.1, 71, or 357 mg/kg-day) for 4 weeks (Hollingsworth et al., 1956). Appearance, behavior, growth, mortality, hematology, and gross histopathology were evaluated. Effects were observed only in the high-dose group, consisting of histologic changes in the kidneys (marked cloudy swelling of the tubular epithelium with cast formation) and liver (marked cloudy swelling and necrosis in the centrilobular region). This study is limited by the small number of animals and a lack of additional relevant information on the design or results of this study (e.g., use of a control group, number of affected animals).

In a longer subchronic study by the same investigators (Hollingsworth et al., 1956), groups of 10 young adult white female rats (strain not specified) were administered 1,4-dichlorobenzene in an olive oil-gum arabic emulsion by gavage at doses of 0, 18.8, 188, or 376 mg/kg, 5 days/week for 138 doses in 192 days (adjusted: 0, 13.5, 135, or 270 mg/kg-day). Organ weights, histology, hematology, bone marrow values, and presence of cataracts were evaluated. No adverse effects were reported for the low dose. Slight increases in average liver and kidney weights occurred at 135 mg/kg-day, but these effects were not considered adverse due to lack of any accompanying histopathologic changes. Effects at 270 mg/kg-day included changes in average organ weights (liver moderately increased, kidneys slightly increased, spleen slightly decreased) and slight cirrhosis and focal necrosis in the liver. No quantitative data (e.g., organ weights and lesion incidences) or other relevant information were reported.

Hollingsworth et al. (1956) also investigated the oral toxicity of 1,4-dichlorobenzene in 7 rabbits that were treated with 500 mg/kg for a total of 263 doses in 367 days (adjusted: 358 mg/kg-day), and in 5 rabbits that were treated with 1000 mg/kg for 92 doses in 219 days (adjusted: 420 mg/kg-day). The chemical was administered by gavage in olive oil, and the rabbits were white and colored (strain not specified) and of mixed sex. A group of vehicle control rabbits (number and additional information not provided) were used for comparative purposes. Clinical signs, body weight, hematology, histology, and presence of cataracts were

evaluated. Observed effects included weight loss, definite to marked tremors, weakness, and slight liver histopathology (cloudy swelling, very few areas of focal, caseous or coagulation necrosis) at ≥358 mg/kg-day, and some deaths at 420 mg/kg-day. No quantitative data or other relevant information were reported.

Two 13-week studies in F344/N rats were performed to determine doses to be used in a chronic rat bioassay (NTP, 1987). The second 13-week study was conducted using reduced doses because a no-effect level had not been achieved in the first study. In both 13-week studies, groups of 10 animals of each sex per dose were treated with technical grade 1,4-dichlorobenzene (>99% pure) in corn oil by gavage, 5 days/week. Evaluations in the first 13-week study included body weight, hematology, urinalysis, clinical chemistry, organ weights, and necropsy on all animals, and histology on selected dose groups, as detailed below. Evaluations in the second 13-week study were limited to body weight, necropsy on all animals, and histology on selected dose groups, as detailed below.

The doses in the first 13-week rat study were 0, 300, 600, 900, 1200, or 1500 mg/kg (adjusted: 0, 214, 429, 643, 857, or 1071 mg/kg-day) (NTP, 1987). Comprehensive histologic exams were performed in the control and three highest dose groups; at lower doses, histology assessment was limited to kidneys and lungs in both sexes at 429 mg/kg-day and in males at 214 mg/kg-day. Body weight gain was reduced in males at ≥214 mg/kg-day (11–32% lower final weight than controls) and in females at 1071 mg/kg-day (11–20%). Mortality apparently related to chemical exposure (no deaths due to gavage error reported) was found in males at 857 mg/kg-day (5/10 died) and 1071 mg/kg-day (8/10 died), and in females at 1071 mg/kg-day (9/10 died). The only clinical signs observed in the exposed rats were tremors, poor motor response, and ocular discharge before death. Kidney histopathology was the main finding at lower doses, occurring in most males at all dose levels (9/10 or 10/10 at 214–857 mg/kg-day, 3/10 at 1071 mg/kg-day). The renal lesions occurred in the proximal convoluted tubules and were characterized by multifocal degeneration or necrosis of the cortical epithelial cells. The lumens of the affected tubules contained an amorphous eosinophilic material, and the numbers and size of eosinophilic droplets in the cytoplasm of the tubular epithelial cells were increased. Other renal effects observed only in male rats included increased kidney weight/brain weight ratio at ≥429 mg/kg-day and increased BUN levels at ≥643 mg/kg-day.

Serum chemistry changes included significantly increased AP in males at ≥214 mg/kg-day and in females at 857 mg/kg-day, reduced triglycerides in males at ≥214mg/kg-day (not changed in females), increased cholesterol in males at ≥429 mg/kg-day and in females at ≥643 mg/kg-day, and reduced total protein at ≥214 mg/kg-day in males and ≥643 mg/kg-day in

females (NTP, 1987). These modest clinical chemistry changes probably reflected hepatic effects of this compound. No alterations in serum AST occurred in either sex. Liver weight/brain weight ratio was significantly increased in both sexes at ≥643 mg/kg-day, and incidences of rats with hepatocyte degeneration and necrosis were increased in both sexes at 857 and/or 1071 mg/kg-day. Liver porphyrin levels were not increased in either sex at any dose, although small increases in urinary uroporphyrin (males) and coproporphyrin (both sexes) occurred at 857 and/or 1071 mg/kg-day. The changes in serum triglycerides, serum cholesterol, and liver weight at the lower dose levels are consistent with the hepatotoxic effects of the chemical indicated by the histopathology at higher doses. Slight, but statistically significant, decreases in erythrocyte count, HCT, and hemoglobin occurred in males at ≥214 mg/kg-day (not found in females). Other effects included bone marrow hypoplasia, spleen and thymus lymphoid depletion, and nasal turbinate epithelial necrosis in both sexes at ≥857 mg/kg-day. The lowest effect level in this study was 214 mg/kg-day, based on changes in liver-associated serum indices and RBC parameters.

The doses in the second 13-week rat study were 0, 37.5, 75, 150, 300, or 600 mg/kg (adjusted: 0, 27, 54, 107, 214, or 429 mg/kg-day) (NTP, 1987). This study was performed because renal lesions had occurred at all dosages in males in the first 13-week study. Comprehensive histologic examinations were performed in the control and three highest dose groups; at the lower doses, histology assessment was limited to kidneys and lungs in both sexes at 54 mg/kg-day and in males at 27 mg/kg-day. No treatment-related effects on body weight gain or survival in either sex, or histopathologic changes in females were observed. An increase in the incidence and severity of kidney cortical tubular degeneration occurred in males at the high dose (control: 7/10, mild; 107 mg/kg-day: 5/10, mild to moderate; 214 mg/kg-day: 3/10, moderate; 429 mg/kg-day: 9/10, moderate).

Monsanto Company (1996) conducted a study of the chronic, systemic effects of 1,4-dichlorobenzene on male and female beagle dogs (5 dogs/sex/group). 1,4-Dichlorobenzene (99.9% pure) was administered to dogs in gelatin capsules at initial dose levels of 0, 10, 50, or 150 mg/kg, 5 days/week, for 1 year (Monsanto Company, 1996). Controls received empty gelatin capsules. Since unexpectedly severe toxicity occurred at the highest dose level, the high dose was adjusted to 100 mg/kg-day during the third week of exposure for males and further reduced to 75 mg/kg-day for both sexes at the beginning of week 6. Both males and females at the highest dose level were untreated during the fourth and fifth weeks to allow for recovery, while lower dose animals were administered the test compound continuously, yielding time

adjusted doses of 0, 7, 36, and 54[1] mg/kg-day. The authors stated that one high-dose male (day 12) and one high-dose female (day 24) dog may have died due to inflammatory lung lesions and/or pulmonary hemorrhages while the cause of death of another high-dose male (day 25) remained undetermined. One control male dog died on day 83 and the cause of death may have been due to a physical displacement of the small intestine, with secondary aspiration pneumonia. Blood and urine were collected pretest, at approximately 6 months and at study termination for hematology, urine analysis, and serum chemistry analyses. Ophthalmoscopic examinations were also conducted pretest and at study termination. All surviving dogs were sacrificed at 12 months and selected organs were examined for gross pathology and histopathology. Pathology examinations included terminal body weights and absolute and relative weights of adrenals, brain, heart, kidneys, liver, pituitary, testes, and thyroid/parathyroid. Histopathologic examinations were conducted on tissues obtained from the adrenals, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, gallbladder, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes, muscle, nerve (sciatic), ovaries, pancreas, parathyroids, pituitary, prostate, rectum, salivary gland, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid, trachea, urinary bladder, and uterus.

Absolute and relative liver weights were statistically significantly increased in both sexes at the two highest doses (36 and 54 mg/kg-day) (see Table 4-1). Increases in absolute and/or relative weights were observed in both sexes at the two highest doses in the following organs: adrenal (absolute: 125 and 130% of control in males; 135 and 141% in females; relative: 143 and 158% of control in males; 138 and 153% in females), and thyroid (absolute: 118 and 123% of control in males; 139 and 132% in females; relative: 133 and 149% of control in males; 143 and 141% in females). These changes were considered possibly treatment-related effects, although no histopathologic lesions were found to explain the increase in the weights of the adrenals and thyroid (Monsanto Company, 1996).

[1] The time-weighted average dose was calculated as follows: [(150 mg/kg-day x 2 weeks) + (100 mg/kg-day x 1 week) + (0 mg/kg-day x 2 weeks) + (75 mg/kg-day x 47 weeks)]/52 weeks = 75 mg/kg-day. This weighted average dose was the same as that administered from week 6 to study termination (time-adjusted: 75×5÷7=54).

Table 4-1. Absolute and relative liver weights of female and male beagle dogs exposed to 1,4-dichlorobenzene in gelatin capsules

Effect	Dose in mg/kg-day (standard deviation)						
	0	7	% Control	36	% Control	54	% Control
Absolute liver weight (gm), male	379.8 (60.58)	318.64 (40.41)	84	473.22 (97.94)	125	531.9[a] (51.28)	140
Absolute liver weight (gm), female	261.8 (45.28)	291.42 (45.22)	111	388.68 (25.32)	148	407.4[b] (41.24)	156
Relative liver weight (%), male	2.7738 (0.49)	2.8821 (0.44)	104	3.9663[b] (0.35)	143	4.726[b] (0.61)	170
Relative liver weight (%), female	2.7078 (0.17)	3.0504 (0.63)	113	4.2028[b] (0.47)	155	4.6040[b] (0.70)	170

[a]Significantly different from control ($p \leq 0.05$; Dunnett's test).
[b]Significantly different from control ($p \leq 0.01$; Dunnett's test).

Source: Monsanto Company, 1996.

Histopathologic examination revealed several liver lesions only in the dosed groups that were considered either direct or indirect/adaptive effects of 1,4-dichlorobenzene and were consistent with gross necropsy findings, organ weight data, and clinical results (see Table 4-2). Liver lesions of mild to moderate severity were observed in all mid- and high-dose male and female dogs. Hepatocellular hypertrophy, multifocal to diffuse with increasing dose level, was statistically significant ($p \leq 0.01$, Fisher's Exact test, one-tailed) in all male and female dogs at mid and high doses, and was also observed in a single female at the lowest dose level. Hepatocellular pigment deposition was observed in two male and one female from each of the mid and high dose groups. Bile duct/ductile hyperplasia was observed at the highest dose level in one male and one female dog. Additional hepatic effects included nodular hyperplasia, bile stasis, chronic active inflammation, and hepatic portal inflamation (Monsanto Company, 1996).

In addition to liver lesions, chronic active interstitial inflammation, pleural fibrosis, and/or pleural mesothelial proliferation was also observed in the lungs of males at all dose levels and females at the mid and high dose levels (36 and 54 mg/kg-day). Although these changes were not observed in the control groups, the lung lesions were not considered to be treatment-related since their occurrence was rare and there was not much difference in severity among the treated groups. Kidney collecting duct epithelial vacuolation was reported in one high dose male and in four females (one in the low-dose group, one in the mid-dose group, and two in the high-dose group). The authors concluded that the lesion could be associated with the test chemical at

the mid and high doses in the females since it was accompanied by increased kidney weights and macroscopic renal discoloration (Monsanto Company, 1996).

Table 4-2. Summary of liver histopathology incidence in female and male beagle dogs exposed to 1,4-dichlorobenzene in gelatin capsules

Liver histopathology	Dose group (mg/kg-day)							
	0		7		36		54	
	M	F	M	F	M	F	M	F
Number of animals examined	5	5	5	5	5	5	5	5
Multifocal bile stasis	0	0	0	0	0	0	0	1
Diffuse congestion	0	0	0	0	0	0	1	0
Bile duct/ductile, multifocal hyperplasia	0	0	0	0	0	0	1	1
Diffuse hepatocellular hypertrophy	0	0	0	0	3	2	5[a]	4[b]
Multifocal hepatocellular hypertrophy	0	0	0	1	2	3	0	1
Focal periportal mononuclear infiltrate	1	0	1	0	1	2	1	0
Multifocal periportal mononuclear infiltrate	0	1	0	0	1	0	1	0
Multifocal chronic active inflammation	0	0	0	0	0	0	0	1
Focal chronic inflammation	0	0	1	0	0	1	0	0
Multifocal chronic inflammation	2	5	3	4	5	3	4	3
Focal portal inflammation	0	0	0	1	0	0	0	0
Multifocal portal inflammation	0	0	0	0	0	0	2	1
Nodular multifocal hyperplasia	0	0	0	0	0	0	0	1
Multifocal hepatocytes pigment deposition	0	0	0	0	2	1	2	1
Multifocal Kupffer cells pigment deposition	1	1	0	1	1	0	1	1

[a]Statistically significant at $p \leq 0.01$, Fisher's exact test, one-tailed.
[b]Statistically significant at $p \leq 0.05$, Fisher's exact test, one-tailed.

Source: Monsanto Company, 1996.

Clinical pathology results revealed a few statistically significant differences in hematology and clinical chemistry parameters and were considered to be related to 1,4-dichlorobenzene exposure (Monsanto Company, 1996). At the 6-month sampling time point, hematological changes included a reduction in basophils at the high dose level and increases in platelet count at the mid and high doses in female dogs. The number of RBCs was significantly reduced in both sexes at the high dose level, while HCT was lowered in the high dose males. At the terminal sampling time point, numbers of large unstained blood cells were reduced in both sexes, platelet count was increased in high-dose females, and MCV was elevated in mid-dose males. Statistically significant differences were observed in various clinical chemistry parameters at the mid and/or high dose levels. AP, ALT, and to a lesser extent AST and GGT were elevated at the mid- and high-dose levels in males and females. AP and ALT levels in mid- and high-dose dogs were 2 to 9 fold higher than control and AST and GGT were no more than 3-fold higher than control. These increased liver enzyme levels support the finding of liver hypertrophy in the dog. Direct and total bilirubin, glucose, and potassium were elevated, while creatinine, albumin, and cholesterol were decreased in the high-dose female dogs. Albumin levels were reduced in males at the mid and high dose levels. No compound-related changes were observed in serum chemistry parameters at the lowest dose. No adverse changes were observed in the urine of males or females at any dose level.

In the chronic NTP (1987) study, groups of 50 male and 50 female F344/N rats were treated with 1,4-dichlorobenzene (>99% pure) in corn oil by gavage, 5 days/week for 103 weeks. The doses in this study were 0, 150, or 300 mg/kg (adjusted: 0, 107, or 214 mg/kg-day) in males and 0, 300, or 600 mg/kg (adjusted: 0, 214, or 429 mg/kg-day) in females. Evaluations comprised body weight, clinical signs, necropsy, and histology in all animals.

Mean body weights of the high-dose males and females were generally slightly lower than those of the controls (5–8% after week 38 and 5–7% after week 55, respectively). Survival of the high-dose males was similar to controls for most of the study, but decreased towards the end of the study (30% lower than controls after week 97). No significant effects on survival were observed for low-dose males or any of the female treatment groups. Nonneoplastic lesions and tumors were observed in the kidneys of the male rats. The incidence of nonneoplastic renal lesions was increased in male rats at $\geq$107 mg/kg-day and included epithelial hyperplasia of the renal pelvis (1/50, 30/50, 31/50 in the control to high dose groups), mineralization of the collecting tubules in the renal medulla (4/50, 46/50, 47/50), and focal hyperplasia of the renal tubular epithelium (0/50, 1/50, 9/50). Incidences of nephropathy were similar in the control and treated male groups, although the severity of lesions was increased in the treated males. Hyaline

droplets, presumably α_{2u}-globulin, were identified in the epithelial cells of the proximal convoluted tubules of male rats. In females, increased nephropathy was the only renal lesion that was treatment-related (21/49, 32/50, 41/49). The nephropathy in the female rats was characterized by the occurrence of several interrelated changes, including degeneration and regeneration of the tubular epithelium, tubular dilatation with thinning and atrophy of the epithelium, granular casts in tubules, thickening of basement membranes, and minimal accumulation of interstitial collagen, but no kidney tumors.

Other histologic lesions included hyperplasia of the parathyroid gland, which was increased in male rats (4/42, 13/42, 20/38). NTP (1987) concluded that the parathyroid hyperplasia was likely secondary to renal effects (i.e., related to a decrease in functional renal mass with subsequent alteration in serum phosphate and calcium excretion by the kidney, followed by stimulation of the parathyroid gland to release parathyroid hormone). The male rat-specific hyaline droplet (α_{2u}-globulin) nephropathy syndrome likely contributed to the kidney effects observed in the males. Based on the observed renal lesions in the male rat, the LOAEL for the male rat is 107 mg/kg-day, the lowest dose tested. Because α_{2u}-globulin-associated renal toxicity is specific to the male rat, the LOAEL associated with this histopathologic finding is not relevant to an assessment of human health. Based on the renal histopathology in the female rats, the chronic LOAEL is 214 mg/kg-day, the lowest dose tested in the females.

Kidney tumors that were induced in the male rats included dose-related increases in tubular cell adenocarcinomas (1/50, 3/50, 7/50), and combined tubular cell adenoma and adenocarcinoma (1/50, 3/50, 8/50) that were statistically significant in the high-dose group relative to controls (NTP, 1987). A dose-related increase in the incidence of mononuclear cell leukemia was also observed in male rats (5/50, 7/50, 11/50) that was significant in the high-dose group. However, even in the high-dose group, the incidence of the leukemia (22%) was comparable to historical vehicle control incidences (14 $\pm$ 8%) in previous NTP studies. No evidence of carcinogenesis was seen in female F344 rats at either dose level. Based on these data, NTP (1987) concluded that there was clear evidence of carcinogenicity in male F344 rats, as shown by an increased incidence of renal tubular cell adenocarcinomas, and no evidence of carcinogenicity in female F344 rats. The renal tumors in male rats are consistent with male rat-specific hyaline droplet (α_{2u}-globulin) nephropathy.

Two 13-week studies in $B6C3F_1$ mice were performed to determine doses to be used in a chronic mouse bioassay (NTP, 1987). The second 13-week study was conducted at reduced dosages because a no-effect level was not achieved in the first study. In both 13-week studies, groups of 10 mice of each sex per dose were treated with technical-grade 1,4-dichlorobenzene

(>99% pure) in corn oil by gavage, 5 days/week. Endpoints in the 13-week mouse studies were the same as those evaluated in the NTP (1987) subchronic rat studies summarized above.

The doses in the first 13-week mouse study were 0, 600, 900, 1000, 1500, or 1800 mg/kg (adjusted: 0, 429, 643, 714, 1071, or 1286 mg/kg-day) (NTP, 1987). Comprehensive histologic examinations were performed in the control and two highest dose groups; at lower doses, histology assessment was limited to the liver and gall bladder in males. Body weight gain was decreased in males (11–14% lower final weight than controls) at ≥429 mg/kg-day, but not clearly affected in females. Chemical exposure-related mortality was found in both sexes at ≥1071 mg/kg-day (3–9 deaths per group); no gavage error deaths were reported. Incidences of centrilobular hepatocellular degeneration were increased in all dose groups and both sexes (7/10 males and 9/10 females at 429 mg/kg-day, 10/10 males and females at 643–1071 mg/kg-day, and 5/10 males and 6/10 females at 1286 mg/kg-day). The severity of the hepatocellular degeneration was dose-related. Other effects included significantly increased serum cholesterol in males and (liver weight):(brain weight) ratios in both sexes at ≥643 mg/kg-day, increased serum protein and triglycerides in males at ≥1071 mg/kg-day, and increased serum AST in males at 1286 mg/kg-day. Serum ALT values were not significantly affected in either sex. Liver porphyrins were slightly increased in both sexes at ≥714 mg/kg-day, but the magnitude was considered to have little biologic significance and was not indicative of porphyria. White blood cell counts were reduced in males (34–50%) at ≥429 mg/kg-day and in females (27–33%) at ≥714 mg/kg-day. The LOAEL was 429 mg/kg-day based on hepatocellular degeneration in both sexes, and decreased white blood cell count in males.

The doses in the second 13-week mouse study were 0, 84.4, 168.8, 337.5, 675, or 900 mg/kg (adjusted: 0, 60, 121, 241, 482, or 643 mg/kg-day) (NTP, 1987). This study was performed because liver lesions had occurred in both sexes at all doses in the first 13-week study. Comprehensive histologic examinations were performed in the control and two highest dose groups; at lower doses, histology assessment was limited to the liver and gall bladder in males. In the second study, no treatment-related effects on body weight gain or survival were observed in either sex. Incidences of centrilobular to midzonal hepatocytomegaly were increased at 482 mg/kg-day (8/10 males and 4/10 females, minimal to mild severity) and 643 mg/kg-day (9/10 males and 10/10 females, mild to moderate severity), indicating that the NOAEL and LOAEL for liver pathology were 241 and 482 mg/kg-day, respectively.

In the chronic NTP (1987) study in $B6C3F_1$ mice, groups of 50 males and 50 females were administered 0, 300, or 600 mg/kg (adjusted: 0, 214, or 429 mg/kg-day) 1,4-dichlorobenzene (>99% pure) in corn oil by gavage, 5 days/week for 103 weeks. Evaluations

comprised body weight, clinical signs, necropsy, and histology in all animals. Body weight and survival in controls and treated mice were comparable. Nonneoplastic lesions and tumors in the liver were prominent effects of exposure in both sexes, as summarized in Table 4-3. The nonneoplastic liver lesions were increased at both dose levels and included hepatocellular degeneration with cell size alteration (cytomegaly and karyomegaly) and individual cell necrosis. No increases in hepatic or bile duct hyperplasia were found in either sex. Hepatocellular adenoma, hepatocellular carcinoma, and combined hepatocellular adenoma or carcinoma occurred with positive dose-related trends in both male and female mice, with the incidences in the low-dose males and high-dose groups of both sexes being significantly greater than those in the control groups. In addition, four cases of hepatoblastoma, an extremely rare type of hepatocellular carcinoma, were observed in high-dose males. No hepatoblastomas were found in the control or low-dose male mice or in any of the female groups. The increased incidence rate for hepatoblastoma was not quite statistically significant (p=0.074), but comparison to historical incidence rates in previous NTP studies (0/1091 in vehicle control and 0/1784 in untreated control males, and 0/1092 in vehicle control and 1/2080 in untreated control females) suggested that the lesion was probably related to treatment. Based on the increased incidences of hepatocellular neoplasms, NTP concluded that there was clear evidence of carcinogenicity in male and female B6C3F_1 mice.

Table 4-3. Liver lesions in B6C3F$_1$ mice treated with 1,4-dichlorobenzene by gavage for two years

	Male mice			Female mice		
Lesion	**Vehicle control**	**214 mg/kg-day[a]**	**429 mg/kg-day[a]**	**Vehicle control**	**214 mg/kg-day[a]**	**429 mg/kg-day[a]**
Number of mice examined	50	49	50	50	48	50
Hepatocellular adenoma	5	13	16	10	6	21
Hepatocellular carcinoma	14	11	32	5	5	19
Hepatocellular adenoma or carcinoma	17	22	40	15	10	36
Hepatoblastoma[b]	0	0	4	0	0	0
Hepatocellular degeneration	0	36	39	0	8	36
Cell size alteration	0	38	40	0	4	27
Focal necrosis	1	35	37	1	4	30

[a]Duration-adjusted dose.
[b]All hepatoblastomas were observed in mice that had hepatocellular carcinomas.

Source: NTP, 1987.

Other histopathologic effects observed in the mice included increased incidences of nephropathy in males (primarily cortical tubular degeneration, with thickening of tubular and glomerular basement membranes and increased interstitial collagen: 6/50, 12/50, 15/47), and renal tubular regeneration in females (4/50, 7/47, 13/46); tubular regeneration was not increased in males. Male mice also showed increased incidences of thyroid gland follicular cell hyperplasia (1/47, 4/48, 10/47), adrenal medullary hyperplasia (2/47, 4/48, 4/49), and adrenal capsular focal hyperplasia (11/47, 21/48, 28/49). The combined incidences of adrenal pheochromocytomas and malignant pheochromocytomas in male mice displayed a significant positive trend (0/47, 2/48, 4/49), but the incidence rates were lower than the historical NTP control values for this tumor. Increased incidences of lymphoid hyperplasia of the mandibular lymph node were observed in male mice (1/46, 12/41, 10/47) and female mice (3/46, 8/43, 10/44). There was a slightly increased incidence of alveolar/bronchiolar carcinomas in low-dose male mice (0/50, 5/50, 0/50), but these tumors were not observed in the high-dose male mice, and the incidence of combined alveolar/bronchiolar adenomas or carcinomas was not significantly increased in either the low- or high-dose male mice (6/50, 13/50, 2/50).

Considering the occurrence of nonneoplastic lesions in the liver, kidneys, thyroid, adrenals, and lymph nodes in both dose groups, this study identified a LOAEL of 214 mg/kg-day.

Several subchronic oral studies, presented below, were conducted to examine possible mechanisms underlying the carcinogenicity of 1,4-dichlorobenzene, particularly the observed species and tissue differences in tumor formation in the NTP (1987) chronic bioassay (i.e., kidney tumors in male rats and liver tumors in both sexes of mice) (Umemura et al., 2000, 1998; Gustafson et al., 1998; Lake et al., 1997; Eldridge et al., 1992; Bomhard et al., 1988). As discussed in Section 4.4 and detailed below, the results include findings indicating that 1,4-dichlorobenzene does not act as a tumor initiator in rat kidneys or as a tumor promoter in mouse liver. Some of the data provide conclusive evidence that 1,4-dichlorobenzene induced renal tubular tumors in male rats by a non-DNA-reactive mechanism, through a male rat-specific α_{2u}-globulin-related response. Other findings suggested that the mechanism leading to the formation of mouse liver tumors by 1,4-dichlorobenzene may be based on sustained mitogenic stimulation and proliferation of the hepatocytes.

1,4-Dichlorobenzene was studied for its ability to induce oxidative DNA damage or initiate carcinogenesis in the kidneys of male F344 rats (Umemura et al., 2000). The potential for generating oxidative stress was assessed by determining the formation of 8-oxodeoxyguanosine (8-oxodG) adducts in kidney nuclear DNA of groups of five rats that were administered 0 or 300 mg/kg of 1,4-dichlorobenzene by gavage, 5 days/week, for 13 weeks (214 mg/kg-day). There was no exposure-related increase in 8-oxodG levels in the kidney DNA. Assessment of cell proliferation in the renal tubules following uptake of injected bromodeoxyuridine (BrdU) showed that, in the exposed rats, the replicating fraction was significantly increased in the proximal convoluted tubules, but not the proximal straight tubules or distal tubules. The kidney tumor-initiating activity of 1,4-dichlorobenzene was evaluated using a two-stage renal carcinogenesis model. Groups of 11 rats were treated with 0 or 300 mg/kg of 1,4-dichlorobenzene by gavage, 5 days/week for 13 weeks (214 mg/kg-day), followed by exposure to 1000 ppm trisodium nitrilotriacetic acid (NTA, a known kidney tumor promoter) in the drinking water for 26 or 39 weeks. Histologic examinations showed that promotion by NTA did not induce renal neoplastic lesions in the rats given 1,4-dichlorobenzene.

Groups of 10 male and 10 female F344 CDF rats were treated with 1,4-dichlorobenzene in corn oil by gavage at daily doses of 0, 75, 150, 300, or 600 mg/kg (Bomhard et al., 1988). Five animals of each sex and dose group were sacrificed after 4 weeks, and the remaining animals after 13 weeks of treatment. Evaluations included clinical observations, body weight, food and water consumption, HCT, blood chemistry (creatinine, urea, testosterone),

comprehensive urinalysis, gross examination of all organs and tissues, kidney weight, and kidney histology and ultrastructure. No compound-related effects on clinical signs, body weight, or food consumption were observed in either sex. Water consumption was increased 20% at 75 mg/kg-day and 40% at 600 mg/kg-day in males, and 23% in females at 600 mg/kg-day. Other effects observed in male rats included significantly increased urinary excretion of LDH (day 9–week 12) and protein (weeks 4–12) at ≥75 mg/kg-day, and increased β-N-acetylglucosaminidase (NAG) excretion (week 12) at 600 mg/kg-day. Urinary LDH, total protein and NAG values were generally decreased in treated females. Absolute and relative kidney weights were significantly increased in males at ≥150 mg/kg-day and in females at 600 mg/kg-day at 13 weeks, but histologic signs of renal damage were observed only in males. Renal histopathologic changes in the males included hyaline droplet accumulation in the cortical tubular epithelia and lumina at ≥75 mg/kg-day, dilated tubules with granular cast formation in the outer zone of the medulla and tubular single-cell necrosis at 150–600 mg/kg-day, and occasional epithelial desquamation of the longer parts of tubules at ≥300 mg/kg-day. The female rats showed no comparable renal histopathology. The renal effects in male rats were a consequence of male rat specific α_{2u}-globulin nephropathy, and not predictive for effects in humans. No toxic effects were seen in females at any dose.

Effects of 1,4-dichlorobenzene on replicative DNA synthesis in the liver and kidney and hepatic xenobiotic metabolism were investigated in rats and mice (Lake et al., 1997). Groups of 6–8 male F344 rats were treated with 0, 25, 75, 150, or 300 mg/kg doses in corn oil by gavage, 5 days/week for 1, 4, or 13 weeks (adjusted: 0, 18, 54, 107, or 214 mg/kg-day). Groups of 6–8 male B6C3F_1 mice were similarly exposed to 0, 300, or 600 mg/kg (adjusted: 0, 214, or 429 mg/kg-day) of compound for 1–13 weeks. Toxicity endpoints evaluated at all dose levels and durations in both species included body weight, relative liver and kidney weights, hepatocyte and renal proximal tubule cell BrdU labeling indices, hepatic microsomal CYP P450 content, and 7-pentoxyresorufin O-dependentylase (PROD) activity (a marker for induction of CYP P450 isoenzyme CYP2B). Rats dosed with 107 or 214 mg/kg-day and mice dosed with 429 mg/kg-day for 1 week were evaluated for hepatic microsomal protein content and activities of 7-ethoxyresorufin O-deethylase (EROD) and erythromycin N-demethylase (markers for CYP1A and CYP3A, respectively). Rats dosed with 54 or 214 mg/kg-day and mice dosed with 214 or 429 mg/kg-day for 1 week were additionally assayed for induction of hepatic microsomal CYP2B1/2 and CYP3A using Western immunoblotting analysis. Liver histology was evaluated in the control and high-dose groups of rats and mice exposed for 13 weeks.

Toxic effects in the rats included significantly increased liver weights at ≥54 mg/kg-day for 4 weeks and ≥107 mg/kg-day for 4 and 13 weeks; increased hepatocyte labeling index at 214 mg/kg-day for 1 week (not increased at ≤214 mg/kg-day for 4 and 13 weeks); increased CYP P450 at ≥107 mg/kg-day for 1 week, ≥25 mg/kg-day for 4 weeks and ≥54 mg/kg-day for 13 weeks; increased PROD activity at ≥54 mg/kg-day for 1 and 4 weeks and ≥18 mg/kg-day for 13 weeks; increased CYP2B1/2 at ≥54 mg/kg-day for 1 week; increased CYP3A at 214 mg/kg-day for 1 week; increased hepatic EROD and erythromycin N-demethylase activities at ≥107 mg/kg-day for 1 week; increased microsomal protein at 214 mg/kg-day for 1 week; and mild centrilobular hypertrophy at 214 mg/kg-day for 13 weeks (Lake et al., 1997). Renal effects in rats included increased kidney weights at ≥107 mg/kg-day for 4 and 13 weeks, and increased BrdU labeling indices in renal proximal tubule cells at 214 mg/kg-day for 1 week, ≥54 mg/kg-day for 4 weeks, and ≥107 mg/kg-day for 13 weeks. A LOAEL of 214 mg/kg-day was derived from this study based on centrilobular hepatocellular hypertrophy in rats. A NOAEL was not identified because histopathology was not performed at the lower doses.

Hepatic effects in mice included significantly increased liver weight and PROD activity at ≥214 mg/kg-day for 1–13 weeks; increased hepatocyte labeling index at ≥214 mg/kg-day for 1 and 4 weeks (not increased at 13 weeks); increased CYP P450 at 429 mg/kg-day for 1–13 weeks; increased EROD and erythromycin N-demethylase activities and microsomal protein at 429 mg/kg-day for 1 week; increased CYP2B1/2 at ≥214 mg/kg-day for 1 week; and marked centrilobular hypertrophy at 429 mg/kg-day for 13 weeks. Renal effects in mice included increased BrdU labeling in renal proximal tubule cells at ≥214 mg/kg-day for 4 weeks (not increased at ≤429 mg/kg-day for 1 or 13 weeks) with no changes in relative kidney weight. Induction of hepatic enzymes and increased liver weight are considered adaptive effects of 1,4-dichlorobenzene. The LOAEL was 429 mg/kg-day based on marked centrilobular hypertrophy; a NOAEL was not identified for the same reason as with the rat study.

Hepatocellular proliferation was investigated in groups of 5–7 B6C3F_1 mice of both sexes and female F344 rats that were administered 1,4-dichlorobenzene by gavage, 5 days/week for 13 weeks in doses of 0, 300, or 600 mg/kg (adjusted: 0, 214, or 429 mg/kg-day) (mice) or 0 or 600 mg/kg (adjusted: 0 or 429 mg/kg-day) (rats) (Eldridge et al., 1992). Toxicity endpoints included body weight, absolute liver weight, hepatocyte BrdU labeling index, plasma enzyme activities (ALT, AST, LDH, and sorbitol dehydrogenase), and liver histology. Significant increases in hepatocyte labeling index were only observed in male and female mice at 429 mg/kg-day after 1 week of exposure, in male mice at 429 mg/kg-day after 3 weeks, and female rats at 429 mg/kg-day after 1 and 6 weeks. The increase in labeling index was relatively small in rats at 6

weeks and was not observed at 12 weeks, and there were no significant increases in mice after 6 or 13 weeks. Absolute liver weights were significantly increased in male and female mice at 214 mg/kg-day at weeks 6 and 13, as well as in male and female mice and female rats at 429 mg/kg-day at weeks 1–13. No exposure-related changes in body weight or liver-associated plasma enzyme levels were observed. There was no histopathologic evidence of hepatocellular necrosis in either species, although centrilobular hepatocytes were hypertrophic with enlarged hyperchromatic nuclei in male and female mice at 429 mg/kg-day after 13 weeks. None of the reported changes in rats are considered adverse. In mice, the 429 mg/kg-day dose was a LOAEL for hypertrophic liver lesions and 214 mg/kg-day was a NOAEL because none of the changes reported for this dose were considered adverse.

Liver cell proliferation was also evaluated in groups of 5 male $B6C3F_1$ mice and male F344 rats that were gavaged with 1,4-dichlorobenzene in corn oil, 5 days/week for 1 or 4 weeks in doses of 0, 150, 300, or 600 mg/kg (adjusted: 0, 107, 214, or 429 mg/kg-day) (mice) or 0, 75, 150, or 300 mg/kg (adjusted: 0, 54, 107, or 214 mg/kg-day) (rats) (Umemura et al., 1998). Toxicity endpoints included relative liver weight, BrdU-based hepatocyte cumulative replicating fraction (CFR), and liver injury based on immunohistochemical detection of glutamine synthetase (GS)-expressing centrilobular hepatocytes. Liver histology was not evaluated. Relative liver weights were significantly increased after 1 and 4 weeks in mice at $\geq$429 mg/kg-day and rats at $\geq$107 mg/kg-day. The CFR was increased after 1 week in mice at $\geq$214 mg/kg-day and rats at $\geq$107 mg/kg-day, but was elevated only in mice at 429 mg/kg-day at week 4. Hepatocyte injury (reduced size of hepatic GS area) was detected in mice exposed to $\geq$107 mg/kg-day for 1 or 4 weeks, but not in rats. None of the endpoints observed were clearly adverse, so the high doses of 429 mg/kg-day in mice and 214 mg/kg-day in rats were NOAELs.

The potential for 1,4-dichlorobenzene to promote liver tumors in rats was evaluated in a subchronic initiation-promotion bioassay (Gustafson et al., 1998). Male F344 rats were given a single intraperitoneal (i.p.) injection of 0.9% saline (12 animals) or 200 mg/kg of diethylnitrosamine (DEN) in saline (18 animals), followed by oral administration of 1,4-dichlorobenzene beginning 2 weeks later. Rats promoted with 1,4-dichlorobenzene were treated with doses of 0.1 or 0.4 mmol/kg-day (14.7 or 58.8 mg/kg-day) in corn oil by gavage for 6 weeks. Control rats were similarly treated with corn oil alone or DEN in corn oil. All animals were partially hepatectomized 1 week after the start of 1,4-dichlorobenzene exposure. The study was terminated at the end of week 8, and immunohistochemical analysis was performed to identify preneoplastic glutathione *S*-transferase-expressing foci in the liver. No 1,4-dichloro-

benzene-related increased incidence of hepatic foci was found, suggesting that the compound was not a liver tumor promoter in rats.

4.2.2. Inhalation Exposure

4.2.2.1. *1,2-Dichlorobenzene*

Groups of male and female albino rats (20/sex) and guinea pigs (8/sex) were exposed to 0, 49, or 93 ppm (0, 290, or 560 mg/m^3, respectively) of 1,2-dichlorobenzene (99% pure) vapor for 7 hours/day, 5 days/week for 6-7 months (Hollingsworth et al., 1958). In addition, groups of male and female albino rabbits (2/sex) and 2 female monkeys were similarly exposed to 93 ppm, and groups of 10 female mice (strain not reported) were similarly exposed to 49 ppm. Study parameters included gross appearance, behavior, final body weight, absolute organ weights (lungs, heart, liver, kidneys, spleen, and testes), gross pathology, and histopathology. Relative organ weights were not determined and the scope of the histopathologic examinations was not specified. Hematology evaluations (in rabbits and monkeys), qualitative urine tests (sugar, albumin, sediment and blood in females of all species), and BUN determinations were also performed, but appear to have been limited to the 93 ppm group. Effects observed at 93 ppm consisted of statistically significant ($p \leq 0.05$) decreases in absolute spleen weight in male guinea pigs (20% lower than controls) and final body weight in male rats (8.9% lower than controls). No lesions in any tissues were reported. No compound-related changes occurred in any of the species exposed to 49 ppm 1,2-dichlorobenzene. No additional relevant information on the design and results of this study, including possible respiratory system effects, was reported. Based on the available information, this study identified a NOAEL of 49 ppm and LOAEL of 93 ppm based on decreased body weight gain in rats and decreased spleen weight in guinea pigs.

A short-term study compared the histologic effects of various inhaled chemicals, including 1,2-dichlorobenzene, on the respiratory tract (Zissu, 1995). Groups of 10 male Swiss OF_1 mice were exposed to 1,2-dichlorobenzene at actual mean concentrations of 0, 64, or 163 ppm (0, 385, or 980 mg/m^3) for 6 hours/day, 5 days/week for 4, 9, or 14 days. The upper and lower respiratory tracts were the only tissues examined in the study. Histopathologic lesions were observed in the olfactory epithelium of the nasal cavity at ≥64 ppm. The olfactory epithelial lesions were graded as very severe following the 4-day exposure and moderate after the 14-day exposure, indicating to the authors that a repair mechanism may have taken place despite continued exposure. The more severe cases were characterized by a complete loss of olfactory epithelium, which left only the partially denuded basement membrane. No histologic alterations were observed in the respiratory epithelium of the nasal cavity, or in the trachea or lungs. The

results suggest that the upper respiratory tract was a target for inhalation exposure to 1,2-dichlorobenzene at concentrations below those that caused systemic effects in rats in the Hollingsworth et al. (1958) study summarized above.

4.2.2.2. *1,3-Dichlorobenzene*

No subchronic or chronic inhalation studies were located for 1,3-dichlorobenzene.

4.2.2.3. *1,4-Dichlorobenzene*

A subchronic toxicity study (Asio et al., 2005a) exposed BDF_1 mice and F344 rats of both sexes (6 h/d and 5 d/wk) to inhalation of 25, 55, 120, 270 or 600 ppm (v/v) 1,4-dichlorobenzene vapor for 13 weeks. The exposure to 1,4-dichlorobenzene vapor retarded the growth rate in the male mice, and induced hepatotoxicity in the mice and rats of both sexes and renal and hematological toxicity in the male rates. Hepatotoxicity was characterized by increased liver weight, hepatocellular hypertrophy, and increased serum levels of total cholesterol. Liver necrosis and increased serum levels of AST and ALT were observed in the exposed mice, whereas these changes, which indicate hepatocellular death, did not occur in any of the exposed rats. Renal lesions occurred only in 1,4-dichlorobenzene-induced male rats. The NOAEL was 120 ppm for the hepatic endpoint in mice and for the renal endpoint in rats.

In another subchronic toxicity groups of 20 rats (10/sex), 16 guinea pigs (8/sex), 10 mice (males or females), 2 rabbits (1/sex), and 1 monkey (female) were exposed to 96 or 158 ppm (580 or 950 mg/m^3) 1,4-dichlorobenzene ($\geq$99% pure) vapor for 7 hours/day, 5 days/week for 5-7 months (Hollingsworth et al., 1956). Similar numbers of animals were used as control groups for each species and exposure level, except for the 158 ppm rats and rabbits, which had control groups that comprised approximately double the number of exposed animals. Other groups of animals were exposed for 7 hours/day, 5 days/week to 173 ppm (1040 mg/m^3) for 16 days (5 rats/sex, 5 guinea pigs/sex and 1 rabbit/sex) or 341 ppm (2050 mg/m^3) for 6 months (20 male rats and 8 guinea pigs/sex). In addition, groups of rats (19 males, 15 females), guinea pigs (16 males, 7 females), and rabbits (8 males, 8 females) were exposed to 798 ppm (4800 mg/m^3) for 8 hours/day, 5 days/week for up to 69, 23, and 62 exposures, respectively.

In this subchronic study (Hollingsworth et al., 1956) clinical signs, organ weights, gross pathology, and histopathology were examined following all exposures. Additional toxicity endpoints reported for the 96, 158, and 173 ppm groups included final body weights and relative organ weights (lungs, heart, liver, kidneys, spleen, and testes). Hematology evaluations (in rabbits and female rats), qualitative urine tests (sugar, albumin, sediment, and blood in females of

all species) and BUN determinations (rabbits and female guinea pigs) were performed, but appeared to have been limited to the 96 ppm exposure groups. Relative liver weights were significantly increased ($p \leq 0.05$) in female guinea pigs exposed to 96 ppm for 199 days and 158 ppm for 157 days (9–10% higher than controls), and in rats of both sexes exposed to 158 ppm for 198–199 days or 173 ppm for 16 days (10–27% higher than controls). Relative kidney weights were significantly increased in male rats exposed to 158 ppm for 199 days (12.5% higher than controls). Histopathology included slight liver changes in the rats at 158 and 173 ppm (cloudy swelling, congestion or granular degeneration of questionable significance in the parenchymal cells of the central zones), and hepatic effects in male guinea pigs at 341 ppm (cloudy swelling, fatty degeneration, focal necrosis, and slight cirrhosis). Effects observed in the animals exposed to 798 ppm included frank signs of toxicity (marked tremors, weakness, weight loss, eye irritation, scruffy appearance, unconsciousness, and a few deaths) and histopathologic changes in the liver (cloudy swelling and central necrosis), kidneys (slight cloudy swelling of the tubular epithelium in female rats), and lungs (slight congestion and emphysema in two rabbits). No additional relevant information on the design and results of this study was reported. The NOAEL and LOAEL were most appropriately identified as 96 and 158 ppm, respectively, based on the increases in liver weight accompanied by hepatic histopathology in rats.

Chronic inhalation studies were conducted with 1,4-dichlorobenzene in rats and mice (Imperial Chemical Industries [ICI], 1980; Riley et al., 1980). In the rat study, groups of 76–79 Wistar rats of each sex were chamber exposed to 0, 75, or 500 ppm 1,4-dichlorobenzene for 5 hours/day, 5 days/week for up to 76 weeks (ICI, 1980). Five rats/sex/group were sacrificed at 26–27, 52–53, and 76–77 weeks, and the remaining animals were sacrificed after a 32-week recovery period (at week 112). Endpoints evaluated throughout the study included clinical condition, body weight, and food and water consumption. Blood chemistry (urea, glucose, ALT, and AST), urinalysis (pH, glucose, bilirubin, specific gravity, protein, and coproporphyrin) and hematology (red cell count, total and differential white cell counts, hemoglobin, HCT, MCHC, packed cell volume, platelet count, and bone marrow abnormalities) were assessed in 5 or 10 rats/sex/group at weeks 5, 14, 26–27, 40, and/or 52–53. Hepatic aminopyrine demethylase activity was evaluated in 5 rats/sex/group at 52–53 weeks. Pathological examinations that included absolute organ weight measurements (liver, kidney, adrenal, spleen, gonads, heart, lung, brain, and/or pituitary) and comprehensive histology (including nasal sinuses, trachea, and lung) were performed on all rats found moribund or dead, or killed at the interim or terminal sacrifices.

There were no exposure-related effects on clinical signs, survival, food or water consumption, blood chemistry, or hematology in either sex (ICI, 1980; Riley et al., 1980). Body

weight gain was slightly reduced (~3-5% less than controls) in both groups of male rats during the first few weeks of the study, but was comparable to controls by week 10 and throughout the rest of the study. Changes in urinalysis values were observed at 500 ppm and included increases in urinary protein and coproporphyrin excretion. Mean urinary protein levels were 2.9- to 3.3-fold higher than control values in 500 ppm males after 27, 40, and 52 weeks of exposure; no clear exposure-related changes were observed in females. Mean urinary coproporphyrin levels were 1.2- to 5.4-fold higher than control values in 500 ppm males throughout the exposure period but were unaffected in females. The urinalysis values were statistically not significantly different from the controls, but were based on a small number of measurements (5 per interval). Absolute kidney weights were significantly increased at 500 ppm in males at weeks 26–27 and 76–77, but were similar to those of controls at 109–112 weeks (i.e., after the recovery period). In females, absolute kidney weights were significantly increased in the 500 ppm group at 109–112 weeks. Absolute liver weights were significantly higher than controls in males at 500 ppm after 76–77 weeks, and in females at ≥75 ppm after 26–27 weeks and 500 ppm after 109–112 weeks, but not in 500 ppm females after 76–77 weeks. Hepatic aminopyrine demethylase activity at 52 weeks was slightly increased (1.8-fold higher than controls) in males at 500 ppm but unaffected in females.

There was no clear histologic evidence of any treatment-related toxic or carcinogenic effects in any tissues, including those of the respiratory system. Examination of the nasal passages showed lesions that included olfactory epithelial degeneration, respiratory epithelial hyperplasia, subacute rhinitis, squamous metaplasia and adentitis of nasal glands, but similar changes were also observed in the control groups and the effects were generally considered to be incidental or age-related. Effects considered to be minimal and age-related were also found in the lungs of control and exposed rats (e.g., peribronchial/perivascular lymphoid accumulation and infiltration, chronic interstitial inflammatory infiltration, and alveolar histiocytosis). An effect level of 500 ppm has been identified based on increases in liver and kidney weights, but the toxicological significance of these changes is unclear due to the lack of related clinical chemistry and histopathology findings. The adequacy of this study for carcinogenicity evaluation is limited by the failure to reach a MTD, as well as the less-than-lifetime exposure duration and short observation period.

In the mouse study, groups of 75 female SPF Swiss mice were exposed by inhalation to 1,4-dichlorobenzene at vapor concentrations of 0, 75, or 500 ppm for 5 hours/day, 5 days/week, for 57 weeks, followed by observation for 18–19 weeks (Riley et al., 1980). The study originally included similar groups of male mice, but was terminated because of high mortality attributed to

fighting and probable respiratory infection. A high background incidence of respiratory disease was observed in all groups of males as well as females. Study endpoints appeared to be the same as in the ICI (1980) rat inhalation study summarized above. There was no histologic evidence of compound-related toxic or carcinogenic effects, but the exposure and observation durations were insufficient for adequate assessment of carcinogenic potential. Evaluation of this study is complicated by the lack of a primary report; unlike the rat study summarized above, the mouse study was reviewed from a secondary source (Loeser and Litchfield, 1983) because the complete report was not available (i.e., not submitted to EPA under TSCA).

In a study conducted by the Japan Bioassay Research Center (JBRC) (1995) groups of 50 male and female F344/DuCrj rats and Crj:BDF1 mice were exposed to target concentrations of 0, 20, 75, or 300 ppm 1,4-dichlorobenzene for 6 hours/day, 5 days/week for 104 weeks. This study was subsequently published by Aiso et al. (2005b). Investigators conducted the study in accordance with Organization for the Economic Cooperation and Development (OECD) guidelines and in conformity with the OECD Principles of Good Laboratory Practice. Animals were observed daily for clinical signs and mortality. Body weight and food consumption were determined weekly for the first 14 weeks, and every 4 weeks thereafter. Urinalysis was performed in study week 104. Fasting blood samples were taken from all animals at sacrifice and evaluated for hematological and blood biochemical parameters. A complete gross examination was performed on all animals, and organ weights were determined for adrenal glands, testis, ovaries, heart, lungs, kidneys, spleen, liver, and brain. Thirty-seven organs or tissues were fixed in formalin and examined histologically for the presence of nonneoplastic and neoplastic changes.

For rats, actual mean chamber concentrations were 0, 19.8, 74.8, or 298.4 ppm over the duration of the study. The number of rats surviving to scheduled termination was significantly reduced in 300 ppm male rats, but not in 20 or 75 ppm male rats (control: 33/50, 20 ppm: 34/50, 75 ppm: 29/50, 300 ppm: 18/50); survival began to be noticeably lower than controls at approximately week 80 of the study. Survival in female rats was not affected by treatment (control: 38/50, 20 ppm: 34/50, 75 ppm: 38/50, 300 ppm: 36/50). No treatment-related changes in body weight, food consumption, or clinical signs were reported at any exposure level with either sex. The study authors reported a number of changes in organ weights (liver in both sexes, kidney in males) and biochemical and hematological parameters (mean cell volume, blood total cholesterol, phospholipids, BUN, creatinine, and calcium in males; total protein, total bilirubin, BUN, and potassium in females) at 300 ppm; however, absence of quantitative data or statistical

analysis within the report precludes interpretation of these results. No treatment-related changes in urinary parameters were reported for any groups of male or female rats.

Exposure to 1,4-dichlorobenzene resulted in increased incidence of eosinophilic changes (moderate or greater severity) in the olfactory epithelium of both sexes of rats (1/50, 2/50, 2/50, and 7/50 in males, and 27/50, 29/50, 39/50, and 47/50 in females in the 0, 20, 75, and 300 ppm groups, respectively); the increases were statistically significant in 300 ppm males and 75 and 300 ppm females. In high-dose females only, significantly increased incidences of eosinophilic changes in the respiratory epithelium, and respiratory metaplasia of the nasal gland were also seen. In 300 ppm male rats, statistically significant increases in mineralization of the renal papilla, and in hyperplasia of the urothelium were reported; no treatment-related kidney changes were seen in female rats. The incidence of centrilobular hepatocellular hypertrophy was increased in 300 ppm males (5/50 versus 0/50 in the other groups of male rats). In rats, Aiso et al. (2005b; JBRC, 1995) identified a NOAEL of 20 ppm and a LOAEL of 75 ppm for increased incidence of eosinophilic changes in the olfactory epithelium of females. Increases in neoplastic lesions were not seen in either sex of rats despite apparently reaching the MTD, as evidenced by decreased survival in 300 ppm male rats.

In mice, mean chamber concentrations were 0, 19.9, 74.8, or 298.3 ppm over the duration of the study (Aiso et al., 2005b; JBRC, 1995). Survival was slightly reduced in exposed males of all groups (39/49, 31/49, 32/50, and 30/49 for the 0, 20, 75, and 300 ppm groups, respectively), but was statistically significantly decreased only in the 300 ppm males; survival in exposed females was comparable to controls. At study termination, body weights were significantly decreased in 300 ppm male mice only (12% at the end of the 2-year exposure period compared to controls). No treatment-related changes in food consumption or clinical signs were noted. Absolute and relative liver weights were increased in 300 ppm male and female mice, but not in other treated groups. Absolute and relative kidney weights were significantly increased in 300 ppm females; relative kidney weight was significantly increased in 300 ppm males. As with rats, 300 ppm animals showed changes in a number of biochemical and hematological parameters (total cholesterol, serum ALT, AST, LDH, and ALP activity in males; platelet numbers, serum total protein, albumin, total cholesterol, BUN, calcium, ALT, AST, LDH, and ALP activity in females), but a lack of numerical data or statistical analysis precludes interpretation of these findings. No changes in urinary parameters were reported in male or female mice at any exposure level.

On histologic examination, male mice showed a statistically significant increase in centrilobular hepatocellular hypertrophy in the 300 ppm group only (0/49, 0/49, 0/50, and 34/49

in the 0, 20, 75, and 300 ppm groups, respectively), and a significant increase in mineralization of the testis in the 75 and 300 ppm groups (27/49, 35/49, 42/50, and 41/49 in the 0, 20, 75, and 300 ppm groups, respectively). No nonneoplastic changes in histologic endpoints were reported in female mice. The NOAEL and LOAEL for nonneoplastic effects were 20 and 75 ppm, respectively, based on mineralization of the testis in males.

The incidences of several tumor types were elevated, as shown in Table 4-4 below. Hepatocellular carcinomas and histiocytic sarcomas were statistically significantly increased in 300 ppm male mice, and both showed a significant dose-response trend. In female mice, adenomas and carcinomas were increased at 300 ppm; both showed a positive dose-response trend. The combined incidence of bronchoalveolar adenomas and carcinomas showed a significant positive trend; the incidence at 300 ppm (7/50) was at the upper end of the range of historical control data from the JBRC (minimum for a given bioassay: 1/50; maximum: 7/50). The hepatocellular carcinomas in a number of the 300 ppm males (8/38) and females (6/41) displayed hepatoblastoma-like features. Hepatoblastoma is a malignant neoplasm composed of tissue resembling embryonal or fetal epithelium, or mixed epithelial and mesenchymal tissues. Hepatoblastoma-like features in mouse hepatocellular carcinoma were rare in the laboratory's historical control data (10/1496 for male BDF_1 mice and 0/1498 for females) (JBRC, 1995). The significance of the lesion is unclear, but the authors considered it to be a subtype of hepatocellular carcinoma, because almost all hepatoblastoma-like features were found within or adjacent to the hepatocellular adenoma carcinoma, and there was a continuity between the hepatocellular carcinoma and hepatoblastoma-like feature. In this study, 1,4-dichlorobenzene treatment resulted in increased incidence of liver tumors at 300 ppm in both male and female mice; the relevance of the increases in hepatocellular adenomas in 20 ppm females is questionable, given the lack of an increase in the 75 ppm animals.

Table 4-4. Incidence of neoplastic lesions in mice treated with 1,4-dichlorobenzene via inhalation

		Incidence of tumors (# tumors/# animals)			
Tumor type	**Gender**	**Control**	**20 ppm**	**75 ppm**	**300 ppm**
Hepatocellular carcinoma	Male	12/49[a]	17/49	16/50	38/49[b]
Hepatic histiocytic sarcoma	Male	0/49[a]	3/49	1/50	6/49[b]
Hepatocellular carcinoma	Female	2/50[a]	4/50	2/49	41/50[b]
Hepatocellular adenoma	Female	2/50[a]	10/50[b]	6/49	20/50[b]
Hepatocellular adenoma or carcinoma	Female	4/50[a]	13/50[b]	7/49	45/50[b]
Bronchoalveolar adenoma and carcinoma	Female	1/50[a]	4/50	2/49	7/50[b]

[a] Significant positive linear trend by Peto test.
[b] Statistically significantly different (p<0.05) from control, using Fisher's Exact test.
Historical control incidence rates for the above tumors were as follows: hepatocellular adenoma: 119/700 male, 33/699 female; histiocytic sarcoma: 22/700 male; hepatocellular carcinoma: 171/700 male, 15/699 female; bronchoalveolar adenoma: 26/699 female; bronchoalveolar carcinoma: 23/699 female.

Source: Aiso et al., 2005b, JBRC, 1995.

No effects were found in a subchronic immunotoxicity study of inhaled 1,4-dichlorobenzene in guinea pigs (Suzuki et al., 1991). This study was reported in the Japanese literature and relevant information was obtained from the English abstract, and data in tables. Groups of 10 male SPF Hartley guinea pigs were exposed to concentrations of 0, 2, or 50 ppm for 12 weeks (exposure schedule not specified). The animals were sensitized with ovalbumin twice during the exposure period (4 and 8 weeks after exposure commencement) to evaluate effects on immunoglobulin [Ig]E, IgG, and IgM antibody production. IgE antibody titers (passive cutaneous anaphylaxis test) and IgG and IgM antibody titers (determined by enzyme-linked immunosorbent assay) against ovalbumin in serum collected 1 and 2 weeks after the first sensitization and 1, 2, and 4 weeks after the second sensitization, showed no significant differences between the exposed and control groups. The passive cutaneous anaphylaxis test was also conducted with antiserum from the 50 ppm exposure group (collected 1 and 2 weeks after the first sensitization and 1, 2, and 4 weeks after the second sensitization) to determine if IgE antibodies were produced against 1,4-dichlorobenzene; no antibodies against the compound were

detected. Active systemic anaphylaxis was also evaluated in the 0 and 50 ppm exposure groups. An antigen mixture of 1,4-dichlorobenzene and guinea pig serum albumin did not cause an anaphylactic reaction when injected intravenously in the animals 14 days after the last exposure. There were no exposure-related effects on other study endpoints, including body weight, hematology (including total and differential leukocyte counts), and absolute and relative weights of selected organs (thymus, spleen, liver, kidneys, lungs, and heart), indicating that 50 ppm was the subchronic NOAEL for immunological and other systemic effects in guinea pigs.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Exposure

4.3.1.1. *1,2-Dichlorobenzene*

No oral reproductive toxicity studies of 1,2-dichlorobenzene were located.

An oral developmental toxicity study of 1,2-dichlorobenzene is available only as an abstract with little detailed information available concerning the methods used. In this study (Ruddick et al., 1983), pregnant female Sprague-Dawley rats were administered 50, 100, or 200 mg/kg-day doses of 1,2-dichlorobenzene by gavage on gestational days 6–15 (use of controls not reported). Maternal body weight gain, 15 unspecified biochemical parameters, and histology were used to evaluate maternal toxicity. The fetuses were evaluated for litter size, fetal weight, deciduoma (intrauterine mass composed of decidual cells), skeletal and visceral changes, and histopathology. No teratologic effects were reported. No other information regarding developmental or maternal toxicity was noted. Based on the limited available information, 200 mg/kg-day was a NOAEL for maternal and developmental toxicity of 1,2-dichlorobenzene in rats.

4.3.1.2. *1,3-Dichlorobenzene*

No oral reproductive toxicity studies of 1,3-dichlorobenzene were located.

An oral developmental toxicity study of 1,3-dichlorobenzene is available only as an abstract with little detailed information available concerning the methods used. In this study (Ruddick et al., 1983), pregnant female Sprague-Dawley rats were administered via gavage 50, 100, or 200 mg/kg 1,3-dichlorobenzene on gestational days 6–15 (use of controls not reported). Maternal body weight gain, 15 unspecified biochemical parameters, and histology were used to evaluate maternal toxicity. The fetuses were evaluated for litter size, fetal weights, deciduoma, skeletal and visceral changes, and histopathology. No teratologic effects were reported. No other information regarding developmental or maternal toxicity was noted. Based on the limited

available information, 200 mg/kg-day was a NOAEL for maternal and developmental toxicity of 1,3-dichlorobenzene in rats.

4.3.1.3. *1,4-Dichlorobenzene*

A 2-generation reproduction study was conducted in which 1,4-dichlorobenzene (99% pure) in olive oil was administered daily by gavage to male and female Sprague Dawley rats at dose levels of 0, 30, 90, or 270 mg/kg-day (Bornatowicz et al., 1994). Groups of 24 F_0 rats/sex/dose were treated for 77 days (males) and 14 days (females) before mating, followed by exposure of both sexes for 21 days during mating and females during gestation (21 days). The males were exposed for a longer duration (77 days) than females (14 days) to expose the sperm through all stages of spermatogenesis. Exposure of the F_0 females continued throughout lactation until weaning of the F_1 pups on postnatal day 21. Groups of 24 F_1 weanlings/sex/dose were treated for 84 days before mating, followed by exposure of both sexes for 30 days during mating and females during gestation (21 days) and lactation (21 days). The study was ended following weaning of the F_2 pups on postnatal day 21. The F_0 and F_1 males were sacrificed 21 days after the end of the mating period, although it is unclear whether their exposure continued post-mating. The F_0 and F_1 females were sacrificed after their pups were weaned. Study endpoints included clinical observations in adults and pups, body weight and food consumption in maternal animals (during gestation and lactation) and pups (from birth to day 21), reproductive indices (including duration between mating and successful copulation, number of pregnancies, gestation length, and litter size), numbers of live and dead pups, postnatal survival, postnatal developmental milestones (times to erect ears and eyelid separation), and neurobehavioral effects in pups at weaning (auricle reflex, orientation reaction, grasping, and draw-up reflexes). Necropsies were performed on adult males and females at the scheduled sacrifices, on apparently non-pregnant F_0 and F_1 females and spontaneously dead animals, and on pups that died during the first 4 days or were sacrificed on day 4 (i.e., those not selected for continuation in the study). Liver, kidney, and spleen weights were measured in males and females of both generations; it was not indicated if additional organs were weighed. Histopathologic examinations were limited to selected tissues (liver, kidneys, spleen, vagina, cervix, uterus, ovaries, mammary gland, testes, epididymides, penis, prostate, seminal vesicles, and spermatic cord) from adult F_0 and F_1 animals that had no living young, died prematurely, or were sacrificed as moribund, as well as gross lesions in all animals.

No reproductive or other exposure-related changes were found at 30 mg/kg-day in adults or pups (Bornatowicz et al., 1994). Effects occurring at $\geq$90 mg/kg included statistically

significantly reduced (method of analysis and *p*-values not reported) average birth weight in F_1 pups (4.4, 5.7, and 22.6% lower than control group at 30, 90, and 270 mg/kg-day, respectively). Significant reductions in body weight were also observed at 270 mg/kg-day in F_1 pups on postnatal days 7, 14, and 21, as well as at 270 mg/kg-day in F_2 pups at birth and on postnatal days 4, 7, 14, and 21. The total number of deaths from birth to postnatal day 4 was significantly increased in F_1 pups at 270 mg/kg-day (3/214, 6/310, 7/273, and 62/231 at 0, 30, 90, and 270 mg/kg-day, respectively), and F_2 pups at ≥90 mg/kg-day (3/294, 4/296, 17/330, and 31/253 at 0, 30, 90, and 270 mg/kg-day, respectively). None of the data in this study were reported on a per-litter basis or analyzed for dose-related trends. Other significant effects on offspring survival indices were observed at 270 mg/kg-day, including reduced total number of live F_1 and F_2 pups at birth, increased total dead F_1 and F_2 pups at birth, and increased total dead F_1 and F_2 pups during postnatal days 5-21. Additional exposure-related effects included delayed eye opening (first day of appearance or day shown in all pups) in F_1 and F_2 pups at 270 mg/kg-day, delayed ear erection (day shown in all pups) in F_2 pups at 270 mg/kg-day, and reduced percentage of pups per litter with a positive reaction in the draw-up test in the F_1 pups at 270 mg/kg-day and in F_2 pups at ≥90 mg/kg-day (3.3, 7.4, and 22.3% less than controls at 30, 90, and 270 mg/kg-day, respectively). The draw-up test evaluated whether pups that were hanging from a horizontal wire by the front paws could grasp the wire with at least one hind leg within 5 seconds.

Clinical manifestations were evident in pups of both generations at ≥90 mg/kg-day, including dry and scaly skin until approximately postnatal day 7 (0, 0, ca. 70, and 100% of the litters at 0, 30, 90, and 270 mg/kg-day, respectively). Tail constriction appeared between days 4 and 21 in all or nearly all litters (percentages not reported) and, in isolated cases, led to loss of parts of the tail (Bornatowicz et al., 1994). Annular constriction of the tail results from a lack of hydration in the skin of the tail. Additionally, the number of F_1 pups described as cyanotic after birth was increased (not quantified) at 270 mg/kg-day. Effects observed in adult animals were generally not quantified, but included reduced average body weight in F_1 males and females at 270 mg/kg-day (approximately 20 g [males] or 10 g [females] lower than control groups at all time points during gestation and lactation [no other data reported]), increased relative liver weight in F_1 males at ≥90 mg/kg-day, and changes in absolute and/or relative kidneys weights (increased) and spleen weights (reduced) in F_1 males at 270 mg/kg-day. There were no effects on organ weights in females of either generation. The only histopathologic finding attributed to exposure was unspecified kidney damage in both generations (effect levels, possible male specificity, and other information not reported). This study identified a NOAEL and LOAEL of

30 and 90 mg/kg-day, respectively, for developmental toxicity based on increased mortality and other effects in F_1 and F_2 pups during the preweaning period. There were no effects on mating and fertility indices in any group.

Developmental toxicity was evaluated in groups of 13–17 pregnant CD rats that were administered 1,4-dichlorobenzene (99% pure) in corn oil by gavage in dosages of 0, 250, 500, 750, or 1000 mg/kg-day on gestational days 6–15 (Giavini et al., 1986). Sacrifices were performed on gestational day 21. Maternal evaluations included clinical signs, survival, food consumption, body weight, gross necropsy, and liver weight. Uteri were examined for numbers of corpora lutea, implantations, live fetuses, and resorptions. Fetal evaluations included body weight, visceral abnormalities (one-half of the fetuses), and skeletal abnormalities (remaining fetuses). Maternal deaths due to gavage error occurred at 500 and 1000 mg/kg-day. Dose-related decreases in mean maternal weight gain and food consumption were observed during the treatment period. At 250 mg/kg-day, maternal weight gain and food consumption were decreased 18.3% (not statistically significant) and 11.1% ($p<0.05$), respectively; decreases in weight gain were statistically significant at ≥500 mg/kg-day. The decreases in maternal weight gain and food intake returned to control levels after the treatment period. There were no exposure-related changes in maternal liver weight. Numbers of fetuses with extra ribs were significantly increased in a dose-related manner at ≥500 mg/kg-day; data for this endpoint were not reported on a per litter basis. Incidences of fetuses with any skeletal anomaly were significantly increased at ≥750 mg/kg-day, although there was no change in incidences of affected litters. Mean fetal body weights were significantly reduced (8.1%) at 1000 mg/kg-day. No other exposure-related fetal effects were observed. This study identified a NOAEL and LOAEL of 250 and 500 mg/kg-day, respectively, for developmental toxicity based on skeletal variations. These doses were also a NOAEL and LOAEL for maternal toxicity based on decreased body weight gain.

One oral developmental toxicity study of 1,4-dichlorobenzene is available only as an abstract with little detailed information available concerning the methods used. In this study (Ruddick et al., 1983), pregnant female Sprague-Dawley rats were administered via gavage 50, 100, or 200 mg/kg 1,4-dichlorobenzene on gestational days 6–15 (use of controls not reported). Maternal body weight gain, 15 unspecified biochemical parameters, and histology were used to evaluate maternal toxicity. The fetuses were evaluated for litter size, fetal weights, deciduoma, skeletal and visceral changes, and histopathology. No teratologic effects were reported. No other information regarding developmental or maternal toxicity was noted. Based on the limited

available information, 200 mg/kg-day was a NOAEL for maternal and developmental toxicity of 1,4-dichlorobenzene in rats.

Another oral developmental toxicity study of 1,4-dichlorobenzene exposed eighteen pregnant rats to 2 mg/kg 1,4-dichlorobenzene and/or 10 mg/kg 1,1-dichloro-2,2 bis(p-chlorophenyl)ethylene (p,p′-DDE) by mixing these compounds with rat chow to form food pellets (Makita, 2005). In this study, ingestion of 1,4-dichlorobenzene and/or p,p′-DDE did not result in any overt sign of maternal toxicity, and every pregnant dam delivered offspring. There were no adverse reproductive outcomes in dams (as measured by fertility, average litter size, fetal sex ratio, and number of live pups). No gross malformations were observed at birth in any of the offspring. No differences in growth were recognized in any group (controls; 1,4-dichlorobenzene only; 1,4-dichlorobenzene and p,p′-DDE). There were no marked differences in food consumption in the groups studied. Male offspring was followed until 6 weeks of age after perinatal exposure to approximately 2 mg/kg 1,4-dichlorobenzene and/or 10 mg/kg p,p′-DDE and exhibited no developmental effects, as measured by viability, body weight gain, anogenital distance (PND 4 and 7), time of eye opening, organ weights, and serum hormones (testosterone, luteinizing hormone and follicle stimulating hormone).

4.3.2. Inhalation Exposure

4.3.2.1. *1,2-Dichlorobenzene*

A two-generation inhalation reproduction study was conducted in which groups of Charles River CD (Sprague-Dawley derived) rats (30/sex/generation) were exposed by inhalation to 1,2-dichlorobenzene (99.2% pure) in vapor concentrations of 0, 50, 150, or 394 ppm (0, 301, 902, and 2370 mg/m^3, respectively) (Bio/dynamics, 1989). F_0 adults were exposed for 6 hours/day, 7 days/week for a 10-week premating period and during mating. Following mating, F_0 males were exposed 6 hours/day, 7 days/week until sacrifice at 3–4 weeks post-mating. Pregnant F_0 females were exposed for 6 hours/day on gestation days 0–19 and lactation days 5–28, then sacrificed post-weaning. F_1 pups (29 days old) received similar exposures throughout an 11-week premating period, continued through mating, gestation, and lactation. Although the respiratory tract was not examined, a comprehensive range of toxicological responses was evaluated including mortality, clinical signs, body weight, food consumption, organ weights, reproductive parameters, gross necropsy, and histologic examination of selected tissues (all the selected tissues in the high-exposure group as well as kidney in males and liver of both sexes in low- and mid-exposure groups). Parameters used to evaluate toxicity in pups included mortality,

clinical signs, body weight (measured on lactation days 0, 4, 14, 21, and 28), sex ratio, gross necropsy (all tissues), and histologic examination of grossly abnormal tissues.

There were no exposure-related effects on reproductive performance or fertility indices in either generation, indicating that the NOAEL for reproductive toxicity was 394 ppm (Bio/dynamics, 1989). Statistically significant changes in F_0 and F_1 adults exposed to 150 and 394 ppm included decreased body weights relative to controls at some intervals during the premating period, increased absolute (males) and relative (both sexes) kidney weights, and increased absolute and relative liver weights (both sexes). Because information on food consumption was not available, it could not be established whether reduced body weight was related to 1,2-dichlorobenzene exposure or a consequence of reduced food consumption. Histopathologic examination revealed hypertrophy of central lobular hepatocytes in adult F_0 and F_1 rats of both sexes exposed to 150 and 394 ppm. Histopathologic lesions of the kidney at these exposure levels featured dilated renal tubular lumina with intraluminal granular casts, predominantly at the corticomedullary junction. Adult F_0 and F_1 males from all exposure groups had cytoplasmic granules/droplets in the proximal convoluted tubular epithelium of the kidney; the severity of this condition increased with exposure levels. The description of the renal lesions, the histochemical staining characteristics of the granules/droplets, and their occurrence only in the males are consistent with hyaline droplet (α_{2u}-globulin) nephropathy. The NOAEL and LOAEL for systemic toxicity were 50 and 150 ppm, respectively, based on decreased body weight; the increases in liver weight were not considered adverse in the absence of degenerative histopathologic changes.

The developmental toxicity following inhalation of 1,2-dichlorobenzene was investigated in rats and rabbits. A range-finding study was conducted (Dow Chemical Company, 1981) to establish the maximum tolerated maternal exposure levels used in a complete developmental toxicity study of these species (Hayes et al., 1985). In the range-finding study, groups of 10 female F344 rats and 7 female New Zealand rabbits were exposed to 1,2-dichlorobenzene (98.81% pure) in measured concentrations of 0, 200, 400, or 500 ppm for 6 hours/day on gestational days 6–15 (rats) or 6–18 (rabbits), and animals were sacrificed on the day following the last exposure (Dow Chemical Company, 1981). Examinations were limited to the maternal animals and included clinical signs, food and water consumption, body weight, liver and kidney weights, gross pathology, corpora lutea, number and position of live, dead, and resorbed fetuses, implantation sites in nonpregnant animals, and pregnancy incidence. There were no reported effects on the respiratory system or exposure-related changes in the reproductive and fetal endpoints in either species. Effects in the maternal rats included decreased food consumption

and increased relative liver and kidney weights at ≥400 ppm. Additional effects observed in maternal rats at 500 ppm included clinical signs (e.g., slight eye irritation, severe perineal staining); decreased body weight, weight gain and food consumption; gross pathologic signs of systemic toxicity (particularly enlargement or slight paleness of the liver); and embryolethality among the animals showing the most severe signs of maternal toxicity (3 of 10 animals had severe vaginal bleeding and totally resorbed litters). Slight toxicity was observed in the maternal rabbits at 500 ppm, as indicated by nonsignificant but consistent decreases in body weight gain, and liver weight and slight gross hepatic changes (generalized paleness or accentuated lobular pattern in 5 of 7 animals).

The developmental toxicity of inhaled 1,2-dichlorobenzene (98.81% pure) was more completely investigated in groups of 30–32 pregnant female F344 rats and 28–30 pregnant New Zealand White rabbits that were exposed to 0, 100, 200, or 400 ppm (0, 600, 1200, or 2400 mg/m^3) for 6 hours/day on days 6–15 (rats) or 6–18 (rabbits) of gestation, with termination on gestational day 21(rats) or 29 (rabbits) (Hayes et al., 1985). Maternal toxicity endpoints included clinical signs, body weight, food and water consumption, and liver and kidney weights. Fetal observations included number and position of fetuses *in utero*, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, implantation sites in nonpregnant animals, sex, body weight, crown-rump length, and external, visceral, head, and skeletal abnormalities. Maternal effects in the rats included significantly reduced body weight gain on gestational days 6–8, 12–15, and 6–20 at ≥100 ppm, increased liver weight at 100 ppm (relative) and 400 ppm (absolute and relative), and urine soaking of the perineal area at 400 ppm. No respiratory system effects were reported in either species. Exposure-related developmental effects in the rats comprised a statistically significant increase in the incidence of fetuses with delayed ossification of cervical vertebral centra at 400 ppm (not significantly increased on a per litter basis). Maternal effects in the rabbits were essentially limited to body weight loss during the first 3 days of exposure (gestation days 6–8) in all exposed groups at ≥100 ppm. The lowest concentration, 100 ppm, was a LOAEL for maternal toxicity in both species based on body weight effects. No exposure-related developmental effects were observed in rabbits, indicating that 400 ppm was a NOAEL for developmental effects in this species. The NOAEL and LOAEL for developmental toxicity in rats were 200 and 400 ppm, respectively, based on the increase in skeletal variations.

4.3.2.2. *1,3-Dichlorobenzene*

No inhalation reproductive or developmental studies were located for 1,3-dichlorobenzene.

4.3.2.3. *1,4-Dichlorobenzene*

A two-generation inhalation reproduction study of 1,4-dichlorobenzene was conducted in which groups of 28 Sprague-Dawley rats of each sex were exposed to vapor concentrations of 0, 50, 150, or 450 ppm for 6 hours/day, 5 days/week for 10 weeks (Tyl and Neeper-Bradley, 1989). Mean analytical concentrations (±SD) in the three exposure groups were 66.3±8.47, 211±8.0, and 538±50.5 ppm (398, 1268, or 3233 mg/m^3) (see discussion in the following paragraph). Additional groups of 10 females were similarly exposed for 10 weeks in a satellite study. The animals in the main study were paired within groups for a 3-week mating period to produce the F_1 generation. Main study males that did not mate successfully during the first 10 days of the mating period were paired with satellite females for 10 days. Main study females that did not mate successfully during the first 10 days of the mating period were paired with proven males for the remaining 11 days of the mating period. Exposure of the main study F_0 females was continued throughout the mating period and the first 19 days of gestation, discontinued from gestational day 20 through postnatal day 4, and then resumed until sacrifice at weaning on postnatal day 28. Exposure of the satellite F_0 females was continued through mating until sacrifice on gestation day 15. Exposure of F_0 males continued until sacrifice at the end of the study and satellite mating periods. Groups of 28 F_1 weanlings/sex and satellite groups of 10 F_1 female weanlings were exposed for 11 weeks and mated as described above to produce the F_2 generation. Additionally, 20 F_1 weanlings/sex from the control and high exposure groups served as recovery animals that were observed without exposure for 5 weeks prior to sacrifice. Complete necropsies were performed on all F_0 and F_1 adult (parental) animals, F_1 recovery animals, F_1 weanlings not used in the rest of the study, and F_2 weanlings, and histology was evaluated in the F_0 and F_1 parental animals. Histologic examinations were conducted on the liver and kidneys in all groups and on selected other tissues (pituitary, vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, and tissues with gross lesions) in the control and high exposure groups. The kidney evaluation included examination for the presence of α_{2u}-globulin droplets. Additional endpoints evaluated in the parental generations included clinical observations, mortality, body weight, and food consumption. Mating and fertility indices were determined for F_0 and F_1 males and females, and gestational, live birth, postnatal survival (4-, 7-, 14-, 21-, and 28-day), and lactation indices were determined for the F_1 and F_2 litters.

The initial analytical method was determined to be inadequate by the investigators due to problems associated with sampling (syringe draw from stainless steel tubes extending into the breathing zone), such that there was an underestimation of the vapor concentrations during the first 80 days of the study. Analyses obtained by charcoal absorption methods during the last third of the study indicated chamber concentrations that were in good agreement with nominal concentrations. Mean charcoal tube analytical/nominal ratios and the original nominal data were used to recalculate actual chamber atmosphere concentrations for exposure days 1–171. The mean chamber concentrations (±SD) for the 284 days of exposure were determined to be 66.3±8.47, 211±8.0, and 538±50.5 ppm (398, 1268, and 3233 mg/m^3) in the three exposure groups.

There were no effects on reproductive parameters in either generation in the absence of parental toxicity. Systemic toxicity occurred at all dose levels in F_0 and F_1 adult rats (Tyl and Neeper-Bradley, 1989). Hyaline droplet nephropathy was found in F_0 and F_1 adult males at ≥66 ppm. Manifestations of this male rat-specific renal syndrome included α_{2u}-globulin accumulation and increased kidney weights at ≥66 ppm and other characteristic histologic changes (e.g., tubular cell hyperplasia) at 538 ppm. Body weights and weight gain were significantly reduced in F_0 and F_1 adult males and F_1 adult females during the premating exposure period at 538 ppm. Relative liver weights were significantly (p<0.05 or p<0.01) increased in F_0 adult males at ≥66 ppm, F_0 adult females and F_1 adult males at ≥211 ppm, and F_1 adult females at 538 ppm. Absolute liver weights were significantly increased in F_0 adult males at ≥211 ppm, and in F_0 adult females and F_1 adult males and females at 538 ppm. Liver weight changes were more pronounced in males than in females. Mean relative liver weights in the 66, 211, and 538 ppm adult male groups of the F_0 generation (sacrificed at week 15) were 4.8, 13.9, and 52.1% , respectively, higher than controls, and 0, 6.7, and 43.7% higher than controls in the F_1 generation (sacrificed at week 17). Hepatocellular hypertrophy was observed in the livers of F_0 and F_1 males and females at 538 ppm; no hepatic histologic changes were induced at the lower exposure concentrations. The increases in liver weight and hepatocellular hypertrophy were considered to be adaptive and not adverse liver effects because there were no accompanying degenerative lesions.

Other effects also occurred in the F_0 and F_1 males and females at 538 ppm, indicating that there was a consistent pattern of adult toxicity at the high exposure level, including reduced food consumption and increased incidences of clinical signs (e.g., tremors, scruffy appearance, urine stains, salivation, and nasal and ocular discharges); these effects occurred only sporadically at 211 ppm. Other effects at 538 ppm included reduced gestational and lactational body weight

gain, and postnatal toxicity, as evidenced by increased number of stillborn pups, reduced pup body weights and reduced postnatal survival in F_1 and/or F_2 litters. A NOAEL of 211 ppm and a LOAEL of 538 ppm were identified in the Tyl and Neeper-Bradley study (1989) based on clinical signs and postnatal developmental toxicity.

Information on male reproductive toxicity of inhaled 1,4-dichlorobenzene is also available from an unpublished mouse dominant lethal test (Anderson and Hodge, 1976) that was summarized by Loeser and Litchfield (1983). Groups of 35 (control) or 16 (exposed) fertile male CD-1 mice were exposed to 0, 75, 225, or 450 ppm of 1,4-dichlorobenzene for 6 hours/day for 5 days, and then mated with unexposed virgin females each week for 8 weeks during all stages of the spermatogenic cycle (Anderson and Hodge, 1976). Females were killed 13 days after fertilization and the uteri were examined for live implantations and early and late fetal deaths. No exposure-related effects on male reproductive performance were observed, as evaluated by endpoints that included percentages of males that successfully mated each week and females that became pregnant, early fetal deaths per pregnant female, females with one or more early deaths, percentage of total implantations per pregnancy, or total implantations per pregnant female, making the high exposure level of 450 ppm a NOAEL in this study. Positive responses were produced in groups of concurrent positive control mice exposed to ethyl methane sulfonate or nitrogen mustard.

The developmental toxicity of inhaled 1,4-dichlorobenzene was investigated in rats and rabbits. The rats were investigated in an unpublished study (Hodge et al., 1977) that was summarized by Loeser and Litchfield (1983). Groups of $\geq$20 SPF rats were exposed to 0, 75, 200, or 500 ppm of 1,4-dichlorobenzene for 6 hours/day on days 6–15 of gestation. Study endpoints included clinical signs, maternal weight gain, number of viable fetuses, resorptions and corpora lutea, fetal sex and body weights, and external, visceral, and skeletal abnormalities. There were no exposure-related indications of maternal toxicity, embryotoxicity, fetotoxicity, or teratogenicity, indicating that 500 ppm was a NOAEL for these endpoints. No additional relevant information was provided in the available study summary.

A range-finding study was conducted in rabbits (Dow Chemical Company, 1982) to establish the maximum tolerated maternal exposure levels used in a complete developmental toxicity study in rabbits (Hayes et al., 1985). Groups of seven New Zealand rabbits were exposed to 1,4-dichlorobenzene (99.97% pure) at concentrations of 0, 300, 600, or 1000 ppm for 6 hours/day on days 6–18 of gestation and sacrificed on the following day (Dow Chemical Company, 1982). Examinations were limited to the maternal animals and included clinical signs, body weight, liver and kidney weights, gross pathology, corpora lutea, number and position of

live, dead and resorbed fetuses, implantation sites in nonpregnant animals, and pregnancy incidence. The only exposure-related effects were observed at 1000 ppm and indicative of slight maternal toxicity (e.g., slight decreases in body weight gain and decreased hepatocellular vacuolation suggestive of decreased glycogen deposition).

The developmental toxicity of inhaled 1,4-dichlorobenzene (99.9% pure) was more completely evaluated in groups of 29–30 pregnant New Zealand rabbits that were exposed to 0, 100, 300, or 800 ppm (0, 590, 1770, or 4720 mg/m^3) of 1,4-dichlorobenzene vapor (99.9% pure) for 6 hours/day on gestation days 6–18, and sacrificed on day 29 (Hayes et al., 1985). Maternal toxicity endpoints included clinical signs, body weight, food and water consumption, and liver and kidney weights. Fetal observations included number and position of fetuses *in utero*, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, implantation sites in nonpregnant animals, sex, body weight, crown-rump length, and external, visceral, head, and skeletal abnormalities. Effects were observed at 800 ppm that included maternal body weight loss on gestational days 6–8 and a slight, nonsignificant increase in the incidence of retroesophageal right subclavian artery in the offspring (p>0.05, Fisher's Exact test) on a fetal or litter basis. Maternal weight gain was not significantly reduced at other time periods in the study, and the 800 ppm group gained only slightly (4.25%) less weight than controls over the total period of exposure. The fetal effect was considered to be a minor variation of the circulatory system rather than an abnormality indicative of a teratogenic response, and was previously observed in 2% of control litters in the same laboratory. The only other statistically significant findings in this study were increased percentages of resorbed implantations and litters with resorptions in the 300 ppm group only.

4.4. OTHER STUDIES

4.4.1. Mechanistic Considerations

4.4.1.1. *Renal Effects of Dichlorobenzenes*

In a previous Health Effects Assessment for *p*-dichlorobenzene, U.S. EPA (1987) indicated that the relevance of the male rat kidney tumors to human carcinogenicity was an ongoing scientific debate, and concluded that the available bioassay data were equivocal as a basis for extrapolating to humans. Of primary concern was the possibility that the renal tumors observed in male rats in the NTP study were the result of what has been called "α_{2u}-globulin nephropathy," a condition that results in kidney lesions, including tumors, in male rats, but not in female rats, by a mechanism that is not present in other species, including humans. (For a more complete discussion of the α_{2u}-globulin nephropathy, see U.S. EPA, 1991b.) Both

1,4-dichlorobenzene and its major metabolite, 2,5-dichlorophenol, have been shown to bind reversibly to α_{2u}-globulin in a manner similar to that of 2,2,4-trimethylpentane (TMP), a component of unleaded gasoline that has been shown to elicit α_{2u}-globulin-related effects (Charbonneau et al., 1989). Animals treated with 1,4-dichlorobenzene develop kidney lesions characteristic of α_{2u}-globulin-related toxicity, including hyaline droplet formation and cellular damage and proliferation of the P1/P2 proximal tubule regions (Lake et al., 1997; Bomhard et al., 1988). Additionally, NBR rats, a strain that does not synthesize α_{2u}-globulin, showed no renal effects following a gavage exposure to 500 mg/kg of 1,4-dichlorobenzene for 4 days, whereas F344 rats showed clear evidence of α_{2u}-globulin accumulation and toxicity at the same dose levels (Dietrich and Swenberg, 1991). Thus, the available evidence supports the development of α_{2u}-globulin-related lesions following exposure to 1,4-dichlorobenzene.

The evidence for the involvement of α_{2u}-globulin in the development of renal lesions following subchronic or chronic exposure to 1,2- or 1,3-dichlorobenzene is less strong. The available subchronic data for 1,2-dichlorobenzene offer some evidence of effects on the kidney, with the strongest evidence coming from the 2-generation inhalation study by Bio/dynamics (1989), which reported the presence of hyaline droplets, consistent with α_{2u}-globulin nephropathy, in both F_0 and F_1 male rats. Other studies of 1,2-dichlorobenzene toxicity (Robinson et al., 1991; NTP, 1985; Hollingsworth et al., 1958) presented evidence of renal toxicity, but not of effects consistent with α_{2u}-globulin nephropathy. For example, Hollingsworth et al. (1958) and Robinson et al. (1991) both reported increased kidney weights in both male and female rats, while NTP (1985) reported increased renal tubular regeneration in male mice chronically-exposed to 1,2-dichlorobenzene. Since α_{2u}-globulin-related effects are specific to male rats, these observed renal effects must occur via another mechanism, possibly the metabolism-based mechanism discussed below for hepatic effects (Valentovic et al., 1993). Available data do not indicate that renal lesions are a sensitive endpoint for exposure to 1,3-dichlorobenzene (McCauley et al., 1995), and do not suggest an involvement of α_{2u}-globulin.

4.4.1.2. *Hepatic Effects of Dichlorobenzenes*

4.4.1.2.1. ***Role of metabolism***. The initial step in the acute toxicity of at least two of the dichlorobenzene isomers, particularly following oral exposure, appears to be metabolic activation by CYP P450 enzymes within the liver (see Figures 3-1 to 3-3). However, the degree of involvement of the P450 enzymes appears to vary greatly from one dichlorobenzene isomer to the other, with the more acutely hepatotoxic isomers, 1,2- and 1,3-dichlorobenzene, showing greater involvement of CYP P450-based metabolism than the hepatocarcinogenic

1,4-dichlorobenzene (Nedelcheva et al., 1998). The initial metabolism likely results in a reactive intermediate, most likely an epoxide, that can bind covalently to cellular proteins or react with GSH, resulting in a depletion of cellular GSH stores. The typical hepatotoxic effects of dichlorobenzenes, including the release of intracellular enzymes and degenerative and necrotic changes, are presumed to be a result of the accumulation of reactive metabolites. However, while these mechanisms are potentially involved in the subchronic and/or chronic toxicity of the dichlorobenzenes, their contribution has not been established conclusively.

4.4.1.2.1.1. ***1,2-Dichlorobenzene***. Considerable evidence exists supporting the hypothesis that the toxicity of 1,2-dichlorobenzene results from initial P450-related metabolism to an epoxide, followed by reaction of that epoxide with cellular molecules. Stine et al. (1991) treated F344 rats with 0.9–5.4 mmol/kg (132–794 mg/kg) of 1,2-dichlorobenzene by i.p. injection, resulting in a dramatic hepatotoxic response at all doses, as measured by increases in plasma ALT, with the greatest peak occurring at 24 hours postexposure, and a gradual decrease throughout 72 hours postexposure. Pretreatment with SKF-525A, a CYP P450 inhibitor, effectively blocked the increase in ALT caused by 1,2-dichlorobenzene treatment, while pretreatment with phenobarbital resulted in a considerable increase in hepatotoxicity. Valentovic et al. (1993) similarly reported that pretreatment with piperonyl butoxide (another CYP P450 inhibitor) significantly decreased the hepatotoxicity of 1,2-dichlorobenzene.

Additional evidence for the involvement of a reactive intermediate in the hepatotoxicity of 1,2-dichlorobenzene comes from studies involving depletion of cellular oxidant defenses, or measuring indicators of oxidative stress. Pretreatment with phorone, which depletes hepatic GSH, resulted in greatly enhanced serum ALT levels after 1,2-dichlorobenzene administration (Stine et al., 1991). In a later study by Younis et al. (2000), pretreatment of F344 or Sprague-Dawley rats with 1-aminobenzotriazole, a noncompetitive inhibitor of CYP P450, completely eliminated the decrease in hepatic GSH levels and increased the level of oxidized glutathione (GSSG) in the bile following oral exposure to 1,2-dichlorobenzene.

4.4.1.2.1.2. ***1,3-Dichlorobenzene***. In the study mentioned above, Stine et al. (1991) exposed F344 rats to a single i.p. dose of 0.9–5.4 mmol/kg (132–794 mg/kg) of 1,3-dichlorobenzene, and reported increased levels of plasma ALT activity 12–72 hours post-exposure at doses of 3.6 mmol/kg (529 mg/kg) or higher. The increased ALT levels were dramatically enhanced by pretreatment with phenobarbital, to a level equivalent to that seen with 1,2-dichlorobenzene, which normally produces a much greater toxicity. Pretreatment with phorone to deplete hepatic

GSH resulted in a substantial increase in the amount of plasma ALT observed following 1,3-dichlorobenzene exposure (Stine et al., 1991). Thus, similar to 1,2-dichlorobenzene, 1,3-dichlorobenzene appears to be biotransformed by CYP P450 enzymes to a hepatotoxic intermediate, evidenced by the increase in ALT following phenobarbital administration. The fact that GSH depletion enhanced the toxicity of 1,3-dichlorobenzene is further evidence of biotransformation to a reactive intermediate, likely an epoxide, that can react with cellular GSH. No other data on the involvement of CYP P450 enzymes on the hepatotoxicity of 1,3-dichlorobenzene or data examining the possible role of GSH conjugation or covalent binding in the toxicity of 1,3-dichlorobenzene are available.

4.4.1.2.1.3. ***1,4-Dichlorobenzene***. 1,4-Dichlorobenzene appears to be the least acutely hepatotoxic dichlorobenzene isomer, and the isomer whose acute toxicity is least likely to be influenced by CYP P450-based metabolism. Exposure of male F344 rats and male B6C3F$_1$ mice to 1,4-dichlorobenzene resulted in both an increase in general CYP P450 activity and an induction of microsomal CYP P4502B1/2 protein levels, as assessed by Western blotting (Lake et al., 1997). However, while exposure to 1,4-dichlorobenzene can induce CYP P450 enzymes, induction of CYP P450 enzymes by pretreatment with phenobarbital did not result in an acute toxic response, as measured by plasma ALT levels, after a single i.p. injection of 0.9 mmol/kg (132 mg/kg) of 1,4-dichlorobenzene (Stine et al., 1991). In contrast to the results with 1,2- and 1,3-dichlorobenzene, i.p. injection of doses as high as 5.4 mmol/kg (794 mg/kg) had no effect on plasma ALT levels in F344 rats (Stine et al., 1991).

While not as convincing as the evidence for 1,2-dichlorobenzene, evidence exists supporting a mechanism of toxicity of 1,4-dichlorobenzene based on metabolism to a reactive or oxidative metabolite. Incubation of microsomes with radiolabeled 1,4-dichlorobenzene and later treatment with antioxidants (i.e., ascorbic acid) resulted in a decrease in in vitro covalent binding to macromolecules (Hissink et al., 1997b), suggesting that in vitro metabolism resulted in the formation of reactive oxygen species. Additional studies demonstrated that depletion of GSH levels resulted in an acute hepatotoxic response following administration of 100–132 mg/kg of 1,4-dichlorobenzene (Mizutani et al., 1994; Stine et al., 1991). However, unlike 1,2-dichlorobenzene, 1,4-dichlorobenzene treatment did not appear to increase oxidized glutathione levels in liver (Gustafson et al., 2000), suggesting that if a reactive intermediate was formed, it occurred at a low concentration or did not tend to oxidize GSH. In vivo studies did not report covalent binding to DNA after treatment with 1,4-dichlorobenzene.

4.4.1.2.2. ***Role of cell proliferation***. An issue that has been subject to considerable discussion is the potential mechanism behind the appearance of liver tumors in mice, but not in rats, in the 2-year bioassays of 1,4-dichlorobenzene, particularly given the fact that other isomers were much more acutely hepatotoxic but did not show evidence of hepatocarcinogenicity at similar doses. One hypothesis suggested an effect of 1,4-dichlorobenzene on regulation of hepatic cell proliferation.

Exposure of male F344 rats to 1,4-dichlorobenzene by gavage for 7 days resulted in a decrease in the proportion of hepatic tetraploid cells, an increase in hepatic octoploid cells, and an increase in hepatic labeling index following BrdU administration (Hasmall and Roberts, 1997). Umemura et al. (1992) likewise reported an increase in proliferating cells in both sexes of rats and mice exposed to 1,4-dichlorobenzene by gavage for 4 days. In a 4-week study of male F344 rats and B6C3F$_1$ mice, using the same doses as the NTP bioassay, Umemura et al. (1998) reported increased hepatic proliferation, as measured by an increase in the CRF, in both species at 1 week. The increase was observed only in high-dose mice (the only dose at which a statistically significant increase in tumor incidence was seen in the chronic study) in week 4 of the study. Similar increases in labeling index were reported in the 13-week subchronic studies of B6C3F$_1$ mice (Lake et al., 1997; Eldridge et al., 1992) and F344 rats (Lake et al., 1997).

The observed hepatic cell proliferation did not appear to be the result of post-necrotic regeneration, as evidenced by a lack of histologic evidence for necrosis in the NTP chronic study (NTP, 1987) and data reporting that 1,4-dichlorobenzene exposure did not induce unscheduled DNA synthesis in the livers of rats and mice (Sherman et al., 1998; Perocco et al., 1983). Rather, the proliferation was believed to result from an increase in the rate of cell division, a decrease in the rate of apoptosis, or a combination of the two. Additional data will be required to fully evaluate the role of this mechanism in 1,4-dichlorobenzene-induced carcinogenesis.

Differences in the mitogenic response to 1,4-dichlorobenzene appear to exist between rats and mice. In both the rat and the mouse, 1,4-dichlorobenzene induced both increased DNA synthesis and a suppression of apoptosis; however, the magnitude of growth perturbation in the mouse was greater than in the rat (James et al., 1998). Sherman et al. (1998) similarly reported an increase in replicative DNA synthesis in both rats and mice following exposure to 1,4-dichlorobenzene. In F344 rats exposed to 300 mg/kg-day, an increase in hepatocyte BrdU labeling was seen after 1 week of treatment, but not after 4 or 13 weeks (Lake et al., 1997). By contrast, male B6C3F$_1$ mice showed an increased BrdU labeling index at 1 and 4 weeks, but not at 13 weeks, after exposure to 300 or 600 mg/kg-day (Lake et al., 1997). Similar results were reported by Umemura et al. (1998), who noted that in B6C3F$_1$ mice exposed to up to 600

mg/kg-day of 1,4-dichlorobenzene for up to 4 weeks, the CRF of cells in the liver was increased 16-fold at 1 week, but only fourfold at 4 weeks; F344 rats exposed to up to 300 mg/kg-day for 4 weeks showed an elevated CRF only at week 1 but not at week 4. Eldridge et al. (1992) reported that following 5 days of exposure of $B6C3F_1$ mice to 600 mg/kg-day or 3 weeks of exposure of F344 rats to 300 mg/kg-day, the BrdU and ^{3}H-thymidine labeling indices were similar; however, the authors did not examine both species at the same time point (i.e., rats were evaluated after 3 weeks, and mice only after 5 days). Thus, while the study presented evidence that a proliferative response occurred in both rats and mice, a direct interspecies comparison cannot be made. In summary, cellular proliferation following exposure to 1,4-dichlorobenzene occurs in both rats and mice, but appears to be a more sustained phenomenon in mice.

4.4.2. Genotoxicity

The genotoxic effects of the dichlorobenzenes are summarized in Tables 4-5 to 4-7. The results of in vitro examinations of genotoxicity of the dichlorobenzenes have mostly been negative; in vivo studies are limited, but have suggested potential genotoxic effects of exposure to dichlorobenzenes.

4.4.2.1. *1,2-Dichlorobenzene*

1,2-Dichlorobenzene was negative for reverse mutations in *Salmonella typhimurium*, either with or without metabolic activation (Connor et al., 1985; NTP, 1985; Shimizu et al., 1983; Waters et al., 1982). 1,2-Dichlorobenzene treatment gave negative results for reverse mutations in *Escherichia coli* without metabolic activation (Waters et al., 1982), but positive results in *Saccharomyces cerevisiae* with metabolic activation (Paolini et al., 1998). In mouse lymphoma cells, 1,2-dichlorobenzene was negative for forward mutations without metabolic activation, but was positive (predominantly small colonies) in the presence of S9 mixture (Myhr and Caspary, 1991). 1,2-Dichlorobenzene treatment resulted in damage to DNA in *E. coli* and *S. cerevisiae*, but not in *Bacillus subtilis* (Waters et al., 1982). No induction of the *umu* gene in *S. typhimurium* (Nakamura et al., 1987) or prophage lambda in *E. coli* (DeMarini and Brooks, 1992) was seen following 1,2-dichlorobenzene treatment. Exposure to 1,2-dichlorobenzene did not result in changes in replicative DNA synthesis in cultured human lymphocytes (Perocco et al., 1983) or increased DNA repair in primary rat hepatocytes (Williams et al., 1989). 1,2-Dichlorobenzene did not cause chromosomal aberrations, either with or without metabolic activation, in Chinese hamster ovary (CHO) cells, but did result in increased levels of

sister-chromatid exchanges when treatment was performed with metabolic activation; no changes were seen when S9 was omitted from the experiment (Loveday et al., 1990).

Micronucleated polychromatic erythrocytes were induced in the bone marrow of mice following i.p. injection of 1,2-dichlorobenzene (Mohtashamipur et al., 1987). The compound was administered in two equal doses of 93.5, 187.5, 281, or 375 mg/kg, 24 hours apart, and the highest total dose (750 mg/kg) was approximately 70% of the single dose LD_{50}. All doses levels caused a significant increase in micronuclei compared to unexposed controls. No other in vivo genotoxicity studies of 1,2-dichlorobenzene were located in the examined literature.

Table 4-5. Results of selected genotoxicity studies of 1,2-dichlorobenzene

Test system	Results		
In vitro	**Without metabolic activation**	**With metabolic activation**	**Reference**
Reverse mutation in *S. typhimurium* (strains TA1535, TA1537, TA1538, TA98, and TA100)	-	ND	Waters et al., 1982
Reverse mutation in *S. typhimurium* (strains TA98, TA100, UTH8414, and UTH8413)	-	-	Connor et al., 1985
Reverse mutation in *S. typhimurium* (strains TA98, TA100, TA1535, and TA1537)	-	-	NTP, 1985
Reverse mutation in *S. typhimurium* (strains TA98, TA100, TA1535, TA1538, and TA1538)	-	-	Shimizu et al., 1983
Reverse mutation in *E. coli* WP2 *uvra*	-	ND	Waters et al., 1982
Reverse mutation in *S. cerevisiae*	ND	+	Paolini et al., 1998
Forward mutation in mouse lymphoma cells	-	+	Myhr and Caspary, 1991
DNA damage in *polA⁻ E. coli*	+	ND	Waters et al., 1982
DNA damage in *recA⁻ B. subtilis*	-	ND	Waters et al., 1982
DNA damage in *S. cerevisiae* D3	+	ND	Waters et al., 1982
umu gene induction in *S. typhimurium*	-	-	Nakamura et al., 1987
Induction of prophage lambda in *E. coli*	-	-	DeMarini and Brooks, 1992
Chromosomal aberrations in CHO cells	-	-	Loveday et al., 1990
Sister-chromatid exchange in CHO cells	-	+	Loveday et al., 1990
Replicative DNA synthesis in human lymphocytes	-	-	Perocco et al., 1983
Increased DNA repair in primary rat hepatocytes	-	ND	Williams et al., 1989
In vivo			
Micronucleus formation in mice	+		Mohtashamipur et al., 1987

- : Negative; + : Positive; ND: Not determined

4.4.2.2. *1,3-Dichlorobenzene*

Exposure to 1,3-dichlorobenzene did not cause an increase in reverse mutations, either with or without S9 mixture, in *S. typhimurium* (Connor et al., 1985; Shimizu et al., 1983; Waters et al., 1982) or *E. coli* (Waters et al., 1982). Treatment with 1,3-dichlorobenzene resulted in DNA damage in *E. coli*, but not in *B. subtilis* or *S. cerevisiae* (Waters et al., 1982). 1,3-Dichlorobenzene did not result in an increase in replicative DNA synthesis in cultured human lymphocytes (Perocco et al., 1983).

Micronucleated polychromatic erythrocytes were induced in the bone marrow of mice following i.p. injection of 1,3-dichlorobenzene (Mohtashamipur et al., 1987). The compound was administered in two equal doses of 87.5, 175, 262.5, or 350 mg/kg, 24 hours apart, and the highest total dose (700 mg/kg) was approximately 70% of the single dose LD_{50}. All doses levels caused a significant increase in micronuclei compared to unexposed controls. No other in vivo genotoxicity studies of 1,3-dichlorobenzene were located in the examined literature.

Table 4-6. Results of selected genotoxicity studies of 1,3-dichlorobenzene

Test system	Results		
In vitro	**Without metabolic activation**	**With metabolic activation**	**Reference**
Reverse mutation in *S. typhimurium* (strains TA1535, TA1537, TA1538, TA98, and TA100)	-	ND	Waters et al., 1982
Reverse mutation in *S. typhimurium* (strains TA98, TA100, UTH8414, and UTH8413)	-	-	Connor et al., 1985
Reverse mutation in *S. typhimurium* (strains TA98, TA100, TA1535, TA1538, and TA1538)	-	-	Shimizu et al., 1983
Reverse mutation in *E. coli* WP2 *uvra*	-	ND	Waters et al., 1982
DNA damage in *polA⁻ E. coli*	+	ND	Waters et al., 1982
DNA damage in *recA⁻ B. subtilis*	-	ND	Waters et al., 1982
DNA damage in *S. cerevisiae* D3	-	ND	Waters et al., 1982
Replicative DNA synthesis in human lymphocytes	-	-	Perocco et al., 1983
In vivo			
Micronucleus formation in mice	+		Mohtashamipur et al., 1987

- : Negative; + : Positive; ND: Not determined

4.4.2.3. *1,4-Dichlorobenzene*

The in vitro data on 1,4-dichlorobenzene genotoxicity are predominantly negative. Evaluation of 1,4-dichlorobenzene for reverse mutations yielded negative results in both *S. typhimurium* (NTP, 1987; Connor et al., 1985; Shimizu et al., 1983; Waters et al., 1982) and *E. coli* (Waters et al., 1982), but positive results in *S. cerevisiae* (Paolini et al., 1998). Assays for DNA damage in *E. coli*, *B. subtilis*, and *S. cerevisiae* were all negative (Waters et al., 1982). Evaluations for chromosomal aberrations or sister chromatid exchanges in CHO cells, either with or without metabolic activation, reported both negative (Anderson et al., 1990; NTP, 1987) and positive (Carbonell et al., 1991) results. 1,4-Dichlorobenzene gave equivocal results, both for colony number and colony size, following examination for forward mutations in mouse lymphoma cells (McGregor et al., 1988; NTP, 1987), but was negative in assays for induction of replicative DNA synthesis (Perocco et al., 1983) and DNA strand breaks in both rat and human hepatocytes (Canonero et al., 1997). In vitro evaluations of induction of micronucleus formation by 1,4-dichlorobenzene were equivocal in human and rat hepatocytes (Canonero et al., 1997), but were positive in human and rat kidney cells (Robbiano et al., 1999). Robbiano et al. (1999) also noted increased damage to DNA in rat and human kidney cells following in vitro exposure to 1,4-dichlorobenzene.

In vivo, 1,4-dichlorobenzene predominantly tested negative for micronucleus formation in both sexes of mice (Tegethoff et al., 2000; Morita et al., 1997; NTP, 1987), although positive results have been reported (Mohtashamipur et al., 1987). Exposure to 1,4-dichlorobenzene resulted in increased micronucleus formation and damage to nuclear DNA in male rat kidney (Robbiano et al., 1999). Exposure of male mice to 1,4-dichlorobenzene resulted in increases in replicative DNA synthesis (Miyagawa et al., 1995).

Table 4-7. Results of selected genotoxicity studies of 1,4-dichlorobenzene

Test system	Results		
In vitro	**Without metabolic activation**	**With metabolic activation**	**Reference**
Reverse mutation in *S. typhimurium* (strains TA1535, TA1537, TA1538, TA98, and TA100)	-	ND	Waters et al., 1982
Reverse mutation in *S. typhimurium* (strains TA98, TA100, UTH8414, and UTH8413)	-	-	Connor et al., 1985
Reverse mutation in *S. typhimurium* (strains TA98, TA100, TA1535, and TA1537)	-	-	NTP, 1987
Reverse mutation in *S. typhimurium* (strains TA98, TA100, TA1535, TA1538, and TA1538)	-	-	Shimizu et al., 1983
Reverse mutation in *E. coli* WP2 *uvra*	-	ND	Waters et al., 1982
Reverse mutation in *S. cerevisiae*	ND	+	Paolini et al., 1998
DNA damage in *polA⁻ E. coli*	-	ND	Waters et al., 1982
DNA damage in *recA⁻ B. subtilis*	-	ND	Waters et al., 1982
DNA damage in *S. cerevisiae* D3	-	ND	Waters et al., 1982
Chromosomal aberrations in CHO cells	-	-	Anderson et al., 1990
Chromosomal aberrations in CHO cells	-	-	NTP, 1987
Sister-chromatid exchange in CHO cells	-	-	Anderson et al., 1990
Sister-chromatid exchange in CHO cells	-	-	NTP, 1987
Sister-chromatid exchanges in human lymphocytes	+	ND	Carbonell et al., 1991
Forward mutation in mouse lymphoma cells	+/-	+	McGregor et al., 1988
Forward mutation in mouse lymphoma cells	-	+/-	NTP, 1987
Replicative DNA synthesis in human lymphocytes	-	-	Perocco et al., 1983
DNA strand breaks in primary rat hepatocytes	-	ND	Canonero et al., 1997
DNA strand breaks in human hepatocytes	-	ND	Canonero et al., 1997
Micronucleus formation in human hepatocytes	-	ND	Canonero et al., 1997
Micronucleus formation in primary rat hepatocytes	+/-	ND	Canonero et al., 1997

Table 4-7. Results of selected genotoxicity studies of 1,4-dichlorobenzene

Test system	Results		Reference
In vitro	**Without metabolic activation**	**With metabolic activation**	
Micronucleus formation in human kidney cells	+	ND	Robbiano et al., 1999
Micronucleus formation in rat kidney cells	+	ND	Robbiano et al., 1999
Damage to nuclear DNA in human kidney cells	+	ND	Robbiano et al., 1999
Damage to nuclear DNA in rat kidney cells	+	ND	Robbiano et al., 1999
In vivo			
Micronucleus formation in mice	-		NTP, 1987
Micronucleus formation in mice	-		Tegethoff et al., 2000
Micronucleus formation in mice	+		Mohtashamipur et al., 1987
Micronucleus formation in mice	-		Morita et al., 1997
Micronucleus formation in rat kidney	+		Robbiano et al., 1999
Increased replicative DNA synthesis in mice	+		Miyagawa et al., 1995
Damage to nuclear DNA in rat kidney	+		Robbiano et al., 1999

- : Negative; + : Positive; +/- : Equivocal results; ND: Not determined

4.4.3. Short-term Tests of Carcinogenic Potential

1,4-Dichlorobenzene does not appear to function in an initiator/promoter sequence in rats. Exposure of rats to 300 mg/kg of 1,4-dichlorobenzene by gavage 5 days/week for 13 weeks, followed by 26–39 weeks of exposure to NTA, a known promoter, resulted in no neoplastic lesions, indicating the absence of initiating activity of 1,4-dichlorobenzene (Umemura et al., 2000). Pretreatment with DEN, an initiating agent, followed by 6 weeks of treatment with 1,4-dichlorobenzene (0.1 or 0.4 mmol/kg-day) did not lead to increased formation of preneoplastic foci (1,2,4,5-tetrachlorobenzene was used as a positive control), indicating that 1,4-dichlorobenzene did not act as a promoter in rats (Gustafson et al., 1998).

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

4.5.1. Oral

Toxic effects of oral exposure to dichlorobenzene have been investigated in studies with all three isomers. The preponderance of information relevant to noncancer chronic health risk assessment relates to 1,4-dichlorobenzene. Several repeated-dose toxicity investigations of 1,2-dichlorobenzene have been conducted but only two studies are available for 1,3-dichlorobenzene. Information is available on the developmental toxicity of all three isomers (albeit limited in the case of the 1,2- and 1,3-isomers to findings reported in abstract form only), but reproductive toxicity has only been evaluated with 1,4-dichlorobenzene. Potential effects of repeated oral exposure to dichlorobenzene isomers on the nervous, immune, and endocrine systems have not been adequately studied.

Liver toxicity is the main endpoint common to 1,2-, 1,3-, and 1,4-dichlorobenzene and, as such, provides the best basis for comparing differences in oral toxicity between the isomers. Based on the available data concerning subchronic and chronic hepatic effects, and considering differences in sensitivity of endpoints at comparable dose levels between these studies, there is no clear basis for assessing the relative toxicities of the three isomers. The results of mechanistic and short-term studies discussed in Section 4.4 indicate that 1,2- and 1,3-dichlorobenzene are more acutely hepatotoxic than 1,4-dichlorobenzene. The higher acute hepatotoxicity of 1,2- and 1,3-dichlorobenzene seems to be related to greater involvement of CYP P450-based metabolism than with 1,4-dichlorobenzene. This initial metabolism likely results in a reactive intermediate that can bind covalently to cellular macromolecules or react with GSH, resulting in depletion of cellular GSH stores. Although these mechanisms are likely involved in the subchronic and/or chronic hepatotoxicity of the dichlorobenzenes, their contribution has not been conclusively established.

4.5.1.1. *1,2-Dichlorobenzene*

No information is available on the toxicity of ingested 1,2-dichlorobenzene in humans. Available relevant oral studies of 1,2-dichlorobenzene in animals are summarized in Table 4-8. The subchronic and chronic oral toxicity in animals has been investigated in three studies in rats and mice with effects observed principally in the liver.

Table 4-8. Critical effect levels in subchronic, chronic, and developmental oral studies of 1,2-dichlorobenzene

Species, strain, sex	Exposure protocol[a]	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects[b]	Reference
Rat, NR, F	0, 18.8, 188, or 376 mg/kg, 5 days/week for 192 days (0, 13.5, 135, or 270 mg/kg-day)	135	270	Cloudy swelling in liver.	Hollingsworth et al., 1958
Rat, S-D[c], M&F	0, 25, 100, or 400 mg/kg-day for 90 days	ND[1]	ND[1]	Hypertrophy, degeneration and necrosis in liver observed at 400 mg/kg-day (histopathology not evaluated at 25 or 100 mg/kg-day).	Robinson et al., 1991
Rat, F344/N, M&F	0, 30, 60, 125, 250, or 500 mg/kg, 5 days/week for 13 weeks (0, 21.4, 42.9, 89.3, 179, or 357 mg/kg-day)	ND[2]	89.3	Necrosis of individual hepatocytes.	NTP, 1985
Rat, F344/N, M&F	0, 60, or 120 mg/kg, 5 days/week for 103 weeks (0, 42.9, or 85.7 mg/kg-day)	85.7	ND	No histopathology in liver or other organs.	NTP, 1985
Rat, S-D, F	50, 100, or 200 mg/kg-day, gestation days 6-15	200	ND	No maternal or developmental toxicity. Controls not reported. Abstract only.	Ruddick et al., 1983
Mouse, $B6C3F_1$, M&F	0, 30, 60, 125, 250, or 500 mg/kg, 5 days/week for 13 weeks (0, 21.4, 42.9, 89.3, 179, or 357 mg/kg-day)	89.3	179	Hepatocellular degeneration and necrosis of individual hepatocytes.	NTP, 1985

Table 4-8. Critical effect levels in subchronic, chronic, and developmental oral studies of 1,2-dichlorobenzene

Species, strain, sex	Exposure protocol[a]	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects[b]	Reference
Mouse, $B6C3F_1$, M&F	0, 60, or 120 mg/kg-day, 5 days/week for 103 weeks (0, 42.9, or 85.7 mg/kg-day)	42.9	85.7	Renal tubular regeneration.	NTP, 1985

[a] Doses administered by gavage unless otherwise noted.
[b] Kidney effects not reported for male rats due to the species and sex specificity of the mechanism (α_{2u}-globulin nephropathy).
[c] Sprague-Dawley
NR = Not reported
ND = Not determined
ND^1 = Not determined because histopathology was not conducted at doses lower than 400 mg/kg-day.
ND^2 = Not determined because histopathology was not conducted at doses lower than 89.3 mg/kg-day.

Subchronic studies in rats found indications of liver toxicity (liver lesions) in rats at doses of ≥89.3 mg/kg-day for 13 weeks, 270 mg/kg-day for 192 days, and 400 mg/kg-day for 90 days (Robinson et al., 1991; NTP, 1985; Hollingsworth et al., 1958), as well as in mice exposed to 178.6 mg/kg-day for 13 weeks (NTP, 1985). In addition to liver lesions, tubular degeneration in the kidney was observed at the highest dose (357 mg/kg-day) in male rats only in the 13 week NTP (1985) study. In the only chronic study of 1,2-dichlorobenzene, there were no compound-related increased incidences of lesions in the liver in rats or mice that were exposed to 42.9 or 85.7 mg/kg-day for 103 weeks (NTP, 1985). Contrary to findings in the subchronic study, the incidence of renal tubular regeneration was significantly increased (p<0.05) only in the male mice exposed to 85.7 mg/kg-day (versus male rats in the 13 week study), indicating that at some earlier time point tubular generation must have occurred, which is consistent with an apoptotic response in the kidneys. This indicated that the highest dose in the chronic study in male mice was a LOAEL. The results of the 103-week NTP (1985) study, therefore, showed that 42.9 mg/kg-day was the chronic NOAEL for liver and kidney effects in rats and mice.

Considering the increased incidence of renal tubular regeneration at the highest dose (85.7 mg/kg) in male mice in the chronic NTP study (1985), the induction of renal tubular degeneration in male rats only at the highest dose and the induction of liver effects in rats at doses ≥89.3 mg/kg-day for 13 weeks in the NTP (1985) study, the supporting data for liver lesions in the other subchronic studies at ≥270 mg/kg-day (Robinson et al., 1991; Hollingsworth et al., 1958), the NOAEL was identified as 42.9 mg/kg-day based on the evidence for renal tubular regeneration in male mice in the chronic NTP (1985) study.

The chronic LOAEL of 85.7 mg/kg-day and chronic NOAEL of 42.9 mg/kg-day for renal tubular regeneration in male mice define the critical effect level for 1,2-dichlorobenzene.

4.5.1.2. *1,3-Dichlorobenzene*

No information is available on the toxicity of ingested 1,3-dichlorobenzene in humans. Available relevant oral studies of 1,3-dichlorobenzene in animals are summarized in Table 4-9. Data on effects of repeated oral exposures to 1,3-dichlorobenzene in animals are essentially limited to the results of one subchronic study in which rats were exposed to doses of 0, 9, 37, 147, or 588 mg/kg-day for 90 days (McCauley et al., 1995). Effects in the liver, thyroid, and pituitary occurred at all tested doses.

Table 4-9. Critical effect levels in subchronic and developmental oral studies of 1,3-dichlorobenzene

Species, strain, sex	Exposure protocol[a]	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects	Reference
Rat, S-D[b], M&F	0, 9, 37, 147, or 588 mg/kg-day for 90 days	ND	9	Reduced follicular colloidal density in thyroid. Cytoplasmic vacuolation in pars distalis of pituitary. Increased serum AST and serum cholesterol.	McCauley et al., 1995
Rat, S-D, F	50, 100, or 200 mg/kg-day, gestation days 6–15	200	ND	No maternal or developmental toxicity. Abstract only. Controls not reported.	Ruddick et al., 1983

[a] Doses administered by gavage unless otherwise noted.
[b] Sprague-Dawley
ND = Not determined because histopathology was not conducted at doses lower than 400 mg/kg-day.

Hepatic effects included increased serum levels of AST at ≥9 mg/kg-day and increased incidences of lesions at higher doses, including hepatocellular cytoplasmic alterations of minimal to mild severity at ≥147 mg/kg-day and necrotic hepatocyte foci of minimal severity at 588 mg/kg-day.

Thyroid effects included increased incidences of reduced follicular colloidal density of generally mild or moderate severity at ≥9 mg/kg-day. Incidences of rats with moderate or marked reductions in follicular colloidal density were increased at ≥147 mg/kg-day. The toxicological significance of this lesion is unclear, although chronic data on 1,4-dichlorobenzene support the thyroid as a target of toxicity since follicular gland hyperplasia occurred in mice exposed to 429 mg/kg-day of 1,4-dichlorobenzene for 103 weeks (NTP, 1987). In addition, plasma thyroxine (T_4) concentrations were reduced in rats 24 hours after a single i.p. dose of 1,2-dichlorobenzene (147 or 294 mg/kg) or 1,4-dichlorobenzene (294 mg/kg) (den Besten et al., 1992). This acute injection study also showed that 1,2-dichlorobenzene reduced triiodothyronine (T_3) plasma levels 24 hours after administration.

Pituitary effects in the 1,3-dichlorobenzene study included increased incidences of cytoplasmic vacuolization in the pars distalis of generally minimal to mild severity at ≥9 mg/kg-day (McCauley et al., 1995). Incidences of rats with moderate or marked pituitary cytoplasmic vacuolization were increased at ≥588 mg/kg-day. Though the pituitary lesion occurred only in males and was reportedly similar to "castration cells" found in the pituitary of gonadectomized rats (considered to be an indicator of gonadal deficiency), this is considered to

be a general response to some stimulus (i.e., decreased T_4) to stimulate TSH production and therefore an adaptive response rather than an adverse event.

Serum cholesterol levels were also increased at ≥9 mg/kg-day and could be thyroid-related as well as liver-related. The overall findings in this study suggested a possible disruption of hormonal feedback mechanisms, or target organ effects on the hypothalamus and/or other endocrine organs (McCauley et al., 1995). No information is available on the reproductive toxicity of 1,3-dichlorobenzene, although there was no maternal or developmental toxicity in rats gestationally exposed to 200 mg/kg-day (highest tested dose) (Ruddick et al., 1983). Based on the available data, the thyroid, pituitary, and liver are sensitive targets of 1,3-dichlorobenzene toxicity.

4.5.1.3. *1,4-Dichlorobenzene*

Information on the toxic effects of 1,4-dichlorobenzene in orally exposed humans is limited to two case reports describing hematological changes, particularly anemia, following known or presumed repeated ingestion of unknown doses of the compound in commercial products (Campbell and Davidson, 1970; Hallowell, 1959). Decreases in RBC counts, HCT, and hemoglobin were observed in a subchronic oral study in rats (NTP, 1987), although the 1,4-dichlorobenzene dose level causing these hematologic changes also induced liver and kidney toxicity in chronically exposed rats and mice, as discussed below.

Available relevant oral studies of 1,4 dichlorobenzene in animals are summarized in Table 4-10. The subchronic and chronic oral toxicity of 1,4-dichlorobenzene has been investigated in a number of animal studies conducted predominantly in rats and mice. As discussed below, liver and kidney effects are the best studied and most consistently observed findings. A relatively small amount of information is available indicating that 1,4-dichlorobenzene can affect the hematological system, and the adrenal and thyroid glands, at exposure levels equal to or higher than those causing liver and kidney effects. Reproductive and developmental studies have been performed in rats indicating that offspring are particularly sensitive to 1,4-dichlorobenzene toxicity during the postnatal preweaning period.

Table 4-10. Critical effect levels in subchronic, chronic, developmental and reproductive oral studies of 1,4-dichlorobenzene

Species, strain, sex	Exposure protocol[a]	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects[b]	Reference
Dog, Beagle, M&F	0, 7, 36, or 54 mg/kg-day for 1 year[c]	7	36	Statistically significant increases in liver histopathological changes at the mid and high doses. Kidney collecting duct epithelial vacuolation was present in a high dose male and at all doses in females. The lesion was considered to be a possible effect of the test material at the mid and high doses in females. Statistically significant increases in absolute and relative liver, kidneys, adrenals, and thyroid weights at the mid and high doses.	Monsanto Company, 1996
Rat, NR, F	0, 50, 100, or 200 mg/kg-day for 120 days	200	ND	Transient increase in absolute liver weight and small increase in liver porphyrins with no changes in urinary porphyrins. Liver histology not examined.	Carlson, 1977
Rat, NR, F	0, 18.8, 188, or 376 mg/kg, 5 days/week for 192 days (0, 13.5, 135, or 270 mg/kg-day)	135	270	Slight cirrhosis and focal necrosis in liver.	Hollingsworth et al., 1956
Rat, F344/N, M&F	0, 300, 600, 900, 1200, or 1500 mg/kg, 5 days/week for 13 weeks (0, 214, 429, 643, 857, or 1071 mg/kg-day)	ND	214	Increased serum AP and reduced serum triglycerides and protein. Slightly decreased RBC, HCT and hemoglobin.	NTP, 1987
Rat, F344/N, M&F	0, 37.5, 75, 150, 300, or 600 mg/kg, 5 days/week for 13 weeks (0, 27, 54, 107, 214, or 429 mg/kg-day)	429	ND	No histopathology in liver or other organs. Increased severity (not incidence) of kidney cortical tubular degeneration at 429 mg/kg-day.	NTP, 1987
Rat, F344, M&F	0, 75, 150, 300, or 600 mg/kg-day for 13 weeks	600	ND	No renal histopathology or increased urinary protein, LDH or NAG excretion in females.	Bomhard et al., 1988

Table 4-10. Critical effect levels in subchronic, chronic, developmental and reproductive oral studies of 1,4-dichlorobenzene

Species, strain, sex	Exposure protocol[a]	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects[b]	Reference
Rat, F344, F	0 or 600 mg/kg, 5 days/week for 13 weeks (0 or 429 mg/kg-day)	429	ND	No adverse effects on liver indicated by pathology or serum enzymes.	Eldridge et al., 1992
Rat, F344, M	0, 25, 75, 150, or 300 mg/kg, 5 days/week for 13 weeks (0, 18, 54, 107, or 214 mg/kg-day)	ND	214	Hepatocellular hypertrophy (histopathology not evaluated at 107 mg/kg-day).	Lake et al., 1997
Rat, F344, M	0, 75, 150, or 300 mg/kg, 5 days/week for 4 weeks (0, 54, 107, or 214 mg/kg-day)	214	ND	No adverse effects on liver indicated by immuno-histochemical assay. Histology not evaluated.	Umemura et al., 1998
Rat, F344/N, M&F	0, 150 (M), 300 (M, F), or 600 (F) mg/kg, 5 days/week for 103 weeks (0, 107, 214, or 429 mg/kg-day)	ND	214	Nephropathy, including tubular degeneration and atrophy, in females. No hepatic pathology.	NTP, 1987
Rat, S-D[d], M&F	0, 30, 90, or 270 mg/kg-day for 2 generations. F_0 animals exposed for 77 days (M) or 14 days (F) before mating. F_1 weanlings (M&F) exposed for 84 days before mating.	30	90	Reduced birth weight and postnatal survival, clinical manifestations, neurobehavioral deficits and increased liver weight in F_1 and/or F_2 offspring. Data not reported on a per-litter basis.	Bornatowicz et al., 1994
Rat, CD, F	0, 250, 500, 750, or 1000 mg/kg-day, gestational days 6-15	250	500	Decreased maternal weight gain and increased incidences of extra ribs.	Giavini et al., 1986
Rat, S-D, F	50, 100, or 200 mg/kg-day, gestational days 6-15	200	ND	No maternal or developmental toxicity. Controls not reported. Abstract only.	Ruddick et al., 1983
Mouse, B6C3F_1, M&F	0, 600, 900, 1000, 1500, or 1800 mg/kg 5 days/week for 13 weeks (0, 429, 643, 714, 1071, or 1286 mg/kg-day)	ND	429	Centrilobular hepatocellular degeneration. Reduced white blood cell count.	NTP, 1987

Table 4-10. Critical effect levels in subchronic, chronic, developmental and reproductive oral studies of 1,4-dichlorobenzene

Mouse, $B6C3F_1$, M&F	0, 84.4, 168.8, 337.5, 675, or 900 mg/kg, 5 days/week for 13 weeks (0, 60, 121, 241, 482, or 643 mg/kg-day)	241	482	Hepatocytomegaly	NTP, 1987
Mouse, $B6C3F_1$, M&F	0, 300, or 600 mg/kg, 5 days/week for 13 weeks (0, 214, or 429 mg/kg-day)	214	429	Hepatocellular hypertrophy	Eldridge et al., 1992
Mouse, $B6C3F_1$, M	0, 300, or 600 mg/kg, 5 days/week for 13 weeks (0, 214, or 429 mg/kg-day)	ND	429	Hepatocellular hypertrophy (histopathology not evaluated at 214 mg/kg-day).	Lake et al., 1997
Mouse, $B6C3F_1$, M	0, 150, 300, or 600 mg/kg, 5 days/week for 4 weeks (0, 107, 214, or 429 mg/kg-day)	429	ND	Immunohistochemical assay suggests effect, but not clearly adverse. Histology not evaluated.	Umemura et al., 1998
Mouse, $B6C3F_1$, M&F	0, 300, or 600 mg/kg 5 days/week for 103 weeks (0, 214, or 429 mg/kg-day)	ND	214	Hepatocellular degeneration, adenomas and carcinomas. Nephropathy (mainly renal tubular degeneration). Focal hyperplasia in adrenal capsule. Lymphoid hyperplasia of mandibular lymph node.	NTP, 1987
Rabbit, NR, M&F	0 or 500 mg/kg, 263 doses in 367 days (358 mg/kg-day)	ND	358	Cloudy swelling and minimal focal necrosis in liver. Weight loss, tremors.	Hollingsworth et al., 1956

[a]Doses administered by gavage unless otherwise noted.
[b]Kidney effects not reported for male rats due to the species and sex specificity of the mechanism (α_{2u}-globulin nephropathy).
[c]Doses administered via gelatin capsules.
NR = Not reported
ND = Not determined

Hepatic effects induced by subchronic or chronic oral exposure to 1,4-dichlorobenzene ranged from increased liver weight and hepatocyte enlargement to hepatocellular degeneration, lesions, necrosis, and tumors in dogs, rats, mice, and rabbits. Increases in serum levels of enzymes (e.g., AP and AST) and alterations in other endpoints (e.g., serum cholesterol and triglycerides) indicative of

hepatocellular damage or liver dysfunction have also been observed. Increased liver weight along with histopathologic changes in the liver was the most sensitive effect in a chronic dog study, observed at doses as low as 36 mg/kg-day. Increased liver weight was the most sensitive hepatic endpoint in subchronic studies in rats, observed at doses as low as 107 mg/kg-day for 4–13 weeks and 135 mg/kg-day for 192 days (Umemura et al., 1998; Lake et al., 1997; Hollingsworth et al., 1956), but this effect was not considered adverse without concomitant enzymatic or histopathologic changes. There was no indication of early liver damage in rats exposed to 107 mg/kg-day for 4 weeks using an immunohistochemical marker of centrilobular hepatocyte injury (size of the zone of GS-expressing hepatocytes) (Umemura et al., 1998), and the increase in liver porphyrins in rats exposed to ≥50 mg/kg-day for 120 days was not considered to be toxicologically significant (Carlson, 1977). Hepatocellular hypertrophy and decreased serum triglycerides occurred in rats exposed to ≥214 mg/kg-day for 13 weeks (Lake et al., 1997; NTP, 1987). Degenerative lesions were found in livers of rats exposed to higher doses of 270 mg/kg-day for 192 days (slight cirrhosis and focal necrosis) (Hollingsworth et al., 1956) or 857 mg/kg-day for 13 weeks (hepatocyte degeneration and necrosis) (NTP, 1987), although the findings at 270 mg/kg-day (Hollingsworth et al., 1956) seemed inconsistent with NTP (1987) chronic data showing that exposure to doses as high as 429 mg/kg-day for 103 weeks did not induce liver lesions in rats (NTP, 1987).

Mice were more sensitive than rats to the hepatotoxic effects of 1,4-dichlorobenzene, based on induction of hepatocellular degeneration at doses as low as 429 mg/kg-day for 13 weeks and 214 mg/kg-day for 103 weeks in mice (NTP, 1987). A study in rabbits found cloudy swelling and minimal focal necrosis following exposure to 358 mg/kg-day for 367 days (Hollingsworth et al., 1956), the lowest dose tested in this species, but higher than the chronic LOAEL in mice.

Considering the information summarized above, 36 mg/kg-day was the lowest chronic LOAEL for liver effects in dogs based on a range of liver histopathologic findings and increased absolute and relative liver weights (Monsanto Company, 1996). The chronic LOAEL for liver effects in mice (the most sensitive species in rodent studies) was 214 mg/kg-day based on hepatocellular degeneration (NTP, 1987). There was no chronic NOAEL in mice because 214 mg/kg-day was the lowest tested chronic dose in this species. The only data on liver effects in mice at doses below this chronic LOAEL were the subchronic immunohistochemical findings (increased GS expression) suggestive of early hepatocyte injury following exposure to doses as low as 107 mg/kg-day for 4 weeks (Umemura et al., 1998). The toxicological significance of this marker is unclear because it can reflect neoplastic transformation and progression as well as cell

damage (Osada et al., 2000). Histology was not evaluated, and liver weight was not increased until 429 mg/kg-day in the same study. Subchronic studies in rats found mild histologic alterations (e.g., hepatocellular hypertrophy) at ≥214 mg/kg-day, and necrotic and degenerative effects at ≥270 mg/kg-day (Umemura et al., 1998; Lake et al., 1997; Eldridge et al., 1992; NTP, 1987; Hollingsworth et al., 1956), but no hepatic histopathology occurred at doses ranging from 107 to 429 mg/kg-day in chronic rat studies (NTP, 1987). Considering the histopathologic liver findings in dogs at a dose as low as 36 mg/kg-day, this dose is the most appropriate effect level for assessing the liver toxicity of 1,4-dichlorobenzene.

Kidney collecting duct epithelial vacuolation was reported in a high dose male and at all doses in females in the chronic dog study (Monsanto Company, 1996). It was concluded that the lesion could be associated with the test chemical at the mid and high dose in the females since it was accompanied by increased kidney weights and macroscopically observed renal discoloration. Renal changes, including hyaline droplet accumulation, increased kidney weights, and tubular lesions were characteristic effects of subchronic and chronic oral exposure to 1,4-dichlorobenzene in male rats at doses ≥75 mg/kg-day (Lake et al., 1997; Bomhard et al., 1988; NTP, 1987). These findings are detailed in Section 4.2.1.3 and are not further discussed here or included in Table 4-10 because there is scientific consensus that they are related to the α_{2u}-globulin nephropathy syndrome, which is specific to male rats and not relevant to humans, as discussed in Section 4.4.1.1. Kidney nephropathy was also increased in female rats that were exposed to ≥214 mg/kg-day for 103 weeks (NTP, 1987). There was a high incidence of nephropathy in the unexposed control females, indicating that the effect in the treated animals may represent an increase in normal age-related nephropathy. Subchronic studies found increased kidney weight, but no indications of nephrotoxic action (i.e., no histopathology or effects on urinary indices of renal function) in female rats exposed to ≥135 mg/kg-day for 192 days or 600 mg/kg-day for 13 weeks (Bomhard et al., 1988; Hollingsworth et al., 1956). Kidney lesions, mainly tubular degeneration, were also increased in mice that were chronically exposed to ≥214 mg/kg-day for 103 weeks (NTP, 1987). The results of the NTP (1987) study, therefore, indicated that chronic exposure to 1,4-dichlorobenzene has a nephrotoxic potential in female rats and mice of both sexes, and that the LOAEL for renal effects was 214 mg/kg-day, the lowest tested chronic dose in these species and sexes.

The 36 mg/kg-day LOAEL for liver effects in dogs is the same as the LOAEL for kidney effects and the 214 mg/kg-day LOAEL for liver effects in mice is the same as the LOAEL for nephropathy in mice and female rats. Subchronic or chronic exposure to 1,4-dichlorobenzene caused other effects in dogs, rats and mice at doses equal to or higher than the LOAEL for liver

and kidney effects, including hematological changes (decreased basophils, RBCs, HCT, erythrocyte counts, and hemoglobin, and increased platelet counts and MCV) in dogs at 36 mg/kg-day for 1 year and in rats at ≥214 mg/kg-day for 13 weeks. Increased hyperplasia in the adrenal capsule and mandibular lymph node were observed in mice at ≥214 mg/kg-day for 103 weeks, and increased thyroid follicular gland hyperplasia was observed in mice at 429 mg/kg-day for 103 weeks (NTP, 1987). Developmental toxicity studies provided no indications that 1,4-dichlorobenzene was teratogenic in rats exposed to doses as high as 1000 mg/kg-day during gestation, although fetotoxicity occurred at maternally toxic levels ≥500 mg/kg-day (Giavini et al., 1986; Ruddick et al., 1983). Decreased maternal weight gain and increased incidences of extra ribs, a skeletal variation attributable to maternal toxicity rather than a teratogenic effect of the chemical, occurred in rats at gestational dose levels ≥500 mg/kg-day, but not at 250 mg/kg-day (the lowest tested dose) (Giavini et al., 1986).

Reproductive and developmental toxicity was evaluated in a 2-generation study in which male and female rats were administered 0, 30, 90, or 270 mg/kg-day doses of 1,4-dichlorobenzene (Bornatowicz et al., 1994). No effects on mating and fertility indices were observed at any level, although toxicity occurred in the offspring at doses ≥90 mg/kg-day. Effects observed at ≥90 mg/kg-day included reduced birth weight in F_1 pups and increased total number of deaths from birth to postnatal day 4 in F_1 and F_2 pups, clinical manifestations of dry and scaly skin (until approximately postnatal day 7) and tail constriction (during postnatal days 4–21) in F_1 and F_2 pups, reduced neurobehavioral performance (draw-up reflex evaluated at weaning) in F_2 pups, and increased relative liver weight in adult F_1 males. No exposure-related changes were found at 30 mg/kg-day, indicating that this was the NOAEL for reproductive and developmental toxicity in rats.

In summary, liver, kidney, and perinatal developmental toxicity are the main observed effects of subchronic and chronic oral exposure to 1,4-dichlorobenzene in animals. The rat and mouse are less sensitive to liver toxicity than the dog; the hepatic LOAEL in dogs was 36 mg/kg-day, which is the same as the LOAEL for kidney effects in female beagle dogs (Monsanto Company, 1996). There is sufficient evidence from a two-generation study in rats that oral exposure to 1,4-dichlorobenzene can cause developmental toxicity perinatally and during the later preweaning period, including decreased birth weight and neonatal survival in F_1 and F_2 pups, at doses ≥90 mg/kg-day. The LOAEL of 36 mg/kg-day for hepatotoxicity (Monsanto Company, 1996) is the critical effect level for oral exposure to 1,4-dichlorobenzene.

4.5.2. Inhalation

Toxic effects of inhalation exposure to dichlorobenzene have been investigated in studies with 1,2- and 1,4-dichlorobenzene. The preponderance of information relevant to noncancer chronic health risk assessment is on 1,4-dichlorobenzene. Several repeated exposure toxicity investigations of 1,2-dichlorobenzene have been conducted but no studies are available for 1,3-dichlorobenzene.

4.5.2.1. *1,2-Dichlorobenzene*

Information is available on the inhalation toxicity of 1,2-dichlorobenzene in humans, but the data are not suitable for risk assessment. Workers who were exposed to concentrations ranging from 1 to 44 ppm (average 15 ppm) for unreported durations showed no effects on standard blood or urine indices, as determined from periodic occupational health examinations (Hollingsworth et al., 1958). Five cases of blood disorders (four leukemias and one case of a myeloproliferative syndrome) were described in reports of people who were exposed to 1,2-dichlorobenzene as a solvent for other chemicals or in chlorinated benzene mixtures (IARC, 1982; Girard et al., 1969). Although none of these cases had exposure to unchlorinated benzene (a known human leukemogen), the reports were insufficient to conclude that 1,2-dichlorobenzene was the causative agent. A cohort mortality study was conducted on workers who were exposed to trichloroethylene and a large number of other organic solvents and chemicals, including 1,2-dichlorobenzene, during the cleaning and repairing of small parts at an aircraft maintenance facility (Spirtas et al., 1991). No association was found between exposure to 1,2-dichlorobenzene and mortality from multiple myeloma or NHL, but the risk estimates were based on only a small number of observations. The only information on possible hematological effects of inhaled 1,2-dichlorobenzene in animals comes from a study in which rabbits (2 of each sex) and monkeys (2 females) were exposed to 93 ppm for 7 hours/day, 5 days/week for 6–7 months (Hollingsworth et al., 1958). Hematology evaluations showed no changes in either species, although the numbers of animals were small and the scope of the tests was not indicated.

In the Hollingsworth et al. (1958) study, the aforementioned workers who were exposed to 15 ppm average levels of 1,2-dichlorobenzene did not experience any eye or nasal irritation (Hollingsworth et al., 1958). 1,2-Dichlorobenzene also did not cause eye or nasal irritation in people exposed to approximately 50 ppm (researchers who were exposed during the conduct of inhalation studies in animals), although the odor was perceptible at this level (Hollingsworth et al., 1958). Occupational exposure to higher concentrations (100 ppm) of 1,2-dichlorobenzene was reported to be irritating to the eyes and respiratory passages (Elkins, 1950). This limited

information on irritation effects of 1,2-dichlorobenzene in humans was consistent with histologic findings of nasal olfactory epithelial lesions in mice exposed to 64 or 163 ppm of 1,2-dichlorobenzene for 6 hours/day, 5 days/week for 4–14 days (Zissu, 1995). The lesions were graded as very severe after 4 days of exposure as they were characterized by a complete loss of olfactory epithelium. The severity decreased with time, suggesting that some tissue repair may have occurred despite continued exposure. No histologic alterations were observed in the respiratory epithelium of the trachea or lungs. The mouse data showed that the upper respiratory tract was a sensitive target for inhalation exposures to 1,2-dichlorobenzene, as serious olfactory lesions occurred at exposure concentrations below those that caused systemic effects in rats, as summarized below. The dose of 64 ppm was considered to be the LOAEL for nasal olfactory lesions in the Zissu (1995) study. A NOAEL could not be determined.

Available relevant inhalation studies of 1,2-dichlorobenzene in animals are summarized in Table 4-11. Data on the toxicity of longer-term inhalation exposures to 1,2-dichlorobenzene are available from a multispecies subchronic study (Hollingsworth et al., 1958), a 2-generation reproduction study in rats (Bio/dynamics, 1989), and developmental toxicity studies in rats and rabbits (Hayes et al., 1985; Dow Chemical, 1981). In the subchronic study, rats and guinea pigs were exposed to 49 or 93 ppm for 7 hours/day, 5 days/week for 6–7 months (Hollingsworth et al., 1958). Mice were exposed to 49 ppm only, and rabbits and monkeys to 93 ppm only, but findings in the latter species were compromised by small numbers of animals (2 rabbits/sex and 2 female monkeys). No compound-related histopathologic or other changes occurred in any of the animals exposed to 49 ppm 1,2-dichlorobenzene. The only remarkable finding at 93 ppm was a statistically significant decrease in final body weight (8.9% less than unexposed controls) in male rats, indicating that 93 ppm was the LOAEL in this study. The report did not indicate if respiratory tract examinations were conducted in any species.

Table 4-11. Critical effect levels in subchronic, developmental and reproductive inhalation studies of 1,2-dichlorobenzene

Species, strain, sex	Exposure protocol	NOAEL (ppm)	LOAEL (ppm)	Effects	Reference
Rabbit, NR, M&F	93 ppm for 7 hours/day, 5 days/week, 6–7 months	NA	NA	No effects observed.	Hollingsworth et al., 1958
Monkey, NR, F	93 ppm for 7 hours/day, 5 days/week, 6–7 months	NA	NA	No effects observed.	Hollingsworth et al., 1958
Mouse, OF_1, NR	64 or 163 ppm for 6 hours/day, 5 days/week, 6–14 days	ND	64	Nasal olfactory epithelial lesions	Zissu, 1995
Rat, NR, NR	49 or 93 ppm for 7 hours/day, 5 days/week, 6–7 months	ND	93	Statistically significant decrease in final body weight	Hollingsworth et al., 1958
Guinea pig, NR, NR	49 or 93 ppm for 7 hours/day, 5 days/week, 6–7 months	ND	93	Statistically significant decrease in spleen weight	Hollingsworth et al., 1958
Rat, Charles River, M&F	50, 150, or 394 ppm for 6 hours/day, 7 days/week, 10 weeks before mating and subsequently through the F_1 generation	50	150	Decreased body weight gain, increased absolute and relative liver weights, and centrilobular hepatocyte hypertrophy in adult rats of both sexes and generations	Bio/dynamics, 1989

Table 4-11. Critical effect levels in subchronic, developmental and reproductive inhalation studies of 1,2-dichlorobenzene

Species, strain, sex	Exposure protocol	NOAEL (ppm)	LOAEL (ppm)	Effects	Reference
Rat, F344, F	100, 200, or 400 ppm on days 6–15 of gestation	ND - Maternal toxicity; 200 - Developmental effects	100 - Maternal toxicity; 400 - Developmental effects	Decreased body weight gain in dams. Skeletal variations at high concentrations.	Hayes et al., 1985; Dow Chemical, 1981
Rabbit, New Zealand, F	100, 200, or 400 ppm on days 6–18 of gestation	ND - Maternal toxicity; 400 - Developmental effects	100 - Maternal toxicity; ND - Developmental effects	Decreased body weight gain in dams.	Hayes et al., 1985; Dow Chemical, 1981

NR = Not reported
ND = Not determined from information available in the study
NA = Not applicable

In the reproductive toxicity study, male and female rats were exposed to 50, 150, or 394 ppm of 1,2-dichlorobenzene for 6 hours/day, 7 days/week for 10 weeks before mating and subsequently through the F_1 generation (Bio/dynamics, 1989). α_{2u}-Globulin-related renal changes were found in adult males of both generations at all levels of exposure, but these effects are specific to male rats and are not relevant to humans, as discussed in Section 4.4.4.1. Decreased body weight gain, increased absolute and relative liver weights, and centrilobular hepatocyte hypertrophy occurred in adult rats of both sexes and generations at $\geq$150 ppm. The liver changes were considered to be adaptive and not adverse, indicating that the NOAEL and LOAEL for systemic toxicity were 50 ppm and 150 ppm, respectively, based on decreased weight gain. In the absence of food consumption data, the toxicological significance of reduced body weight gain is uncertain. Evaluation of the respiratory tract was not performed in this study. There were no effects on reproduction in either generation, indicating that the NOAEL for reproductive toxicity was 394 ppm.

The developmental toxicity of inhaled 1,2-dichlorobenzene was evaluated in rats and rabbits that were intermittently exposed to concentrations ranging from 100 to 500 ppm on gestational days 6–15 (rats) or 6–18 (rabbits) (Hayes et al., 1985; Dow Chemical, 1981). A maternal LOAEL of 100 ppm was identified for decreased body weight gain in both species, although decreased food consumption was reported in rats at 400 ppm. A maternal NOAEL was not identifiable because the effects occurred at all levels of exposure. No developmental effects were observed in rabbits at concentrations up to 400 ppm. Skeletal variations occurred in rats exposed to the high concentration, indicating that developmental effects occurred in rats at concentrations that also caused maternal toxicity. Based on these findings, a NOAEL of 200 ppm and LOAEL of 400 ppm, respectively, were identified for developmental toxicity.

The subchronic, reproductive, and developmental toxicity studies all suggested that body weight was a sensitive endpoint of inhalation exposure to 1,2-dichlorobenzene in rats and rabbits. The LOAELs for this effect were similar, ranging from 93 to 150 ppm (Bio/dynamics, 1989; Hayes et al., 1985; Hollingsworth et al., 1958). However, no information was available on respiratory tract histology in any of these studies, while lesions of the nasal olfactory epithelium occurred in mice exposed for 4–14 days to concentrations of 64 or 163 ppm (Zissu, 1995), which are similar to and below the LOAELs identified for the systemic effects. Since the 64 ppm LOAEL for nasal histopathology was a short-term effect level, the most sensitive effect of subchronic or chronic inhalation exposure to 1,2-dichlorobenzene could not be determined reliably.

4.5.2.2. *1,3-Dichlorobenzene*

No information was located regarding the toxicity of inhaled 1,3-dichlorobenzene in humans or animals.

4.5.2.3. *1,4-Dichlorobenzene*

A limited amount of information is available on the toxicity of inhaled 1,4-dichlorobenzene in humans, but the data are insufficient for risk assessment. Periodic occupational health examinations of workers who were exposed to 1,4-dichlorobenzene for an average of 4.75 years showed no changes in standard blood or urine indices (Hollingsworth et al., 1956). Painful irritation of the eyes and nose was usually experienced at 50–80 ppm, although the irritation threshold was higher (80–160 ppm) in workers acclimated to exposure, and no cataracts or other lens changes were observed. Case reports of people who inhaled 1,4-dichlorobenzene provided indication that the liver and nervous system are systemic targets of toxicity in humans, but were limited by lack of adequate quantitative exposure information and/or verification that 1,4-dichlorobenzene was the only factor associated with the observed effects (Reygagne et al., 1992; Miyai et al., 1988; Cotter, 1953). The hepatic, neurologic, and eye/nose irritation findings in humans were consistent with effects observed in exposed animals, as discussed below.

Available relevant inhalation studies of 1,4-dichlorobenzene in animals are summarized in Table 4-12. Information on the inhalation effects of 1,4-dichlorobenzene in animals includes multispecies subchronic toxicity studies (Aiso et al., 2005a; Hollingsworth et al., 1956), a subchronic immunotoxicity study in guinea pigs (Suzuki et al., 1991), and two chronic toxicity studies in rats and mice (Aiso et al., 2005b; JBRC, 1995; ICI, 1980; Riley et al., 1980). A subchronic toxicity study (Asio et al., 2005a) exposed BDF_1 mice and F344 rats of both sexes (6 h/d and 5 d/wk) to inhalation of 25, 55, 120, 270 or 600 ppm (v/v) 1,4-dichlorobenzene vapor for 13 weeks. The exposure to 1,4-dichlorobenzene vapor retarded the growth rate in the male mice, and induced hepatotoxicity in the mice and rats of both sexes and renal and hematological toxicity in the male rates. Hepatotoxicity was characterized by increased liver weight, hepatocellular hypertrophy, and increased serum levels of total cholesterol. Liver necrosis and increased serum levels of AST and ALT were observed in the exposed mice, whereas these changes, which indicate hepatocellular death, did not occur in any of the exposed rats. Renal lesions occurred only in 1,4-dichlorobenzene-induced male rats. The NOAEL was 120 ppm for the hepatic endpoint in mice and for the renal endpoint in rats. In another multispecies subchronic study, rats, mice, guinea pigs, rabbits, and monkeys were exposed to 96 or 158 ppm

for 7 hours/day, 5 days/week for 5–7 months (Hollingsworth et al., 1956). Some of these animals were also similarly exposed to 341 ppm for 6 months (rats and guinea pigs) or 798 ppm for 23–69 exposures (rats, guinea pigs, and rabbits). The experiments with rabbits or monkeys exposed to 96 or 158 ppm were limited by small numbers of animals (1–2/group). Hepatic changes were observed, including increased relative liver weights and slight histologic alterations of questionable toxicological significance in rats at 158 ppm (no effects at 96 ppm), with more severe hepatic histopathology (e.g., cloudy swelling and necrosis) reported in guinea pigs at 341 ppm, and in rats, guinea pigs, and rabbits at 798 ppm. Other effects observed in animals exposed to 798 ppm included eye irritation and frank signs of neurotoxicity (e.g., marked tremors). The subchronic immunotoxicity study found no effects in mice exposed to ≤50 ppm for 12 weeks (highest tested concentration, exposure schedule not specified) (Suzuki et al., 1991).

In one of the chronic studies, rats of both sexes and female mice were exposed to 75 or 500 ppm for 5 hours/day, 5 days/week for up to 76 weeks (rats) or 57 weeks (mice), followed by 32 weeks (rats) or 18–19 weeks (mice) without exposure (ICI, 1980; Riley et al., 1980). There were no exposure-related histopathologic changes in the nasal cavity or other tissues in either species. Liver and kidney weights were increased in rats of both sexes at 500 ppm (in females liver weights were increased at ≥75 ppm after 26–27 weeks of exposure), but the toxicological significance is questionable due to the negative histopathology and lack of related clinical chemistry findings. Evaluation of the mouse data is limited by insufficiencies in the available summary of the study.

In a later chronic evaluation of the toxicity of inhaled 1,4-dichlorobenzene (Aiso et al., 2005b; JBRC, 1995), groups of rats and mice of both sexes were exposed to 0, 20, 75, or 300 ppm for 6 hours/day, 5 days/week for 104 weeks. A large number of biochemical changes occurred in high-dose rats and mice, but could not be evaluated because the study did not present data for these endpoints. Exposure to 1,4-dichlorobenzene heightened the incidence of moderate or greater severity eosinophilic changes in the olfactory epithelium of both sexes of rats (1/50, 2/50, 2/50, and 7/50 in males, and 27/50, 29/50, 39/50, and 47/50 in females in the 0, 20, 75, and 300 ppm groups, respectively); the increases were statistically significant in 300 ppm males and 75 and 300 ppm females. In 300 ppm female rats only, significantly increased incidences of eosinophilic changes of the respiratory epithelium and respiratory metaplasia were seen, and an increase in mineralization of the renal papilla was reported in 300 ppm male rats. On histologic examination, male mice showed a significant increase in centrilobular hepatocellular hypertrophy in the 300 ppm group only (0/49, 0/49, 0/50, and 34/49 in the 0, 20, 75, and 300 ppm groups, respectively), and a significant (p<0.05) increase in mineralization of the testis in the 75 and 300

ppm groups (27/49, 35/49, 42/50, and 41/49 in the 0, 20, 75, and 300 ppm groups, respectively). No nonneoplastic changes in histologic endpoints were reported in female mice. Thus, the chronic inhalation data identified a NOAEL of 20 ppm and a LOAEL of 75 ppm for eosinophilic changes of the olfactory epithelium in female rats, and mineralization of the testis in male mice.

Table 4-12. Critical effect levels in subchronic, chronic, developmental and reproductive inhalation studies of 1,4-dichlorobenzene

Species, strain, sex	Exposure protocol	NOAEL (ppm)	LOAEL (ppm)	Effects	Reference
Rats F344, Mouse, BDF_1, M&F	25,55,120,270 or 600 ppm, 6 hours/day, 5 days/week, 13 weeks	120	ND	Hepatic changes including increased liver weight and hepatocellular hypertrophy	Aiso et al., 2005a
Rat, NR, NR	96, 158 or 341 ppm, 7 hours/day, 5 days/week, 5–7 months	96	158	Hepatic changes including increased relative liver weight and slight histologic alterations	Hollingsworth et al., 1956
Mouse, NR, NR	96 or 158 ppm, 7 hours/day, 5 days/week, 5–7 months	ND	ND	No reported effects	Hollingsworth et al., 1956
Guinea pig, NR, NR	96, 158 or 341 ppm, 7 hours/day, 5 days/week, 5–7 months	ND	341	Hepatic changes including increased relative liver weight and slight histologic alterations	Hollingsworth et al., 1956
Rabbit, NR, NR	96 or 158 ppm, 7 hours/day, 5 days/week, 5–7 months	ND	ND	No effects reported; only 1 rabbit/sex/dose group tested	Hollingsworth et al., 1956
Monkey, NR, NR	96 or 158 ppm, 7 hours/day, 5 days/week, 5–7 months	ND	ND	No effects; only 1 monkey per dose group tested	Hollingsworth et al., 1956
Guinea pig, SPF Hartley, NR	$\leq$50 ppm for 12 weeks	NA	NA	No effects	Suzuki et al., 1991

Table 4-12. Critical effect levels in subchronic, chronic, developmental and reproductive inhalation studies of 1,4-dichlorobenzene

Species, strain, sex	Exposure protocol	NOAEL (ppm)	LOAEL (ppm)	Effects	Reference
Rat, Wistar, Mouse, SPF Swiss, M&F	75 or 500 ppm, 5 hours/day, 5 days/week, 76 weeks (rats) or 57 weeks (mice)	500 - rat ND - mice	ND - rat ND - mice	Increased liver and kidney weights in rats. Mouse study – terminated early because of high mortality.	ICI, 1980
Rat, Sprague Dawley, M&F	66, 211, or 538 ppm, 6 hours/day, 5 days/week, 10 weeks before mating and then through F_1 generation	211	588	Parental clinical signs; postnatal toxicity in the offspring	Tyl and Neeper-Bradley, 1989
Rat, SPF, F	75–500 ppm, 6 hours/day, days 6–15 of gestation	500	NA	No developmental effects	Hodge et al., 1977
Rabbit, New Zealand, F	100–800 ppm, 6 hours/day, days 6–18 of gestation	ND	800	No maternal or developmental effects	Hayes et al., 1985
Rat, F344/DuCrj, mouse, Crj:BDF1, M&F	0, 20, 75, or 300 ppm, 6 hours/day, 5 days/week, 104 weeks	20	75	Eosinophilic changes of the olfactory epithelium in female rats, minerali-zation of the testis in male mice	Aiso et al., 2005b; JBRC, 1995

NR = Not reported
ND = Not determined from information available in the study
NA = Not applicable

Additional data on effects of inhaled 1,4-dichlorobenzene were provided in reproduction studies in rats and mice (Tyl and Neeper-Bradley, 1989; Anderson and Hodge, 1976) and developmental toxicity studies in rats and rabbits (Hayes et al., 1985; Hodge et al., 1977). A two-generation reproductive toxicity study was conducted in male and female rats exposed to 66, 211, or 538 ppm for 6 hours/day, 5 days/week for 10 weeks before mating and subsequently through the F_1 generation (Tyl and Neeper-Bradley, 1989). There were no effects on reproductive parameters in either generation in the absence of parental toxicity. Systemic toxicity occurred at all dose levels in F_0 and F_1 adult rats (Tyl and Neeper-Bradley, 1989). Changes indicative of α_{2u}-globulin nephropathy were found in adult males of both generations at $\geq$66 ppm, but this syndrome is specific to male rats and not relevant to humans (see Section 4.4.4.1). Relative liver weights were increased in adult F_0 males at $\geq$66 ppm, F_1 males and F_0 females at $\geq$211 ppm, and F_1 females at 538 ppm, and absolute liver weights were increased in adult F_0 adult males at $\geq$211 ppm, and in F_1 males and F_0 and F_1 females at 538 ppm. The increase in liver weight was more pronounced in males than females and statistically significant in these groups, but toxicological significance is questionable due to a lack of accompanying degenerative histopathologic effects. The only histopathologic finding in liver was hepatocellular hypertrophy in both sexes and generations at 538 ppm. In the absence of multiple adverse liver effects, the weight increase was considered adaptive. Other effects at 538 ppm included clinical signs (e.g., tremors) in adults and increased stillbirths and perinatal mortality in F_1 and/or F_2 litters. The NOAEL and LOAEL of 211 and 538 ppm, respectively, were based on parental clinical signs and postnatal toxicity in the offspring. Anderson and Hodge (1976) found no effects on reproductive performance in male mice exposed for 6 hour/day for 5 days prior to weekly mating with unexposed females for 8 weeks. No maternal or developmental toxicity occurred in rats that were exposed to 75–500 ppm for 6 hours/day on days 6–15 of gestation (Hodge et al., 1977), indicating that the highest NOAEL for these effects in rats was 500 ppm. A developmental study in which rabbits were exposed to 100–800 ppm for 6 hours/day on gestational days 6–18 found evidence of fetotoxicity (a minor variation of the circulatory system) only at 800 ppm, which was also a maternally toxic exposure level as shown by body weight loss early in gestation (Hayes et al., 1985).

The available animal data identified effects on the respiratory tract (eosinophilic changes of the olfactory epithelium) and the testis (mineralization) as potential critical effects of inhaled 1,4-dichlorobenzene. The NOAEL and LOAEL for these effects were 20 and 75 ppm, respectively, based on findings in rats and mice from a chronic study (Aiso et al., 2005b; JBRC, 1995). There was no evidence that 1,4-dichlorobenzene is a reproductive toxicant in male mice

at concentrations ≤450 ppm (Anderson and Hodge, 1976), or in male and female rats in the absence of parental toxicity (Tyl and Neeper-Bradley, 1989). Developmental toxicity was only found in rabbits exposed to 800 ppm, a level that also caused maternal toxicity and was considerably higher than the LOAEL for systemic effects (Hayes et al., 1985).

4.5.3. Mode of Action Information

4.5.3.1. *1,2-Dichlorobenzene*

While the MOA of the noncancer toxicity of 1,2-dichlorobenzene is not fully understood, its metabolism to reactive metabolites is believed to play a major role in its toxicity (see Section 3.3.1 for a more complete discussion of the metabolism of 1,2-dichlorobenzene, and Section 4.4.1.2.1 for further discussion of the role of metabolism in the toxicity of 1,2-dichlorobenzene). The initial step in 1,2-dichlorobenzene metabolism is oxidation by CYP P450 enzymes to a reactive epoxide; this epoxide is believed to be the source of covalent binding to proteins in in vitro studies of 1,2-dichlorobenzene metabolism (den Besten et al., 1992). Alterations in protein structure and function resulting from covalent binding can have toxic consequences for the cell. Pretreatment with CYP P450 inhibitors blocks the toxicity of 1,2-dichlorobenzene, providing additional evidence for this hypothesis. Also, depletion of GSH results in an enhancement of toxicity, providing further support for this hypothesis. Results from a study by Younis et al. (2003) suggested that the mechanism of 1,2-dichlorobenzene-induced hepatotoxicity involves intercellular communication, used by compromised hepatocytes to signal Kupffer cell activation.

4.5.3.2. *1,3-Dichlorobenzene*

The MOA for the effects of 1,3-dichlorobenzene is not known. Based on limited studies of its metabolism, and a similarity of effects with 1,2-dichlorobenzene, a MOA involving metabolism to a reactive epoxide, and other metabolites downstream, could be hypothesized (see Section 3.3.2 for a more complete discussion of the metabolism of 1,3-dichlorobenzene, and Section 4.4.1.2.1 for a further discussion of the role of metabolism in the toxicity of 1,3-dichlorobenzene). Depletion of GSH enhances the toxicity of 1,3-dichlorobenzene, providing some evidence that its toxicity is the result of a reactive metabolite. The role of metabolism in the chronic toxicity of 1,3-dichlorobenzene is not known. Additional data on the MOA of 1,3-dichlorobenzene are not available.

4.5.3.3. *1,4-Dichlorobenzene*

While not as convincing as the evidence for 1,2-dichlorobenzene, evidence exists to support a mechanism of action of 1,4-dichlorobenzene based on metabolism to a reactive or oxidative metabolite. Incubation of microsomes with radiolabeled 1,4-dichlorobenzene and subsequent treatment with antioxidants (i.e., ascorbic acid) resulted in a decrease in in vitro covalent binding to proteins (Hissink et al., 1997b), suggesting that in vitro metabolism led to the formation of reactive oxygen species. Additionally, it was demonstrated that depletion of GSH levels resulted in an acute hepatotoxic response following administration of 100–132 mg/kg of 1,4-dichlorobenzene (Mizutani et al., 1994; Stine et al., 1991). However, unlike 1,2-dichlorobenzene, 1,4-dichlorobenzene treatment did not appear to raise GSSG levels in the liver (Gustafson et al., 2000), suggesting any reactive intermediate either existed at a low concentration only, or that it had little tendency to oxidize GSH. The potential influence of these pathways on the chronic toxic effects of 1,4-dichlorobenzene has not been elucidated.

A potentially important mechanism of toxic effects involves the action of 1,4-dichlorobenzene on hepatic cell proliferation. Numerous studies in both rats and mice demonstrated that treatment of animals with 1,4-dichlorobenzene for up to 4 weeks resulted in sustained cell proliferation, as measured by BrdU incorporation or an increase in the cumulative replicating cell fraction (Hasmall and Roberts, 1997; Lake et al., 1997; Umemura et al., 1992, 1998). This cellular proliferation does not appear to be the result of postnecrotic regeneration, as evidenced by a lack of histologic evidence for hepatic necrosis reported in the NTP chronic study (NTP, 1987). Rather, the increase is believed to be a result of more complex molecular mechanisms, including alterations in genes regulating cell division, a change in the rate of apoptosis, or other factors. Additional data will be required to fully elucidate the possible role of this mechanism on the toxic effects of 1,4-dichlorobenzene on the liver.

While the predominant effects following oral exposure to 1,4-dichlorobenzene are hepatic in nature, evidence exists that 1,4-dichlorobenzene can function as a respiratory tract irritant. Exposure of humans to 50–80 ppm of 1,4-dichlorobenzene resulted in irritation of the eyes and nose (Hollingsworth et al., 1956), while in a chronic study in rats, similar exposure concentrations (75 or 300 ppm) produced an increase in lesions of the olfactory epithelium (Aiso et al., 2005b; JBRC, 1995). It is not known whether these effects are due to a direct irritant effect of the compound, metabolism of the compound to active metabolites in the target tissues, or a combination of these and other mechanisms.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

4.6.1. 1,2-Dichlorobenzene

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the available carcinogenicity data for 1,2-dichlorobenzene provide *inadequate information to assess carcinogenic potential*. 1,2-Dichlorobenzene could not be assessed for carcinogenicity because of the lack of human data, and questions about the adequacy of the available experimental data to assess carcinogenicity because of uncertainty as to whether or not the MTD had been reached.

No information is available on the carcinogenicity of 1,2-dichlorobenzene in humans. Data on cancer in animals are limited to one chronic oral bioassay, in which no exposure-related tumors were found in male and female rats and mice administered 42.9 or 87.7 mg/kg-day 1,2-dichlorobenzene by oral gavage for 103 weeks (NTP, 1985). This is a well-designed chronic study with respect to exposure duration and scope of histologic examinations, but questions have been raised about dose selection. The oral gavage NTP (1985) study failed to demonstrate compound-related effects on survival and body weight in rats and mice, and there were no nonneoplastic histopathologic lesions in rats. Given the inherent insensitivity of laboratory studies for detecting carcinogenic responses, it is generally agreed that the highest test dose in an animal bioassay should represent an MTD, i.e., a dose that provides some evidence of toxicity or biological effect (Haseman, 1985). In light of the reported findings, questions have been raised as to whether or not the highest dose tested (87.7 mg/kg-day) was an MTD, particularly for the rat. It can be concluded that available bioassay data provides no evidence of carcinogenicity in rats or mice at doses up to 87.7 mg/kg-day, but are not clearly sufficient to evaluate carcinogenicity in experimental animals at higher exposure levels.

Genotoxic effects of 1,2-dichlorobenzene were investigated in various test systems with generally mixed results. Reverse mutation assays were negative in *S. typhimurium* and *E. coli* and positive in *S. cerevisiae*. Tests for DNA damage in *S. typhimurium*, *E. coli*, and *S. cerevisiae* were all negative, but positive in *B. subtilis* (Paolini et al., 1998; NTP, 1987; Connor et al., 1985; Shimizu et al., 1983; Waters et al., 1982). Results of a forward mutation assay in mouse lymphoma cells were positive (Myhr and Caspary, 1991), but tests for replicative DNA synthesis in cultured human lymphocytes and DNA repair in primary rat hepatocytes were negative (Williams et al., 1989; Perocco et al., 1983). Sister-chromatid exchanges were induced in CHO cells with metabolic activation, although chromosomal aberrations were not (Loveday et al., 1990). In vivo exposure induced micronucleus formation in mice (Mohtashamipur et al., 1987).

4.6.2. 1,3-Dichlorobenzene

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the lack of human and animal carcinogenicity data for 1,3-dichlorobenzene provide *inadequate information to assess carcinogenic potential*.

No information is available regarding the carcinogenicity of 1,3-dichlorobenzene in humans or animals.

The genotoxicity of 1,3-dichlorobenzene was evaluated in several in vitro and in vivo tests. Reverse mutations were not induced in assays using *S. typhimurium* or *E. coli* (Connor et al., 1985; Shimizu et al., 1983; Waters et al., 1982). Evidence of primary DNA damage was observed in *E. coli*, but not in *B. subtilis* or *S. cerevisiae* (Waters et al., 1982). 1,3-Dichlorobenzene did not cause an increase in replicative DNA synthesis in cultured human lymphocytes (Perocco et al., 1983). In vivo, micronucleus formation was increased in bone marrow cells of mice that were exposed to 1,3-dichlorobenzene by i.p. injection (Mohtashamipur et al., 1987). Additional data on possible modes of action of carcinogenic effects of 1,3-dichlorobenzene are not available.

4.6.3. 1,4-Dichlorobenzene

4.6.3.1. *Summary of Cancer Evidence*

The carcinogenicity of 1,4-dichlorobenzene in humans has not been investigated. Information on carcinogenicity in animals is available from chronic oral and inhalation studies in rats and mice (Aiso et al., 2005b; JBRC, 1995; NTP, 1987; ICI, 1980; Riley et al., 1980), as well as from subchronic initiation-promotion studies in rats (Umemura et al., 2000; Gustafson et al., 1998).

Chronic oral bioassays were conducted in rats and mice that were exposed to 1,4-dichlorobenzene doses of 107 or 214 mg/kg-day (male rats) or 214 or 429 mg/kg-day (female rats and mice of both sexes) for 103 weeks (NTP, 1987). Kidney tumors were induced in male rats, as shown by a dose-related increase in the incidence of renal tubular cell adenocarcinomas that was statistically significantly higher in the high-dose group than in controls. Male rats additionally had a dose-related increase in the incidence of mononuclear cell leukemia that was statistically significant in the high-dose group, although the increase was considered marginal because it was comparable to the historical control incidence. No indication of carcinogenicity was found in female rats. Findings in mice included liver cancer in both sexes, as shown by positive dose-related trends for hepatocellular adenomas and carcinomas, with incidences in low-dose males and high-dose males and females significantly higher than in the controls.

Hepatoblastoma, an extremely rare form of hepatocellular carcinoma, also occurred in a few of the high-dose male mice, but did not reach statistical significance. Comparison to historical control incidence suggested that the finding was likely related to exposure. Other neoplastic outcomes included a marginal increase in adrenal pheochromocytomas in the male mice.

The only other information regarding the carcinogenicity of 1,4-dichlorobenzene following oral exposure comes from two-stage studies that found no indication of kidney tumor initiation or liver tumor promotion in rats (Umemura et al., 2000; Gustafson et al., 1998). There was no kidney tumor initiating activity of 1,4-dichlorobenzene in rats that were orally administered 214 mg/kg-day for 13 weeks, followed by promotion with NTA for up to 39 weeks (Umemura et al., 2000). Preneoplastic foci in the liver were not increased in rats that were initiated with a single i.p. injection of DEN followed 2 weeks later by oral promotion with $\leq$58.8 mg/kg-day doses of 1,4-dichlorobenzene for 6 weeks (Gustafson et al., 1998).

Effects of chronic inhalation were investigated in rats of both sexes and female mice exposed to 75 or 500 ppm 1,4-dichlorobenzene for 5 hours/day, 5 days/week for up to 76 weeks (rats) or 57 weeks (female mice), followed by 36 weeks (rats) or 19 weeks (female mice) without exposure (ICI, 1980; Riley et al., 1980). There were no neoplastic or any other histopathologic changes in liver, kidneys, or other tissues of rats or female mice. The adequacy of these studies for carcinogenicity evaluation is limited by failure to reach the MTD, less-than-lifetime exposure durations, and short observation periods in both species. The mouse study is further limited by lack of data in males (a group of male mice was terminated due to high early mortality), as well as unavailability of a complete study report.

Data are available from a 2-year inhalation bioassay in rats and mice exposed to 0, 20, 75, or 300 ppm of 1,4-dichlorobenzene for 6 hours/day, 5 days/week for 104 weeks (Aiso et al., 2005b; JBRC, 1995). No evidence of compound-related tumor formation was reported in either sex of rats. In male mice, increased incidences of hepatocellular carcinoma and histiocytic sarcoma were seen in animals exposed to the highest exposure concentration only. In females, increased incidences of hepatocellular carcinoma and combined bronchoalveolar adenoma and carcinoma were seen in high-dose animals only, while the incidence of hepatocellular adenoma was elevated in the low-exposure and high-exposure groups, but not in the mid-exposure group.

No studies are available that investigated genotoxic effects of 1,4-dichlorobenzene in humans, although genotoxicity has been extensively studied in animal systems, as detailed in Section 4.4.2. Negative results were reported in the vast majority of a variety of assays, including gene mutation in *S. typhimurium* and mouse lymphoma cells in vitro; DNA damage in rat and human hepatocytes in vitro; unscheduled DNA synthesis in mouse hepatocytes and rat kidney

cells in vivo, sister chromatid exchange in CHO cells in vitro; mouse bone marrow cells and erythrocytes in vivo; chromosomal aberrations in rat bone marrow cells in vivo; and dominant lethal mutations in mice. Some studies, including assays for chromosomal aberrations, sister-chromatid exchanges, and micronucleus formation in mammalian cells, were equivocal and inconsistent, with findings that were both positive and negative (Tegethoff et al., 2000; Robbiano et al., 1999; Canonero et al., 1997; Morita et al., 1997; Miyagawa et al., 1995; Carbonell et al., 1991; Anderson et al., 1990; Mohtashamipur et al., 1987; NTP, 1987). The minimal evidence for genotoxicity of 1,4-dichlorobenzene is consistent with the IARC (1999) conclusion that there is weak evidence for the genotoxicity of 1,4-dichlorobenzene in mammalian cells in vitro, and that no conclusion can be drawn from the in vivo data. Overall, the available genotoxicity data are insufficient to determine whether 1,4-dichlorobenzene is genotoxic.

4.6.3.2. *Mode of Action Information*

4.6.3.2.1. ***Renal tumors in rats***. There is a widespread scientific consensus that 1,4-dichlorobenzene causes both renal toxicity and tumors through a non-DNA-reactive mechanism involving binding to α_{2u}-globulin, a protein specific to male rats and not present in female rats or other species, including humans (Barter and Sherman, 1999; IARC, 1999; U.S. EPA, 1991b). U.S. EPA (1991b) has concluded that "Male rat renal tubule tumors arising as the result of a process involving α_{2u}-globulin do not contribute to the qualitative weight-of-evidence (WOE) that a chemical poses a human carcinogenic hazard. Such tumors are not included in dose-response extrapolations for the estimation of human carcinogenic risk."

α_{2u}-Globulin nephropathy is characterized by a series of histopathologic changes, including hyaline droplet accumulation in the proximal convoluted tubules and consequent cellular damage and regenerative cell proliferation, which are mechanistically linked to the formation of kidney tumors in rats. To establish that the α_{2u}-globulin process is a factor in observed renal tumors, the following criteria must be met (U.S. EPA, 1991b):

- Increased number and size of hyaline droplets in renal proximal tubule cells of treated male rats.
- Accumulating protein in the hyaline droplets is α_{2u}-globulin.
- Additional aspects of the pathological sequence of lesions associated with α_{2u}-globulin nephropathy are present.

Substantial evidence indicates that the renal effects associated with 1,4-dichlorobenzene exposure are produced by a sequence of events initiated by binding of 1,4-dichlorobenzene to the male rat-specific protein α_{2u}-globulin. Specific evidence for a MOA of 1,4-dichlorobenzene-induced renal carcinogenesis involving α_{2u}-globulin includes the following:

- The results of several subchronic studies in male and female rats(NTP, 1987; Hollingsworth et al., 1956) show that 1,4-dichlorobenzene causes renal damage (tubular degeneration and necrosis) in male rats but not female rats. Bomhard et al. (1988) showed that male F344 CDF rats exposed to 1,4-dichlorobenzene for up to 13 weeks developed kidney lesions characteristic of α_{2u}-globulin-related toxicity, including hyaline droplet formation, cellular damage, and proliferation of the P1/P2 proximal tubule regions. In a two-generation reproductive toxicity study (Tyl and Neeper-Bradley, 1989), hyaline droplet nephropathy was found in F_0 and F_1 adult male rats; manifestations of this male rat-specific renal syndrome included α_{2u}-globulin accumulation, increased kidney weights, and other characteristic histologic changes (e.g., tubular cell hyperplasia).

- In a chronic bioassay (NTP, 1987), nephropathy and renal tumors (together) occurred only in male rats.

- NBR rats, a strain that does not synthesize α_{2u}-globulin, showed no renal effects following gavage exposure to 500 mg/kg of 1,4-dichlorobenzene for 4 days, whereas F344 rats showed clear evidence of α_{2u}-globulin accumulation and toxicity at the same dose levels (Dietrich and Swenberg, 1991).

- Increased cell proliferation in the renal cortex has been demonstrated. Male rats exposed to 1,4-dichlorobenzene for 4 or 13 weeks by gavage showed increased BrdU labeling in renal proximal tubule cells (Lake et al., 1997).

- The protein that accumulated in tubular cells was identified as α_{2u}-globulin (Dietrich and Swenberg, 1991; Tyl and Neeper-Bradley, 1989).

- Both 1,4-dichlorobenzene and its major metabolite, 2,5-dichlorophenol, bound reversibly to α_{2u}-globulin in a manner similar to that of TMP, a component of unleaded gasoline that has been shown to elicit α_{2u}-globulin-related effects (Charbonneau et al., 1989).

The above lines of evidence meet the criteria for establishing the role of 1,4-dichlorobenzene-induced α_{2u}-globulin nephropathy in male rat renal carcinogenesis as described in U.S. EPA (1991b). Accordingly, α_{2u}-globulin-associated kidney tumors induced by 1,4-dichlorobenzene are not considered relevant to humans.

IARC reached a similar conclusion regarding a renal tumor MOA for 1,4-dichlorobenzene. IARC (1999) concluded that: "Overall, the data on genotoxicity do not support a mechanism for renal-cell tumor induction in rats involving direct interaction of *para*-dichlorobenzene with DNA. Therefore, the overall data, including those on genotoxicity, indicate that *para*-dichlorobenzene causes renal tumors in male rats through an α_{2u}-globulin associated response."

4.6.3.2.2. ***Liver tumors in mice***. In contrast to the kidney tumors in male rats, the mechanism by which 1,4-dichlorobenzene induces liver tumors in mice is not well defined. As discussed in Section 4.4.1.2 and other evaluations (Barter and Sherman, 1999; IARC, 1999), available evidence indicates that the mechanism leading to the formation of mouse liver tumors following 1,4-dichlorobenzene exposure is based on sustained mitogenic stimulation and proliferation of hepatocytes. Some of the data indicate that the cell proliferation may be a threshold response to cytotoxicity, which would be consistent with the results of the NTP (1987) bioassay. NTP (1987) found that liver tumor incidence was increased only in high-dose mice that also showed hepatotoxic effects, but not in low-dose female mice, which showed little or no hepatotoxicity. The proliferation may be due to an increase in the rate of cell division, a decrease in the rate of apoptosis, or a combination of the two, based on evidence for decreases in apoptosis and increases in BrdU labeling index, DNA synthesis, or CRF in livers of exposed mice (James et al., 1998; Sherman et al., 1998; Umemura et al., 1998, 1996, 1992; Lake et al., 1997; Eldridge et al., 1992). However, similar effects were found in the livers of exposed rats, even though 1,4-dichlorobenzene did not induce liver tumors in rats (James et al., 1998; Sherman et al., 1998; Umemura et al., 1998, 1996, 1992; Hasmall et al., 1997; Lake et al., 1997; Eldridge et al., 1992). In addition, the mitogenic effects of 1,4-dichlorobenzene may not be sustained throughout long-term exposure (Lake et al., 1997; Eldridge et al., 1992). NTP (1987) did not report significantly elevated hepatic hyperplasia following chronic exposure to 1,4-dichlorobenzene, although other hepatotoxic effects were noted that achieved statistical significance. Hepatocellular hypertrophy co-occurred with liver tumors in male, but not female mice in the inhalation cancer bioassay (Aiso et al., 2005b; JBRC, 1995). Thus, while there is some evidence supporting a sustained proliferative response following 1,4-dichlorobenzene exposure as the MOA for 1,4-dichlorobenzene-induced tumor formation, the evidence at present is not sufficient to warrant a nonlinear mode of action.

4.6.3.3. *Summary of Overall Weight-of-Evidence*

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 1,4-dichlorobenzene is considered *likely to be carcinogenic to humans* by both the oral and inhalation routes based on evidence of cancer in mice at multiple sites and by oral and inhalation exposure; tumors consisted of liver tumors, including hepatoblastomas (a rare type of hepatocellular carcinoma) in male and female mice by both oral and inhalation routes of exposure and bronchoalveolar tumors in female mice exposed by inhalation.

In the NTP (1987) gavage study, there was a positive dose-related trend for hepatocellular adenomas and carcinomas in male and female mice. Hepatoblastoma, an extremely rare form of hepatocellular carcinoma, also occurred in 4/50 high-dose male rats. NTP (1987) reported that only one hepatoblastoma had been observed among 6047 male and female control mice (vehicle and untreated controls combined).

In the 2-year inhalation bioassay of 1,4-dichlorobenzene (Aiso et al., 2005b; JBRC, 1995), investigators reported a statistically significant positive trend in hepatocellular adenomas and carcinomas in male and female mice, liver histiocytic sarcomas in male mice, and bronchoalveolar carcinoma in female mice. Consistent with the findings of the oral gavage NTP bioassay, Aiso et al. (2005b) observed a statistically significant increase in hepatoblastomas in male and female mice; the occurrence of this type of liver tumor was rare in JBRC historical control animals (10/1496 and 0/1498 male and female BDF1 mice, respectively).

The increased incidence of male rat kidney tumors following oral exposure (NTP, 1987) was not considered relevant to humans because the mechanism α_{2u}-globulin nephropathy is specific to male rats.

4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.7.1. Possible Childhood Susceptibility

Limited information regarding possible adverse effects of dichlorobenzenes in children is available from two case reports of 1,4-dichlorobenzene exposure. A 3-year-old boy developed health effects that included acute hemolytic anemia, methemoglobinemia, and jaundice after playing with moth crystals containing 1,4-dichlorobenzene (Hallowell, 1959). Hematological effects also occurred in a woman who consumed toilet air freshener (composed mainly of 1,4-dichlorobenzene) at a rate of one or two blocks per week throughout pregnancy until about 38 weeks of gestation (Campbell and Davidson, 1970). The woman developed severe microcytic, hypochromic anemia (from which she recovered following cessation of exposure), although neonatal examination of the child showed no abnormalities. The limited information from these

case reports provides no indication that the nature of health effects differs in children and adults, although the available data are insufficient to reach conclusions about age-related differences in susceptibility to dichlorobenzenes.

Information on the developmental toxicity of 1,2-, 1,3-, and 1,4-dichlorobenzene is available from oral and inhalation studies in rats and rabbits (Bornatowicz et al., 1994; Bio/dynamics, 1989; Tyl and Neeper-Bradley, 1989; Giavini et al., 1986; Hayes et al., 1985; Ruddick et al., 1983; Hodge et al., 1977). These studies provide no indication that the compounds are teratogenic, although fetotoxicity occurred at exposure levels that were also maternally toxic. A multigeneration study in rats that were orally exposed to 1,4-dichlorobenzene found toxic effects in the pups during the nursing period, including increased neonatal mortality, dermal effects and other clinical manifestations, and reduced neurobehavioral performance (Bornatowicz et al., 1994). The postnatal developmental toxicity occurred at dose levels that were not maternally toxic and below those causing systemic toxicity in other animal studies. The results of this study indicate that postnatal developmental toxicity is the most sensitive endpoint in animals, and suggest a basis for potential concern in exposed children. Effects of dichlorobenzenes on the nervous, immune, and endocrine systems have not been adequately studied.

1,4-Dichlorobenzene-related effects in children versus adults are not sufficiently documented in the open literature to determine whether or not children are more sensitive to 1,4-dichlorobenzene exposure than adults.

4.7.2. Possible Gender Differences

The extent to which men and women may differ in susceptibility to dichlorobenzenes is not known. Available animal data do not provide a clear pattern for gender differences in the toxicity of dichlorobenzenes, although some subchronic and chronic studies found that males were more sensitive than females for some endpoints. For example, a multigeneration inhalation study of 1,4-dichlorobenzene in rats observed increases in adult liver weight that were more pronounced in males than females (Tyl and Neeper-Bradley, 1989). In a subchronic oral study of 1,3-dichlorobenzene in rats, histopathologic changes in the thyroid were generally more severe in males than females (McCauley et al., 1995). This study also found histopathologic changes in the pituitary of male rats, but not in females. The pituitary lesion was reported to be similar to that induced in gonadectomized rats and was considered to be an indicator of gonadal deficiency (McCauley et al., 1995). Though the above-mentioned animal studies provide some indication that males may be more sensitive to dichlorobenzenes exposure, the evidence is insufficient for extrapolation to humans.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. 1,2-Dichlorobenzene

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

No information was located regarding health effects of 1,2-dichlorobenzene in humans following oral exposure.

The systemic toxicity of 1,2-dichlorobenzene in orally exposed animals has been investigated in three subchronic studies in rats and mice (Robinson et al., 1991; NTP, 1985; Hollingsworth et al., 1958) and in one chronic (NTP, 1985) study in rats and mice.

The subchronic studies suggested that the liver may be a sensitive but inconsistent target for repeated oral exposures to 1,2-dichlorobenzene. In rats exposed to doses of 89.3–135 mg/kg-day for ≥13 weeks (Robinson et al., 1991; NTP, 1985; Hollingsworth et al., 1958), the effects were limited to increases in relative liver weight and serum ALT and slight dose-related increases in serum cholesterol, serum protein, and decreases in serum triglycerides. Increased serum ALT was an inconsistent finding because it was induced in rats exposed to ≥100 mg/kg-day for 90 days (Robinson et al., 1991), but not in rats exposed to ≥89.3 mg/kg-day for 13 weeks (NTP, 1985). In addition, the increase in serum ALT was not dose-related, and serum levels of other liver-associated enzymes were not increased in either the Robinson et al. (1991) study (AST, LDH and AP) or the NTP (1985) study (AP and GGT).

In the NTP (1985) subchronic rat study there was an increase in serum cholesterol that was characterized by NTP as "slight" and a decrease in triglycerides. Histopathologic changes in the liver in the 13-week study occurred only at the highest two doses (250 and 500 mg/kg-day) – well in excess of the doses administered in the chronic study, and consisted of necrosis, which may be a general toxicity effect as it was scattered single cell necrosis. The subsequent 2-year NTP bioassay revealed no histopathology in the liver at dose levels of 60 and 120 mg/kg-day. In the absence of functional disturbances and a corresponding increase in serum triglycerides (another risk factor for cardiovascular disease in humans), the serum cholesterol findings are not considered toxicologically significant. In addition, it is uncertain if increases in serum cholesterol in rats following exposure to 1,2-dichlorobenzene are relevant to an assessment of human cardiovascular toxicity, since it is generally acknowledged that the rat is not a good model for predicting cardiovascular effects in humans and because the rat is relatively resistant to hyperlipidemia and atherosclerosis (Sipes et al., 1997; Loeb and Quimby, 1999). The observed

elevation in serum cholesterol following subchronic exposure to 1,2-dichlorobenzene is interesting because a similar subchronic exposure of rats to 1,3-dichlorobenzene (McCauley et al., 1995) was associated with both elevated cholesterol and evidence of thyroid toxicity. It is possible that dichlorobenzene-related changes in serum cholesterol are a secondary effect of some primary physiologic disturbance, one of which could be altered thyroid function.

The incidence of degenerative liver effects was significantly increased in rats exposed to 179–400 mg/kg-day for ≥13 weeks (Robinson et al., 1991; NTP, 1985; Hollingsworth et al., 1958) and mice exposed to 179 mg/kg-day for 13 weeks (NTP, 1985). In the NTP (1985) study, F344 rats (10/sex/group) and B6C3F1 mice (10/sex/group) were administered 1,2-dichlorobenzene in corn oil by gavage at duration-adjusted doses of 0, 21.4, 42.9, 89.3, 179, or 357 mg/kg-day for 13 weeks. Relative liver weight was slightly increased in the rats (~8% higher than controls in both sexes) at 89.3 mg/kg-day, and the incidence of liver necrosis was significantly increased in both species at 179 mg/kg-day.

In the chronic study, groups of F344/N rats (50/sex/group) and $B6C3F_1$ mice (50/sex/group) were administered 1,2-dichlorobenzene in corn oil by gavage in duration-adjusted doses of 0, 42.9, or 85.7 mg/kg-day for 103 weeks (NTP, 1985). Evaluations included clinical signs, body weight, and necropsy and histology on all animals. There was no toxicity observed in rats. In mice, no clinical signs were reported, and mean body weight and survival were comparable in control and dosed mice throughout the study, indicating that it is unclear if an MTD was achieved. The only exposure-related nonneoplastic lesion was a dose-related increased incidence (statistically significant at $p<0.05$, Fisher's Exact test) of renal tubular regeneration in male mice at 85.7 mg/kg-day; incidences in the control, low- and high-dose male groups were 8/48, 12/50, and 17/49, respectively. Though no regeneration or necrotic lesions were found in the kidneys of female mice, a statistically significant increase in renal tubular regeneration in male mice indicated that at some earlier point in the study, tubular degeneration must have occurred. This increase in renal tubular regeneration is consistent with an apoptotic response in the kidneys. In addition, tubular degeneration and regeneration could be considered as precursor events to more chronic nephritis in the dosed animals. This indicates that the high dose of 85.7 mg/kg-day in the chronic study in mice is a minimal LOAEL for kidney effects (male mice) and the low dose of 42.9 mg/kg-day is a NOAEL.

Therefore, the chronic NTP mouse study (NTP, 1985) is the most appropriate study for the development of an oral RfD for 1,2-dichlorobenzene based on the statistically significant kidney effects observed at the high dose in male mice. Renal tubular regeneration is identified as the

critical effect since it was the only nonneoplastic lesion with a statistically significant dose-related increased incidence.

5.1.1.2. *Methods of Analysis—Including Models*

Benchmark dose (BMD) analysis was performed using data for tubular regeneration in the kidneys of male mice from the 103-week NTP (1985) bioassay (detailed data are presented in Appendix B, Table B-1). All dichotomous models in the EPA Benchmark Dose Software (BMDS; version 1.3.2; U.S. EPA, 2004a) were fitted to these incidence data. A 10% extra risk of renal tubular regeneration was selected as the benchmark response (BMR); this BMR was a response rate that fell at the low end of the range of experimental doses in the NTP (1985) study. Selection of this BMR is consistent with the aim of BMD modeling guidelines, viz., to select a BMD at the low end of the observable range to use as the point of departure for deriving quantitative estimates below the range of observation (U.S. EPA, 2000c). The gamma, log-logistic, and Weibull models were not applied to this data set because the number of parameters in the models equaled the number of treatment groups and did not allow sufficient degrees of freedom. The remaining models provided adequate fits to the data (χ^2 goodness-of-fit *p*-value >0.1). The logistic model provided the best fit to the data (as indicated by the lowest Akaike Information Criterion [AIC] and visual inspection of the plot in the range near the point of departure), and was used to derive BMD_{10} and $BMDL_{10}$ values of 43.5 and 29.8 mg/kg-day, respectively. See Section B.1 for detailed model results.

5.1.1.3. *RfD Derivation—Including Application of Uncertainty Factors*

To derive the RfD for 1,2-dichlorobenzene, the $BMDL_{10}$ of 29.8 mg/kg-day was divided by a total uncertainty factor (UF) of 1000—10 for interspecies extrapolation, 10 for interindividual variability, and 10 for database deficiencies.

A 10-fold UF was used to account for the interspecies variability in extrapolating from laboratory animals to humans. The critical effect, renal toxicity, was observed in male mice only in the chronic NTP bioassay (not in female mice or rats of either sex). No information is available on the toxicity of 1,2-dichlorobenzene in orally-exposed humans, and data on toxicokinetic differences between animals and humans in the disposition of ingested 1,2-dichlorobenzene are limited. Therefore, the available information to characterize interspecies differences in response to 1,2-dichlorobenzene exposure are insufficient to move away from the default 10-fold UF for interspecies extrapolation.

A 10-fold UF was used to account for variation in sensitivity within human populations. There is no information on the degree to which humans of varying gender, age, health status, or genetic makeup might vary in the disposition of, or response to, ingested 1,2-dichlorobenzene.

A 10-fold UF was used to account for deficiencies in the database. The database for oral exposure to 1,2-dichlorobenzene includes a chronic oral toxicity study (NTP, 1985) and three subchronic oral toxicity studies. The only information on developmental toxicity comes from a study that is available as an abstract only with limited information on methods and results (Ruddick et al., 1983). In addition, there are no reproductive toxicity studies (including no multigeneration reproductive toxicity study) by the oral route. Therefore, a UF of 10 was used for database deficiencies based on the lack of an adequate developmental study and the absence of a multigeneration reproductive toxicity study.

A subchronic to chronic UF was not necessary because the point of departure was derived from a 2-year chronic bioassay.

A UF to account for the extrapolation from a LOAEL to a NOAEL was not applied because BMD modeling was used to determine the point of departure for derivation of the 1,2-dichlorobenzene RfD.

The RfD for 1,2-dichlorobenzene is calculated as follows:

$$\begin{aligned} \text{RfD} &= \text{BMDL}_{10} \div \text{UF} \\ &= 29.8 \text{ mg/kg-day} \div 1000 \\ &= 0.03 \text{ mg/kg-day} \end{aligned}$$

5.1.1.4. *Previous Oral Assessment*

The previous IRIS assessment utilized the NTP (1985) 2-year rat gavage study as the principal study and a NOAEL of 120 mg/kg-day, 5 days/week, was used for derivation of the RfD (0.09 mg/kg-day). A composite UF of 1000 was applied to account for interspecies and intraspecies extrapolations, as well as uncertainties in the database due to limited reproductive toxicity data.

5.1.2. 1,3-Dichlorobenzene

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

No information is available on the toxicity of ingested 1,3-dichlorobenzene in humans. As discussed in Section 4.5.1.2., the database for toxicity assessment following oral exposure contains only one subchronic toxicity study in rats (McCauley et al., 1995) and one developmental

toxicity study in rats that has only been reported in abstract form (Ruddick et al., 1983). The developmental toxicity study observed no maternal toxicity or developmental toxicity following administration of doses as high as 200 mg/kg-day. In the subchronic toxicity study, rats were exposed to doses of 9, 37, 147, or 588 mg/kg-day 1,3-dichlorobenzene for 90 days and effects in the thyroid and liver occurred at all tested dose levels (Table 5-1).

Table 5-1. Liver and thyroid effects observed in male rats orally exposed to 1,3-dichlorobenzene for 90 days

Effects	Dose (mg/kg-day)				
	0	**9**	**37**	**147**	**588**
Hepatocellular cytoplasmic alterations	1/10	2/10	1/10	6/10[a]	7/9[a]
Serum AST (U/L)[b] Serum cholesterol (mg/dL)[b] Serum ALT (U/L)[b] Serum LDH (U/L)[b]	43.7 ± 37.7 73.5 ± 1.4 46.8 ± 7.7 1762 ± 765	87.6 ± 24.7[c] 96.6 ± 1.7[c] 40.8 ± 9.7 623 ± 466	109.8 ± 9.5[d] 111.1 ± 1.6[c] 43.3 ± 4.5 798 ± 238	88.0 ± 23.3[c] 157.9 ± 2.5[c] 38.5 ± 8.2 778 ± 530	82.8 ± 13.8[c] 89.5 ± 1.5[c] 59.3 ± 11.0 735 ± 288
Thyroid, reduced follicular colloidal density	2/10	8/10[a]	10/10[a]	8/9[a]	8/8[a]

[a] Significantly different (p<0.05) from control; Fisher's Exact Test performed by Syracuse Research Corporation.
[b] Mean±SD.
[c] Reported to be significantly higher (p<0.05) than control mean by study authors.
[d] This value was not reported to be significantly higher than control mean.

Source: McCauley et al., 1995.

The McCauley et al. (1995) study was selected as a candidate study for possible derivation of an RfD for 1,3-dichlorobenzene. Collectively, the data for male rats (which were more sensitive than female rats) in Table 5-1 identified thyroid effects (reduced follicular colloidal density) as the potential critical effect from subchronic exposure. Liver effects (increased incidence of hepatocellular cytoplasmic alterations) occurred at higher dose levels than the lowest doses that induced thyroid effects (Table 5-1). Mean serum levels of AST and cholesterol were statistically significantly increased in all male exposed groups compared with controls, but other serum markers of liver damage such as activities of ALT and LDH were not significantly increased in exposed groups (Table 5-1). Because of this inconsistency, the observed statistically significant changes in AST and cholesterol are not considered to be biologically significant changes indicating liver damage. However, the observed histopathologic changes in the thyroid may be considered to be

adverse. The reduced follicular colloidal density in the thyroid may be indicative of thyroid stimulation (Gershon and Nunez, 1988). In addition, the elevated serum cholesterol concentrations may be related to thyroid damage, rather than liver damage. In the absence of data to indicate otherwise, the thyroid effect is assumed to be the critical effect relevant to humans who may chronically ingest 1,3-dichlorobenzene and is selected as the basis for the chronic RfD. A NOAEL was not identified in this study. However, a LOAEL of 9 mg/kg-day was identified in this study since effects in the thyroid, as well as in the pituitary and liver, occurred at all tested dose levels.

5.1.2.2. *Methods of Analysis - Including Models*

The BMD analysis was performed using data for reduced follicular colloidal density of the thyroid of male rats from the 90-day study by McCauley et al. (1995) (data presented in Appendix B.2, Table B-3). All dichotomous models in the BMDS (version 1.3.2; U.S. EPA, 2004a) were fitted to these incidence data using a benchmark response of 10% extra risk. The χ^2 goodness-of-fit statistics for all models indicated poor statistical fits ($p<0.1$). Data from the highest dose group (588 mg/kg-day) were dropped to see if an improved model fit could be achieved. The χ^2 goodness-of-fit statistic for all dichotomous models indicated inadequate statistical fits ($p<0.1$) using the control and first three dose groups.

Data from the two highest dose groups (147 and 588 mg/kg-day) were dropped to see if adequate model fits could be achieved (i.e., models were run using data for the control, 9 mg/kg-day, and 37 mg/kg-day dose groups only). Model results are provided in Section B.2. The gamma, log-logistic, log-probit, and Weibull models could not be applied to this data set because the number of parameters in the models equaled the number of treatment groups and did not allow sufficient degrees of freedom. Adequate models fits were obtained with the other models in the BMDS package (i.e., χ^2 goodness-of-fit p-value >0.1); however, the dose associated with the BMR of 10% extra risk for reduced thyroid follicular colloidal density was well below the range of experimental doses. As discussed in EPA's BMD modeling guidance (U.S. EPA, 2000c), the aim of BMD modeling is to model the dose-response data for an adverse effect in the observable range and then select a BMD at the low end of the observable range to use as a point of departure for deriving the RfD. In the situation presented by the McCauley et al. (1995) thyroid data, BMD modeling provided little resolution of the dose-response curve in the region of the benchmark response. Accordingly, for thyroid data from the McCauley et al. (1995) study, the conclusion was reached that the data are not suitable for BMD modeling. Since the thyroid data from McCauley et al. were not suitable for BMD modeling, consideration was given to the use of the NOAEL/LOAEL method for the derivation of an oral RfD for 1,3-dichlorobenzene.

5.1.2.3. ***RfD Derivation - Including Application of Uncertainty Factors***

To derive an RfD, the NOAEL/LOAEL approach was considered. For this purpose the LOAEL of 9 mg/kg-day would be divided by 30,000. The UFs were as follows: 3 for interspecies variability, 10 for interindividual variability, 10 for extrapolation from subchronic to chronic exposure, 10 for database deficiencies, and 10 for extrapolation from LOAEL to NOAEL.

A threefold UF was used to account for uncertainty in extrapolating from rats to humans (i.e., interspecies variability). While both rodents and humans have low-affinity protein carriers for thyroid hormone (e.g., albumin), rodents do not have a high-affinity binding protein, thyroxine-binding globulin, which is responsible for binding T_4 in humans (U.S. EPA, 1998c). As a result of the lack of thyroxin-binding globulin in rats, T_4 is bound to proteins with lower affinity leading to a shorter half-life of less than one day in rodents, while in humans the half-life is five to nine days. Serum T_3 levels are also different in rodents and humans, with half-lives of 6 hours in rodents and close to 24 hours in humans (U.S. EPA, 1998c). Because of the low affinity binding of thyroid hormone in rodents, they are more susceptible to agents affecting the metabolism or excretion of thyroid hormones. In addition, the shorter half-life leads to a higher rate of production of these hormones in rodents. Although no information is available on the toxicity of ingested 1,3-dichlorobenzene in humans, rats are generally recognized as markedly more sensitive than adult humans to chemicals that affect thyroid hormone production because of the lack of thyroxin-binding globulin and shorter thyroid hormone half-life (U.S. EPA, 1998c). This supports the use of a threefold UF for interspecies variability.

A 10-fold UF was used to account for interindividual variation in sensitivity to 1,3-dichlorobenzene in human populations. The degree to which humans of varying gender, age, health status, or genetic makeup may vary in disposing of, or responding to ingested 1,3-dichlorobenzene has not been studied. Additional information on possible gender differences in toxicokinetics or toxicodynamics is not available. Therefore, a 10-fold UF for variation in the general population was used.

A 10-fold UF was used to account for extrapolating from subchronic oral exposure to chronic oral exposure. Although the modes of action whereby 1,3-dichlorobenzene stimulates activity of the thyroid are unknown, it is plausible that with longer duration of exposure (i.e., chronic duration), lower exposure levels may induce the same effects.

A 10-fold UF was used to account for deficiencies in the database. The only information on the systemic toxicity of repeated oral exposure to 1,3-dichlorobenzene comes from the subchronic rat study reporting thyroid effects at doses $\geq$9 mg/kg-day (McCauley et al., 1995). This well-designed study investigated a large number of endpoints, including liver-associated enzymes and various other serum chemistry indices, hematology, and comprehensive histology that included

the thyroid, pituitary and other endocrine tissues. A developmental toxicity study in the rat is available, but has been reported in abstract form only (Ruddick et al., 1983). The oral exposure database for 1,3-dichlorobenzene contains no chronic toxicity data and lacks assessments of developmental toxicity in a second animal species, and reproductive toxicity in males or females.

A 10-fold UF was used to account for extrapolating from LOAEL to NOAEL. Typically the highest exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control, is called the NOAEL. When a NOAEL can be identified in a principal study, it becomes the basis of the reference value derivation. If a NOAEL cannot be identified, then a LOAEL is identified instead. To extrapolate from LOAEL to NOAEL a UF of 10 is generally used. Since no NOAEL has been identified in the principal study (McCauley et al., 1995) a UF of 10 was used to derive the RfD.

The composite UF for 1,3-dichlorobenzene comprises five areas of uncertainty. In the report, *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), the RfD/RfC Technical Panel concluded that, in cases where maximum uncertainty exists in four or more areas of extrapolation, it is unlikely that the database is sufficient to derive a reference value. The Panel recommended that no reference value for any particular chemical substance be derived if the composite UF is greater than 3,000. Because the derivation of an oral RfD for 1,3-dichlorobenzene would involve the application of a UF of 30,000 addressing five areas of uncertainty, no reference value is derived, consistent with the recommendations of the RfD/RfC Technical Panel (U.S. EPA, 2002).

In summary, data are inadequate for derivation of a RfD for 1,3-dichlorobenzene.

5.1.2.4. *Previous Oral Assessment*

There was no RfD previously calculated for 1,3-dichlorobenzene.

5.1.3. 1,4-Dichlorobenzene

5.1.3.1. *Choice of Principal Study and Critical Effect—with Rationale and Justification*

Information on the toxic effects of ingested 1,4-dichlorobenzene in humans is limited to two case reports of hematologic changes (anemia) following repeated oral exposure to unknown amounts of 1,4-dichlorobenzene in commercial products (Campbell and Davidson, 1970; Hallowell, 1959).

As discussed in more detail in Section 4.5.1.3, the subchronic and chronic oral toxicities of 1,4-dichlorobenzene have been assessed in a number of studies in animals, predominantly dogs, rats and mice. Liver and kidney effects are the best studied and most consistently observed findings.

Effects on the hematologic system, the adrenals, and the thyroid have been reported as well, but occurred at exposure levels equal to or higher than those causing liver and kidney effects. Results from reproductive and developmental toxicity studies in rats indicate that offspring are particularly sensitive to 1,4-dichlorobenzene during the postnatal preweaning period.

The rat and mouse are less sensitive to 1,4-dichlorobenzene liver toxicity than the dog. The available data indicate that the lowest chronic hepatic LOAEL in dogs is 36 mg/kg-day (Monsanto Company, 1996), which is the same as the lowest chronic LOAEL for kidney effects in dogs. Increased incidence of fetuses with extra ribs, a skeletal variation (not an anomaly or malformation), was observed, along with decreased maternal weight, in pregnant rats that were exposed to doses $\geq$500 mg/kg-day, but not at 250 mg/kg-day (Giavini et al., 1986). These results indicated that developmental effects from gestational exposure, along with changes in maternal weight gain, occurred at higher dose levels than those inducing liver and kidney effects following chronic exposure. Results from a two-generation reproductive and developmental toxicity study in rats (Bornatowicz et al., 1994) indicated that developmental effects, including statistically significantly reduced birth weight in F_1 pups and statistically significantly increased incidence of F_2 pup deaths between birth and postnatal day 4, occurred at doses as low as 90 mg/kg-day. Effects at the high dose included increased number of deaths in F_1 pups at day 4, increased number of deaths in F_1 and F_2 pups later in the postnatal period, and reduced neurobehavioral performance (impaired draw-up reflex) in F_2 pups.

The chronic beagle dog study evaluated the systemic effects of 1,4-dichlorobenzene in 5 male and 5 female beagle dogs per dose group that were administered the chemical (99.9% pure) in gelatin capsules 5 days/week at initial dose levels of 0, 10, 50, or 150 mg/kg-day (Monsanto Company, 1996) for 1 year. Controls received empty gelatin capsules. Since unexpectedly severe toxicity occurred at the highest dose level, the high dose was adjusted to 100 mg/kg-day during the third week of exposure for males and further reduced to 75 mg/kg-day for both sexes at the beginning of week 6. Both males and females at the highest dose level were untreated during the fourth and fifth weeks to allow for recovery, while lower dose animals were administered the test compound continuously. The resulting time-adjusted doses were 0, 7, 36, and 54 mg/kg-day (for details, see Section 4.2.1.3.). The authors stated that one high-dose male (day 12) and one high-dose female (day 24) dog may have died due to inflammatory lung lesions and/or pulmonary hemorrhages while the cause of death of another high-dose male (day 25) remained undetermined. One control male dog died on day 83 and the cause of death may have been due to a physical displacement of the small intestine, with secondary aspiration pneumonia.

Compound-related effects included statistically significant liver lesions and increased absolute and relative organ weights (liver, kidneys, adrenals, and thyroid) at the mid and high dose

levels. In addition to liver lesions, chronic active interstitial inflammation, pleural fibrosis and/or pleural mesothelial proliferation was also observed in the lungs of males at all test levels and females at the mid and high dose (36 and 54 mg/kg-day) level. Although these changes were not observed in the control groups, the lung lesions were not considered to be treatment-related since their occurrence was rare and there was not much difference in severity among the treated groups. Kidney collecting duct epithelial vacuolation was reported in a high-dose male and at all doses in females. The authors concluded that the lesion could be associated with the test chemical at the mid and high doses in females since it was accompanied by increased kidney weights and grossly observed renal discoloration (Monsanto Company, 1996).

In summary, hepatotoxicity was the most critical effect from oral exposure to 1,4-dichlorobenzene. Thus, the chronic study conducted by Monsanto Company (1996) in male and female beagle dogs with a LOAEL of 36 mg/kg-day was selected as the principal study for RfD derivation.

5.1.3.2. *Methods of Analysis—Including Models*

The incidence of compound-related diffuse hepatocellular hypertrophy in male and female beagle dogs was analyzed by BMD modeling.

Male and female dog incidence data for diffuse hepatocellular hypertrophy were combined in the BMD analysis. Combining incidence data was supported because incidence was similar in male and female dogs and because there was no evidence of sex-related differences in response of the liver to exposure to 1,4-dichlorobenzene. The combined incidence data gave a higher sample size for BMD analysis.

Consideration was given to the need to adjust the denominators to account for dogs that died early in the study. Based on the individual pathology results from the Monsanto Company (1996) study report, all 5 animals per sex per dose group were examined for liver histopathology. Early deaths included one control male (day 83), one high-dose female (day 24), and two high-dose males (days 12 and 25). Individual animal pathology results for both of these high-dose males showed diffuse hepatocellular hypertrophy, suggesting that animals were at risk of this liver effect as early as day 12 of the study. Therefore, the four dogs that died before study termination were retained for purposes of dose-response analysis.

All dichotomous models in the EPA BMDS (version 1.3.2; U.S. EPA, 2004a) were fitted to the incidence data for diffuse hepatocellular hypertrophy in male and female beagle dogs (combined). A 10% extra risk of diffuse hepatocellular hypertrophy was selected as the BMR. This BMR fell near the low end of the range of experimental dose levels (i.e., the observable range) and its selection was consistent with BMD modeling guidance (U.S. EPA, 2000c). The log probit

model provided the best fit to the data (as indicated by the lowest AIC with a goodness-of-fit *p*-value >0.1) and was used to derive the BMD_{10} and $BMDL_{10}$ values of 24.0 and 9.06 mg/kg-day, respectively (see Section B.3 for detailed model results).

5.1.3.3. *RfD Derivation—Including Application of Uncertainty Factors*

To derive the RfD, the $BMDL_{10}$ of 9.06 mg/kg-day for liver effects (diffuse hepatocellular hypertrophy) from a 1-year chronic toxicity study in beagle dogs exposed to 1,4-dichlorobenzene was divided by a total UF of 300—10 for interspecies variability, 10 for interindividual variability and 3 for database deficiencies.

A default ten-fold UF was used to account for uncertainty in extrapolating from dogs to humans (i.e., interspecies variability). Glucuronidation and sulfation are substantial detoxification pathways for 1,4-dichlorobenzene. Animal studies have demonstrated that 22–36% of an administered dose of 1,4-dichlorobenzene was eliminated following glucuronidation, and 27–65% of the administered dose was eliminated following sulfation (Hissink et al., 1997a, 1996b; Hawkins et al., 1980; Azouz et al., 1954). Hissink et al. (1997a) studied the biotransformation and kinetics of 1,4-dichlorobenzene in male Wistar rats at three oral dose levels (10, 50 and 250 mg/kg). The authors concluded that 1,4-dichlorobenzene was mainly metabolized to 2,5-dichlorophenol (~90%), which was detected in the urine as its sulfate (50–60%), glucuronide (20–30%) and the free form (5–10%). Minor metabolites were the N-acetyl-cysteine-S-dihydro-hydroxy-1,4-dichlorobenzene and the corresponding dehydrated N-acetyl-cysteine-S-1,4-dichlorobenzene, which comprised ~10% of the total metabolites. No hydroquinones were observed for the male Wistar rat, not even under conditions of induced oxidative metabolism, therefore the potential for redox cycling was limited.

Krishnaswamy et al. (2003) studied the glucuronidation of serotonin in vitro and showed that dogs were among the poorest glucuronidators, based on the order of UGT activities in animal liver microsomes: rat > mouse > human > dog > rabbit. Although glucuronidation of serotonine measured in the above study may not be the same as hydroxylated metabolites of 1,4-dichlorobenzene, this study demonstrates significant differences in rates of glucuronidation between species. Species differences were also found for sulfation. Andersen (1985) studied the degree of tyrosine sulfation in 10 mammalian species. The percentage of sulfation was reported as 24.4 ± 4.2 (mean $\pm$ SEM) in dogs, 46.8 ± 3.3 in humans, 55.9 ± 2.3 in rats, 64.8 ± 2.1 in mice, and 68.2 ± 2.8 in rabbits. Note that dogs were the poorest sulfaters while humans showed an extent of sulfation twice as high as dogs. Sulfation rates of rats and mice were closer to humans than to dogs. Furthermore, Fischer et al. (1995) demonstrated that at 2 and 6 hours the metabolism of 1,4-dichlorobenzene in human liver slices resulted in similar levels of glucuronide and sulfate conjugates as in Sprague-Dawley and F344 rats.

Since sulfation and glucuronidation are major detoxication pathways of 1,4-dichlorobenzene, mice and rats should be the least sensitive species, as they have higher rates of sulfation and glucuronidation than dogs. This observation is consistent with findings from in vivo toxicity studies in rodents and mice. The NTP (1987) 13-week rat study identified a LOAEL of 214 mg/kg-day (based on limited associated serum enzymes). The Eldridge (1992) study identified a LOAEL of 429 mg/kg-day, and NOAEL of 214 mg/kg-day, for the mouse. The Monsanto (1996) 1-year dog study identified a NOAEL of 7 mg/kg-day and a LOAEL of 36 mg/kg-day.

Human metabolism of 1,4-dichlorobenzene is similar to the metabolism in rats and mice, with the activities of glucuronide and sulfate conjugation in humans similar to those in the rat. Like rats and mice, humans are expected to be less sensitive to 1,4-dichlorobenzene toxicity than dogs. Nevertheless the information on species differences in rates of glucuronidation and sulfation is insufficient to warrant a less than 10 default uncertainty factor, particularly without knowledge of the mode of action. This is based on the incomplete characterization of the toxicokinetics of 1,4-dichlorobenzene in dogs and humans. Sulfation and glucuronidation are important in only one portion of the 1,4-dichlorobenzene metabolic pathway (CYP2E1 appears to be the first step, at least in rats) and no comparative data are available to address the potential interspecies differences with respect to other toxicokinetic parameters. Support for the dog as a species more sensitive to 1,4-dichlorobenzene than humans (and thus an UF for interspecies variability smaller than the default of 10) comes from information on the glucuronide and sulfation phase II pathways. However, baseline studies with human liver slices identify glutathione as the predominant phase II metabolite. It is not known to what extent the phase II biotransformation reactions influence the overall metabolism (detoxification) of 1,4-dichlorobenzene. Because the importance of the phase II biotransformation reactions on toxicity is not known, interspecies differences in the toxicity of 1,4-dichlorobenzene cannot be predicted. Therefore, the default 10-fold UF was used to account for uncertainty in extrapolating from dogs to humans (i.e., interspecies variability).

A default 10-fold UF was used to account for interindividual variation in sensitivity to 1,4-dichlorobenzene in human populations. The degree to which humans of varying gender, age, health status, or genetic makeup may vary in disposing of, or responding to ingested 1,4-dichlorobenzene has not been studied. Accordingly, a 10-fold UF for variation in the general population was used.

A subchronic to chronic UF was not necessary because the point of departure was derived from a chronic study.

A UF to account for the extrapolation from a LOAEL to a NOAEL was not applied because BMD modeling was used to determine the point of departure for derivation of the 1,4-dichlorobenzene RfD.

A UF of 3 was used to account for database deficiencies. Although the animal oral toxicity database is substantial and includes a chronic toxicity study in beagle dogs (Monsanto Company, 1996), chronic toxicity/cancer studies in rats and mice (NTP, 1987), several subchronic toxicity studies, a developmental toxicity study in rats (Giavini et al., 1986), and a 2-generation reproductive and developmental toxicity study in rats (Bornatowicz et al., 1994), developmental toxicity has been evaluated in only one species, the rat. In the two generation rat study (Bornatowicz, 1994) increased pup mortality was reported at maternal exposures of greater than or equal to 90 mg/kg-day. This frank effect occurred at a dose that was within an order of magnitude of the point of departure. Therefore, a UF factor of 3 was used to account for the uncertainty associated with the database with respect to both the developmental and reproductive data.

The RfD for 1,4-dichlorobenzene is calculated as follows:

$$\begin{aligned} \text{RfD} &= \text{BMDL}_{10} \div \text{UF} \\ &= 9.06 \text{ mg/kg-day} \div 300 \\ &= 0.0302 \text{ mg/kg-day} \\ &= 0.03 \text{ mg/kg-day} \end{aligned}$$

5.1.3.4. *Previous Oral Assessment*

No previous RfD existed for 1,4-dichlorobenzene.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. 1,2-Dichlorobenzene

5.2.1.1. *Principal Study and Critical Effect—with Rationale and Justification*

Information on the toxicity of inhaled 1,2-dichlorobenzene in humans is limited to results of two industrial hygiene surveys (Hollingsworth et al., 1958; Elkins, 1950), a workplace mortality study (Spirtas et al., 1991), and a series of case reports (IARC, 1982; Girard et al., 1969). Findings included observations that occupational exposure was irritating to the eyes and respiratory passages at 100 ppm, but not at lower levels of approximately 44–50 ppm (Hollingsworth et al., 1958; Elkins, 1950). None of the human data are sufficient for risk assessment, as discussed in Section 4.5.2.1.

The observation of irritation effects of 1,2-dichlorobenzene in occupationally exposed humans was consistent with histologic findings of nasal olfactory epithelial lesions in mice exposed to 64 or 163 ppm for 6 hours/day, 5 days/week for 4–14 days (Zissu, 1995). The lesions were characterized by a complete loss of olfactory epithelium after 4 days of exposure. The severity of the nasal lesions decreased with time, suggesting that some tissue repair may have occurred despite continued exposure. No histologic alterations were observed in the trachea or lungs. Data on the

toxicity of longer-term inhalation exposure to 1,2-dichlorobenzene were available from a multispecies subchronic study (Hollingsworth et al., 1958), a two-generation reproduction study in rats (Bio/dynamics, 1989), and developmental studies in rats and rabbits (Hayes et al., 1985; Dow Chemical, 1981), but none of these studies provided information on possible respiratory tract effects. Body weight changes were the most sensitive measure of maternal toxicity, occurring at 93–150 ppm in rats and rabbits (Bio/dynamics, 1989; Hayes et al., 1985; Hollingsworth et al., 1958). These reductions in body weight were either sporadic, or accompanied by reduced food consumption, or were not accompanied by corresponding food consumption data. There were no effects on reproduction or developmental toxicity in these species at concentrations below 394–400 ppm (Bio/dynamics, 1989; Hayes et al., 1985; Dow Chemical, 1981).

The 14-day mouse study showed that the upper respiratory tract was a sensitive target for inhalation exposures to 1,2-dichlorobenzene, as serious olfactory lesions occurred in mice at concentrations of 64 and 163 ppm (Zissu, 1995), which are similar to and below the lowest subchronic exposure levels that caused systemic effects in rats and rabbits (Hayes et al., 1985; Hollingsworth et al., 1958). The available subchronic inhalation studies of 1,2-dichlorobenzene did not evaluate the respiratory tract, indicating that a critical effect for long-term exposures cannot be identified. In the absence of an identifiable critical effect, derivation of an RfC for 1,2-dichlorobenzene is precluded.

5.2.1.2. *Methods of Analysis—Including Models*

Data are inadequate for derivation of RfC.

5.2.1.3. *RfC Derivation—Including Application of Uncertainty Factors*

Data are inadequate for derivation of RfC.

5.2.1.4. *Previous Inhalation Assessment*

No previous RfC was calculated for 1,2-dichlorobenzene.

5.2.2. 1,3-Dichlorobenzene

5.2.2.1. *Principal Study and Critical Effect—with Rationale and Justification*

No information was located regarding the systemic, reproductive, or developmental toxicity of inhaled 1,3-dichlorobenzene in humans or animals. Consequently, the existing inhalation database is inadequate to support the derivation of an RfC for 1,3-dichlorobenzene.

The feasibility of deriving an RfC from the available oral studies of 1,3-dichlorobenzene toxicity was explored. Comparatively little is known about the mechanisms responsible for the

long-term oral toxicity of 1,3-dichlorobenzene, but the available evidence suggests that hepatic metabolism to a reactive intermediate may be of considerable importance, as discussed in Section 4.4. As the extent of hepatic metabolism is likely to differ dramatically between oral and inhalation exposures, a route-to-route extrapolation from the oral data is precluded.

Derivation of an RfC for 1,3-dichlorobenzene by analogy to 1,2- or 1,4-dichlorobenzene was also considered. Data were inadequate for the derivation of an RfC for 1,2-dichlorobenzene, and available oral data strongly suggest that 1,4-dichlorobenzene is less toxic than either of the other two isomers, and that target sites may vary between the isomers. Derivation of an RfC by analogy to 1,2- or 1,4-dichlorobenzene is therefore precluded.

5.2.2.2. *Methods of Analysis—Including Models*

Data are unavailable for derivation of RfC.

5.2.2.3. *RfC Derivation—Including Application of Uncertainty Factors*

Data are unavailable for derivation of RfC.

5.2.2.4. *Previous Inhalation Assessment*

No previous RfC was calculated for 1,3-dichlorobenzene.

5.2.3. 1,4-Dichlorobenzene

5.2.3.1. *Principal Study and Critical Effect—with Rationale and Justification*

Information on the toxicity of inhaled 1,4-dichlorobenzene in humans is available from limited observations in exposed workers and a few case reports. The only effect described in workers exposed to 1,4-dichlorobenzene was painful irritation of the eyes and nose that was usually experienced at 50–80 ppm, although the irritation threshold was higher (80–160 ppm) in workers acclimated to exposure (Hollingsworth et al., 1956). Case reports of people who inhaled 1,4-dichlorobenzene suggested that the liver and nervous system were systemic targets of toxicity in humans, but were limited by lack of adequate quantitative exposure information and/or verification that 1,4-dichlorobenzene was the sole causal factor (Reygagne et al., 1992; Miyai et al., 1988; Cotter, 1953). The hepatic, neurologic, and eye/nose irritation observations in humans were consistent with effects observed in animals exposed to high concentrations of the chemical.

The inhalation toxicity of 1,4-dichlorobenzene was evaluated in several animal studies, including evaluations of subchronic, chronic, and multigeneration exposure. An early study of rats, guinea pigs, mice, rabbits, and a monkey (Hollingsworth et al., 1956) identified changes in hepatic endpoints in rats, specifically increases in liver weight associated with hepatic histopathology, as a

sensitive endpoint, with a NOAEL of 96 ppm and a LOAEL of 158 ppm. Liver weight was increased in rats exposed to 500 ppm of 1,4-dichlorobenzene for 76 weeks (ICI, 1980), and rats exposed to ≥66 ppm for 15–17 weeks in a two-generation reproductive study (Tyl and Neeper-Bradley, 1989), but an increase in liver weight in the absence of concomitant enzymatic and histopathologic changes was considered to be not adverse. A more recent chronic study in rats and mice (JBRC, 1995) supported the notion of liver as a target of 1,4-dichlorobenzene toxicity in mice, but identified a NOAEL of 20 ppm and a LOAEL of 75 ppm for dose-related eosinophilic changes to the olfactory epithelium in female rats and for dose-related mineralization of the testis in male mice as the most sensitive effects following chronic inhalation exposure. Changes in the nasal cavity were reported in a 76-week rat inhalation study (ICI, 1980), but were not considered to be treatment-related. Additional support for 1,4-dichlorobenzene-induced nasal effects comes from studies in humans, who reported irritation at concentrations between 50 and 80 ppm, which is in the range of the 75 ppm LOAEL identified in the JBRC bioassay (Aiso et al., 2005b; JBRC, 1995). Additional information supporting effects on the testis was not located in the evaluated literature.

At higher exposure levels (538 ppm), reproductive effects began to be seen, including reduced gestational and postnatal body weights in F_0 and/or F_1 parental females, and effects in F_1 and/or F_2 offspring on a total pup basis that included reduced numbers of live pups at birth and postnatal day 4, and decreased body weight gain in pups throughout the lactation period; at this exposure level, clinical signs, including tremors, salivation, and ocular and nasal discharges, were also noted (Tyl and Neeper-Bradley, 1989). There was no evidence that reproductive toxicity or prenatal developmental toxicity were critical effects of inhaled 1,4-dichlorobenzene in rats (Tyl and Neeper-Bradley, 1989; Hayes et al., 1985; Hodge et al., 1977).

The available animal data, therefore, identified a NOAEL of 20 ppm and a LOAEL of 75 ppm for dose-related eosinophilic changes to the olfactory epithelium in female rats and for dose-related mineralization of the testis in male mice.

5.2.3.2. *Methods of Analysis—Including Models*

BMD analysis was performed on the incidence data for both eosinophilic changes in the olfactory epithelium in female rats and for mineralization of the testis in male mice from the JBRC (1995) study. Results of the BMD analysis for the nasal lesions are presented in Section B.4, and the results for the testicular lesions are presented in Section B.5.

In the JBRC study (Aiso et al., 2005b; JBRC, 1995), rats and mice were exposed to nominal 1,4-dichlorobenzene concentrations (Conc) of 0, 20, 75 and 300 ppm for 6 hours/day, 5 days/week, for 104 weeks. In rats, the actual mean chamber concentrations were 0, 19.8, 74.8, or 298.4 ppm over the duration of the study. In mice, the actual mean chamber concentrations were 0, 19.9, 74.8,

or 298.3 ppm over the duration of the study. The actual mean chamber exposure concentrations were duration-adjusted for continuous exposure, using the following equation:

$$Conc_{[continuous]} = Conc \times 5 \text{ days}/7 \text{ days} \times 6 \text{ hours}/24 \text{ hours}$$

The resulting duration-adjusted exposure concentrations were 0, 3.5, 13.4, and 53.3 ppm for rats, and 0, 3.6, 13.4, and 53.3 ppm for mice.

5.2.3.2.1. ***Benchmark dose analysis of eosinophilic changes to the olfactory epithelium***. Human equivalent concentrations (HECs) were estimated following the EPA RfC methodology (U.S. EPA, 1994b). According to this methodology, 1,4-dichlorobenzene behaves as a category 2 gas, water soluble, with systemic and point of contact effects. For category 2 gases, HEC values are calculated using methods for category 1 gases for respiratory tract effects and category 3 methods for remote (extrarespiratory) effects. Thus, for changes to the olfactory epithelium, the HEC was calculated using equations for a category 1 gas with effects in the extrathoracic region as described by U.S. EPA (1994b) and using reference values provided in U.S. EPA (1988, 1994b) as follows:

$$HEC = \text{duration-adjusted Concentration} \times RGDR_{ET}$$

$$RGDR_{ET} = [(V_E/SA_{(ET)})_A/(V_E/SA_{(ET)})_H]$$

$$= (0.24 \text{ m}^3\text{-day}/15 \text{ cm}^2)/(20 \text{ m}^3\text{-day}/200 \text{ cm}^2)$$

$$= 0.16$$

where:

$RGDR_{ET}$ = regional gas dose ratio (extrathoracic region)
V_E = minute volume (mL/min = cm^3/min),
$SA_{(ET)}$ = surface area of the extrathoracic region (cm^2), and
A, H = subscripts denoting laboratory animal (A) and human (H).

The RGDR of 0.16 suggests that because of the physiological differences between animals and humans, effects in humans are expected to occur at an exposure concentration approximately 6-fold lower than in rats. The HECs for the chronic rat study are therefore 0, 0.56, 2.1, and 8.5 ppm. To convert ppm into mg/m^3, a conversion factor of 6.01 was used. (For 1,4-dichlorobenzene 1 ppm equals 6.01 mg/m^3 at 25 °C and 760 mm Hg). The corresponding HECs are 0, 3.4, 12.6, and 51.1 mg/m^3 for the 0, 20, 75, and 300 ppm exposure groups, respectively.

The incidence of olfactory epithelial lesions of moderate or greater severity in female rats was 27/50, 29/50, 39/50, and 47/50 for the 0, 20, 75, and 300 ppm groups, respectively.

Dose-response modeling of the incidence data was performed using dichotomous models available in the EPA BMDS (version 1.3.2; U.S. EPA, 2004a). A 10% extra risk of eosinophilic changes in the olfactory epithelium was used as the BMR. This BMR fell near the low end of the range of experimental dose levels and its selection was consistent with BMD guidance (U.S. EPA, 2000c).

With the exception of the quantal quadratic model, all models provided an adequate fit of the data (as indicated by the χ^2 goodness-of-fit statistic, $p>0.1$). The log probit model provided the best fit to the data (as indicated by the lowest AIC) and was selected to estimate the benchmark concentration (BMC) for 10% extra risk (BMC_{10}) and the lower 95% bound of the BMC (BMCL) for 10% extra risk ($BMCL_{10}$) values as 3.97 and 2.52 mg/m^3, respectively. Complete model results are provided in Section B.4.

5.2.3.2.2. ***Benchmark dose analysis for mineralization of the testes in male mice***. For changes in the testis of male mice, the HEC was calculated using the equations for a category 3 gas that apply to extrarespiratory effects requiring absorption and distribution, as described in Section 5.2.3.2.1 above and U.S. EPA (1994b). The HEC for extrarespiratory effects produced by a category 3 gas is calculated by multiplying the duration-adjusted concentration by the ratio of blood:gas partition coefficients ($H_{b/g}$) in animals and humans (U.S. EPA, 1994b). $H_{b/g}$ values were not available for 1,4-dichlorobenzene in mice and humans. In the absence of data on the blood:gas coefficients, a default value of 1 was used for the ratio of partition coefficients, implying that effects in humans are expected to occur at an exposure concentration comparable to the experimental species tested. As noted in U.S. EPA (1994b), a default value of 1 is based on an analysis of the available blood:air partition coefficient data in rats that shows that the $(H_{b/g})_A$ is greater than the $(H_{b/g})_H$ in most cases. The HEC values for the mouse were 0, 3.6, 13.4, and 53.3 ppm, corresponding to HECs of 0, 22, 81, and 320 mg/m^3 for the 0, 20, 75, and 300 ppm groups, respectively, when the conversion factor of 6.01 was used.

Incidence of the mineralization of the testes in male mice was 27/49, 35/49, 42/50, and 41/49 in the 0, 20, 75, and 300 ppm groups, respectively. Dose-response modeling of these incidence data was performed using the dichotomous models in BMDS (version 1.3.2; U.S. EPA, 2004a), with a 10% extra risk used as the BMR. None of the models provided an adequate fit of the data (based on the goodness-of-fit statistic, $p>0.1$, and visual examination of the models in the low-dose region of the curve).

Data from the high-dose group were dropped to see if improved model fits could be achieved. As assessed by the χ^2 goodness-of-fit test, all models provided adequate fits to the data set that excluded data from the high-exposure group (χ^2 goodness-of-fit p-value > 0.1). A 10% extra risk of mineralization of the testes was selected as the BMR in this revised analysis. The log

logistic model provided the best fit to the data (indicated by the lowest AIC); the BMC_{10} and $BMCL_{10}$ values using this model were 4.78 and 2.26 mg/m^3, respectively. Complete model results are provided in Appendix B.5.

A BMR of 10%, however, was associated with an exposure concentration that fell well below the experimental levels used in this study (i.e., outside the observable range); see Section B.5. To obtain a BMC within the observable range, it would have been necessary to use a BMR of approximately 40% excess risk of mineralization of the testes.

5.2.3.2.3. ***Point of departure for a 1,4-dichlorobenzene RfC***. BMD results for the two endpoints chosen from the JBRC study (Aiso et al., 2005b; JBRC, 1995) are summarized in Table 5-2. The BMC_{10} for mineralization of the testes in male mice fell below the range of experimental doses. BMD guidance (U.S. EPA, 2000c) recommends that a BMC be selected at the low end of the observable range to use as a point of departure for deriving an RfD. For this endpoint, additional analyses demonstrated that a BMC in the low end of the observable range would be associated with a BMR of 40% excess risk. Therefore, the $BMCL_{10}$ of 2.5 mg/m^3 based on eosinophilic changes to the olfactory epithelium was selected as the basis for a point of departure for the 1,4-dichlorobenzene RfC. Because the BMC_{10} for changes to the olfactory epithelium was within the range of observable data, BMD modeling using this endpoint provided better resolution of the dose-response curve in the region of the benchmark response of 10% than did mineralization of the testes. The BMD analysis of the mouse testes data set, however, provides support for the $BMCL_{10}$ of 2.5 mg/m^3 as an appropriate point of departure for the RfC.

Table 5-2. Summary of BMD analyses for 1,4-dichlorobenzene

Endpoint	Model	BMC_{10} (mg/m^3)	$BMCL_{10}$ (mg/m^3)
Eosinophilic changes to the olfactory epithelium	log probit	3.97	2.52
Mineralization of the testes in male mice	log logistic	4.78	2.26

5.2.3.3. ***RfC Derivation—Including Application of Uncertainty Factors***

The RfC was derived by dividing the $BMCL_{10}$ of 2.52 mg/m^3 by a total UF of 30—3 for interspecies extrapolation, and 10 for interindividual variability.

A threefold UF was used to account for the interspecies variability in extrapolating from rats to humans. The interspecies extrapolation factor encompasses two areas of uncertainty: pharmacokinetics and pharmacodynamics. In this assessment, the pharmacokinetic component is

addressed by the dosimetry adjustment (i.e., calculation of the HEC for time and concentration). Accordingly, only the pharmacodynamic area of uncertainty remains as a partial factor for interspecies uncertainty ($10^{0.5}$ or approximately 3).

A 10-fold UF is used to account for variation in sensitivity within human populations. As data are not available on variability within the human population with regard to sensitivity to 1,4-dichlorobenzene inhalation, a default value of 10 was applied.

A UF for database deficiencies was not needed based on a variety of suitable studies. There are chronic inhalation toxicity studies of 1,4-dichlorobenzene in rats and mice (Aiso,et al., 2005b; JBRC, 1995). Prenatal developmental toxicity of inhaled 1,4-dichlorobenzene has been sufficiently studied (Hayes et al., 1985; Hodge et al., 1977). A well-conducted 2-generation reproductive study (Tyl and Neeper-Bradley, 1989) exists.

A subchronic to chronic UF was not necessary because the point of departure was derived from a chronic study.

A UF to account for the extrapolation from a LOAEL to a NOAEL was not applied because BMD modeling was used to determine the point of departure for derivation of the 1,4-dichlorobenzene RfC.

The RfC for 1,4-dichlorobenzene was calculated as follows:

$$\begin{aligned} \text{RfC} &= \text{BMCL}_{\text{HEC}} \div \text{UF} \\ &= 2.52 \text{ mg/m}^3 \div 30 \\ &= 0.08 \text{ mg/m}^3 \end{aligned}$$

5.2.3.4. *Previous Inhalation Assessment*

The previous IRIS assessment used data from the Tyl and Neeper-Bradley (1989) rat multigeneration reproductive study and a NOAEL (HEC) of 75 mg/m^3 to derive an RfC of 0.8 mg/m^3. [The study was previously cited as the Chlorobenzene Producers Association (1986).] The critical effect in this study was increased liver weights in P1 (parental) males. A composite UF of 100 was applied to account for interspecies extrapolation (UF =3), intraspecies extrapolation (UF =10), and extrapolation from subchronic to chronic (UF = 3).

5.3. CANCER ASSESSMENT

5.3.1. 1,2-Dichlorobenzene

There are no quantitative assessments of oral or inhalation cancer risk for 1,2-dichlorobenzene. Available chronic bioassay data did not demonstrate carcinogenicity at the oral doses tested (see Section 4.2.1.1), and no chronic inhalation bioassay data were available.

5.3.2. 1,3-Dichlorobenzene

There are no quantitative assessments of oral or inhalation cancer risk for 1,3-dichlorobenzene. No carcinogenicity data by either route of administration were available.

5.3.3. 1,4-Dichlorobenzene

5.3.3.1. *Oral Exposure*

5.3.3.1.1. ***Choice of study/data with rationale and justification***. Oral cancer bioassays for 1,4-dichlorobenzene were performed in male and female rats and mice by NTP (1987). The rat study found no tumor increases in females but, in males, found a significant increase in the incidence of renal tubular adenomas or adenocarcinomas associated with male rat-specific hyaline droplet (α_{2u}-globulin) nephropathy which is not considered to be relevant to carcinogenicity in humans (U.S. EPA, 1991b). The mouse study found that hepatocellular adenoma, hepatocellular carcinoma, and combined hepatocellular adenoma or carcinoma occurred with positive dose-related trends in both male and female mice, with the incidences in the low-dose males and high-dose groups of both sexes being significantly greater than those in the control groups. In addition, four cases of hepatoblastoma, an extremely rare type of hepatocellular carcinoma, were observed in the high-dose male mice. Based on the increased incidences of hepatocellular neoplasms, NTP concluded that there was clear evidence of carcinogenicity in male and female $B6C3F_1$ mice. This study was used for dose-response analysis of oral exposure.

5.3.3.1.2. ***Dose-response data***. Data on the combined incidence of hepatocellular adenoma or carcinoma in male and female mice from the NTP (1987) study were used for dose-response assessment. These data are shown in Table 5-3. Hepatocellular adenomas and carcinomas are generally considered together because hepatocellular adenomas develop from the same cell lines and can progress to carcinomas. Also, adenomas are often distinguished from carcinomas only on the basis of size, and histopathologic decision criteria may vary between laboratories or over time.

Animals dying before the first appearance of liver tumors during year one of exposure in any group of that sex were censored from the group totals when figuring the denominators. This

adjustment was made so that the denominators included only those animals at risk for developing tumors.

Table 5-3. Tumor incidence data used for dose-response assessment for 1,4-dichlorobenzene

Species/strain/sex	Tumor type and location	0[a] (mg/kg-day)	214[a] (mg/kg-day)	429[a] (mg/kg-day)
Mouse, $B6C3F_1$, male	Hepatocellular adenoma or carcinoma	17/46	22/40	40/42
Mouse, $B6C3F_1$, female	Hepatocellular adenoma or carcinoma	15/48	10/46	36/48

[a] Doses are average daily doses in the gavage study adjusted by 5/7 for a 5 day/week dosing schedule. Denominators were adjusted for early mortality.

Source: NTP, 1987.

5.3.3.1.3. ***Dose conversion***. Because the exposure of mice to 1,4-dichlorobenzene was continuous for the approximate full life span of the animals, no adjustment was needed to account for duration of exposure or duration of study. However, in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a $BW^{3/4}$ scaling factor was used to convert the doses in the animal study to human equivalent doses (HED) to be used for modeling. This conversion assumes that doses (mg/day) in animals and humans are toxicologically equivalent when scaled by body weight raised to the 3/4 power (U.S. EPA, 1992). This may be expressed as follows:

$$\text{HED (mg/kg-day)} = \text{Dose in animals (mg/kg-day)} \times (BW_a / BW_h)^{0.25}$$

where:

HED = human equivalent dose
Dose = average daily dose in animal study
BW_a = animal body weight (kg)
BW_h = reference human body weight (70 kg)

The group mean body weights for animals in each exposure group were used in this equation and the body weight of humans was assumed to be 70 kg (U.S. EPA, 1988). Growth in treated male and female mice was similar to the respective controls. Therefore, time-weighted average body weights in the controls were used to represent animal body weights in the above equation (0.040 kg for males and 0.032 kg for females). The animal doses and corresponding HEDs are shown in Table 5-4.

Table 5-4. HEDs corresponding to average daily doses using a body weight scaling factor and time-weighted average body weights for male and female mice

Dose (mg/kg-day)	0	214	429
HED for use with male incidence data (mg/kg-day)	0	33	66
HED for use with female incidence data (mg/kg-day)	0	31	63

Source: NTP (1987)

5.3.3.1.4. ***Extrapolation method(s)***. Following U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, a linear approach to low-dose extrapolation is taken for agents where the MOA is uncertain, such as 1,4-dichlorobenzene. According to these guidelines, "when the weight-of-evidence evaluation of all available data is insufficient to establish the MOA for a tumor site, and when scientifically plausible based on the available data, linear extrapolation is used as a default approach. . ." As discussed in Section 4.4.1, available evidence indicates that the mechanism leading to the formation of liver tumors in mice following 1,4-dichlorobenzene ingestion is based on sustained mitogenic stimulation and proliferation of hepatocytes, possibly in response to threshold cytotoxicity. The evidence is incomplete, however, as the mitogenic effects of 1,4-dichlorobenzene are not sustained throughout long-term exposure, and similar mitogenic effects are found in the livers of rats, which do not develop liver tumors following 1,4-dichlorobenzene exposure. For the derivation of a quantitative estimate of cancer risk for ingested 1,4-dichlorobenzene, it is therefore appropriate to perform linear extrapolation to determine the cancer slope factor.

The multistage model has been used by EPA in the vast majority of quantitative cancer assessments because it is thought to reflect the multistage carcinogenic process, and it fits a broad array of dose-response patterns. Occasionally the multistage model does not fit the available data, in which case an alternate model should be considered. Use of this decision scheme has contributed to greater consistency among cancer risk assessments.

Consequently, the multistage model was the primary tool considered for fitting the dose-response data, and is given by:

$$P(d) = 1 - exp(-q_0 - q_1 \times d - q_2 \times d^2 - \ldots - q_6 \times d^6)$$

where:

d = exposure level, and

q_i = parameters estimated in fitting the model.

The multistage model in BMDS (version 1.3.2; U.S. EPA, 2004a) was used for all multistage model fits.

A low-dose linear extrapolation approach results in calculation of an oral slope factor that describes the cancer risk per unit dose of the chemical at low doses. The multistage model was fitted to each data set, and a BMD and the 95% lower bound on the BMD (BMDL) were calculated. A slope factor was calculated by linear extrapolation from the BMDL.

5.3.3.1.5. ***Oral slope factor***. The HEDs presented in Table 5-4 were used as the measure of exposure. The results of the BMD modeling and linear analyses are shown in Table 5-5. Background tumor incidence was estimated in the model, and calculations were based on extra risk. For the male mouse data, the lower 95% bound of the BMD_{10} [$BMDL_{10}$] was well below the range of observed data. Consequently, that cancer slope factor was based on a point of departure more consistent with the lower end of the range of observed data, in this case the $BMDL_{50}$. The $BMDL_{50}$ and cancer slope factor were 30.3 mg/kg-day and 1.7×10^{-2} $(mg/kg\text{-}day)^{-1}$, respectively, based on the combined incidences of hepatocellular adenomas or carcinomas in male $B6C3F_1$ mice. The $BMDL_{10}$ and cancer slope factor based on the female data were 25.0 mg/kg-day and 4.0×10^{-3} $(mg/kg\text{-}day)^{-1}$, respectively. More complete model results are given in Appendix B6.

Table 5-5. Cancer slope factor based on combined hepatocellular adenoma or carcinoma in male and female $B6C3F_1$ mice (NTP, 1987)

Species/sex	Benchmark response	BMD[a] (mg/kg-day)	BMDL[a,b] (mg/kg-day)	Slope factor[c] $(mg/kg\text{-}day)^{-1}$
Mouse, male	50%	37.1	30.3	1.7×10^{-2}
Mouse, female	10%	35.4	25	4.0×10^{-3}

[a] BMDs, calculated using BMDS version 1.3.2, associated with a 10% (female mouse) or 50% (male mouse) extra risk.
[b] BMDL = 95% lower confidence limit on the BMD.
[c] Cancer slope factor calculated by dividing the risk at the point of departure by the BMDL at the point of departure.

The value of 1.7×10^{-2} $(mg/kg\text{-}day)^{-1}$ was chosen as the cancer slope factor for 1,4-dichlorobenzene because, of the two data sets modeled, it is based on a clearer dose-response pattern (i.e., the female mouse data were not monotonously increasing). Note that this slope factor should not be used with exposures greater than the point of departure (30 mg/kg-day), because

above this level the fitted dose-response model better characterizes what is known about 1,4-dichlorobenzene oral carcinogenicity.

5.3.3.2. Inhalation Exposure

5.3.3.2.1. ***Choice of study/data with rationale and justification.*** No studies of the carcinogenic effects of inhaled 1,4-dichlorobenzene in humans are available. Only one adequate study of the carcinogenic effects of inhaled 1,4-dichlorobenzene in animals exists. The JBRC (Aiso et al., 2005b; JBRC, 1995) study evaluated both sexes of rats and mice for carcinogenic response following lifetime exposure to 1,4-dichlorobenzene. In this study, groups of 50 rats and 50 mice were exposed to 0, 20, 75, or 300 ppm 1,4-dichlorobenzene for 6 hours/day, 5 days/week for 104 weeks, and increases in the formation of different tumors were reported for male and female mice, respectively. Earlier inhalation bioassays (ICI, 1980; Riley et al., 1980) had not found tumor increases in exposed rats or mice, but both studies were inadequate because of failure to reach the MTD, less-than-lifetime exposure durations, and short observation periods. Therefore, the study by the JBRC was selected as the principal study for dose-response analysis.

5.3.3.2.2. ***Dose-response data***. Inhalation exposure of mice to 1,4-dichlorobenzene resulted in hepatocellular tumors in male and female mice, histiocytic sarcomas in male mice, and bronchoalveolar adenomas/carcinomas in female mice. The incidence data are presented in Table 5-6.

Table 5-6. Incidence data for tumors in mice exposed by inhalation

Tumor type	Incidence of tumors (%)			
	Control	20 ppm	75 ppm	300 ppm
Male mice				
Hepatocellular adenoma	13/49 (27%)	9/49 (18%)	7/50 (14%)	13/49 (26%)
Hepatocellular carcinoma	12/49[a] (24%)	17/49 (34%)	16/50 (32%)	38/49 (76%)
Hepatocellular adenoma or carcinoma	20/49[a] (40%)	21/49 (42%)	18/50 (36%)	41/49 (82%)
Hepatic histiocytic sarcoma	0/49[a] (0%)	3/49 (6%)	1/50 (2%)	6/49 (12%)
Female mice				
Hepatocellular carcinoma	2/50[a] (4%)	4/50 (8%)	2/49 (4%)	41/50 (82%)
Hepatocellular adenoma	2/50[a] (4%)	10/50 (20%)	6/49 (12%)	20/50 (40%)
Hepatocellular adenoma or carcinoma	4/50[a] (8%)	13/50 (26%)	7/49 (14%)	45/50 (90%)
Bronchoalveolar adenoma or carcinoma	1/50[a] (2%)	4/50 (8%)	2/49 (4%)	7/50 (14%)

[a] Significant positive linear trend by Peto test.
Source: Aiso et al., 2005b; JBRC, 1995.

Hepatocellular adenomas and carcinomas are generally considered together, because hepatocellular adenomas develop from the same cell lines as carcinomas and can progress to carcinomas. Also, adenomas are often distinguished from carcinomas only on the basis of size, and histopathologic decision criteria may vary between laboratories or over time. In the JBRC study (Aiso et al., 2005b; JBRC, 1995) there was a statistically significant increasing trend for hepatocellular adenomas or carcinomas in male mice, but there did not appear to be a statistically significant increase in hepatocellular adenomas alone. Therefore, to allow for the possibility that administration of 1,4-dichlorobenzene increased the incidence of hepatocellular carcinomas, but not adenomas, carcinomas were modeled separately. The incidence of adenomas or carcinomas in male mice was also considered, because the NTP (1987) oral bioassay had not demonstrated an increase only in carcinomas for the male mice (see Section 5.3.3.1), and the data base was insufficient to clarify the actual MOA.

The incidence of hepatic histiocytic sarcomas in male mice were also considered. The available bioassay data did not clarify whether these tumors occurred in mice observed with hepatocellular adenomas or carcinomas. Under the assumption that sarcomas occur via a different mechanism than adenomas or carcinomas, they were analyzed separately.

There were statistically significant, increasing trends for bronchioalveolar adenomas, carcinomas, and adenomas plus carcinomas in female mice. Consistent with the discussion above, only the incidence of adenomas or carcinomas was considered for dose-response analysis. The increasing trend in incidence of bronchoalveolar adenomas or carcinomas was similar to that of the sarcomas in the male mice, and was also analyzed separately.

5.3.3.2.3. ***Dose conversion***. For the dose-response analysis of liver tumors, administered concentrations of 1,4-dichlorobenzene were duration-adjusted as described above in Section 5.2.3.2, resulting in mean continuous exposure levels of 0, 3.6, 13.4, and 53.3 ppm. Human equivalent exposures (HECs) were estimated following the U.S. EPA RfC methodology (U.S. EPA, 1994b). According to this methodology, 1,4-dichlorobenzene behaves as a category 2 gas, water soluble, with systemic and point of contact effects. For category 2 gases, HEC values are calculated using methods for category 1 gases for respiratory tract effects and category 3 methods for remote (extrarespiratory) effects. Thus, for liver tumors, the HEC was then calculated using the equations for a category 3 gas with extrarespiratory effects (U.S. EPA, 1994b), which calls for multiplying the duration-adjusted concentration by the ratio of blood:gas partition coefficients ($H_{b/g}$) in animals and humans. Because $H_{b/g}$ values were not available for 1,4-dichlorobenzene in mice and humans, a default value of 1 was used for the ratio of partition coefficients, implying that effects in humans will occur at an exposure concentration comparable to the experimental species tested. The HEC values for the mouse were therefore 0, 3.6, 13.4, and 53.3 ppm, corresponding to 0, 22, 81, and 320 mg/m^3, for the 0, 20, 75, and 300 ppm groups, respectively (1 ppm = 6.01 mg/m^3). The average of the two lower exposures was 8.5 ppm, or 51.5 mg/m^3.

For bronchoalveolar tumors in female mice, HECs were calculated using the rules for a category 1 gas that produces effects in the pulmonary region[2] (U.S. EPA, 1994b). The HECs were calculated using equations described in U.S. EPA (1994b), and using reference values provided in U.S. EPA (1988, 1994b):

---

[2] The dosimetric adjustment for mouse bronchoalveolar tumors assumed that the pulmonary region of the lungs was the most appropriate target of toxicity and thus the surface area of the pulmonary region of the lung was used to calculate the RGDR. It could also be argued that the appropriate target for bronchoalveolar tumors is the thoracic region of the respiratory tract, which includes both the tracheobronchial and pulmonary regions of the lung. The standard defaults surface areas of the thoracic region are 0.05035 m^2 and 54.32 m^2 for mice and humans, respectively (U.S. EPA, 1994b). Use of these surface areas would give a RGDR of 3.4, the same value calculated using the pulmonary region alone. Therefore, use of the species-specific surface areas of the thoracic rather than pulmonary region would have no impact on the estimated inhalation unit risk for 1,4-dichlorobenzenes.

HEC = duration-adjusted concentration × $RGDR_{(PU)}$

$RGDR_{(PU)}$ = $[(V_E/SA_{(PU)})_A/(V_E/SA_{(PU)})_H]$

= (0.041 L/min/0.05 m^2)/(13 L/min/54 m^2)

= 3.4

where:

$RGDR_{(PU)}$ = regional gas dose ratio (pulmonary region)
V_E = minute volume (mL/min = cm^3/min)
$SA_{(pu)}$ = surface area of the pulmonary region (cm^2), and
A, H = subscripts denoting laboratory animal and human, respectively.

This RGDR suggests that effects in humans are expected to occur at an exposure concentration approximately 3-fold higher than in mice. The HEC values for the female mouse lung tumors were therefore 0, 12.2, 45.6, and 181 ppm for the 0, 20, 75, and 300 ppm groups, respectively. These HEC values correspond to 0, 74.8, 275, and 1090 mg/m^3 (based on 1 ppm = 6.01 mg/m^3).

5.3.3.2.4. ***Extrapolation method(s)***. Following U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, a linear approach to low-dose extrapolation is used for agents that are not DNA-reactive and for which a plausible MOA is not established, such as 1,4-dichlorobenzene. As discussed above, available evidence indicates that the mechanism leading to the formation of liver tumors in mice following 1,4-dichlorobenzene ingestion is based on sustained mitogenic stimulation and proliferation of hepatocytes, possibly in response to threshold cytotoxicity. The evidence is incomplete, however, as the mitogenic effects of 1,4-dichlorobenzene are not sustained throughout long-term exposure, and similar mitogenic effects are found in the livers of rats, which do not develop liver tumors following 1,4-dichlorobenzene exposure. While much of this mechanistic information comes from studies of oral exposure, the MOA for hepatic carcinogenesis is likely to be similar following inhalation exposure.

As discussed in Section 5.3.3.1.4, the multistage model in BMDS (version 1.3.2; U.S. EPA, 2004a) has been used in the vast majority of quantitative cancer assessments because it is thought to reflect the multistage carcinogenic process, it fits a broad array of dose-response patterns, and it contributes to greater consistency among cancer risk assessments.

A low-dose linear extrapolation approach resulted in calculation of an inhalation unit risk that describes the cancer risk per unit dose of the chemical at low doses. The multistage model was fitted to each data set, and a 10% response value (BMC_{10}) and its 95% lower bound ($BMCL_{10}$) were calculated for each tumor type. A unit risk was calculated by linear extrapolation from the BMCL.

5.3.3.2.5. ***Inhalation unit risk***. The results of the dose-response analysis of the mouse tumor data are summarized in Section B.7, Table B-14. For male mice, the unit risks resulting from modeling hepatocellular carcinomas only, and from modeling adenomas or carcinomas, differed by approximately twofold, with the unit risk for carcinomas only the higher one at 4.5×10^{-3} $(mg/m^3)^{-1}$. The unit risk resulting from modeling the histiocytic sarcoma data was 7.8×10^{-4} $(mg/m^3)^{-1}$, about sixfold lower than the estimated risk for carcinomas only.

In order to gain some understanding of the total risk from multiple tumor sites in male mice, a sum of risks across tumor sites was considered. This combined risk does not constitute double-counting if it can be assumed that the hepatic adenomas and carcinomas were mechanistically independent from the sarcomas. If there is some dependence between the tumor types, then the combined risk would tend to be an overestimate of the total risk.

A statistically appropriate approach was used to sum the maximum likelihood estimates (MLE) of unit potency across these tumor sites for male mice in the JBRC study (Aiso et al., 2005b; JBRC, 1995), assuming independence of the tumor sites[3]. The resulting upper bound on the summed risks was less than 10% higher than the risk estimated from carcinomas alone. Consequently, adding the risks did not impact the unit risk estimate significantly in this instance.

For female mice, the unit risk estimated from the incidence of adenomas or carcinomas, 4.4×10^{-3} $(mg/m^3)^{-1}$, was very similar to that estimated from male mouse carcinomas. The unit risk corresponding to bronchoalveolar adenomas and carcinomas was the lowest unit risk estimated, at 1.9×10^{-4} $(mg/m^3)^{-1}$. As with the male mouse tumors, summing the risks from these two sites did not change the estimated risk substantially, as the final unit risk is routinely rounded to one significant digit.

---

[3] An estimate of the 95% upper bound on the summed unit risk, corresponding to the region of 10^{-6} extra risk in the two dose-response curves, was calculated by assuming a normal distribution for the individual risk estimates and deriving the variance of the risk estimate for each tumor site from its 95% upper confidence limit (UCL) as defined by the formula

$$95\%\ \text{UCL} = \text{MLE} + 1.645 \times \text{standard error (MLE)}, \quad (1)$$

(where 1.645 is the z-statistic corresponding to a one-sided 95% CI). The standard error is given by

$$\text{standard error (MLE)} = (95\%\ \text{UCL} - \text{MLE})/1.645, \quad (2)$$

then squared to obtain the variance of each MLE. The variances of the MLEs for the two tumor sites were summed to obtain the variance of the sum of the MLEs. Then the standard error of the summed risk was obtained by taking the square root of the variance. The 95% UCL on the sum of the MLEs was then calculated using equation (1) above, substituting the sum of the MLEs for MLE.

The recommended inhalation unit risk for 1,4-dichlorobenzene is $4x10^{-3}$ $(mg/m^3)^{-1}$, based on hepatocellular tumors in male and female mice. Note that this unit risk should not be used with exposures exceeding the point of departure (23 mg/m^3), because above this level the fitted dose-response model better characterizes what is known about 1,4-dichlorobenzene inhalation carcinogenicity.

5.3.4. Sources of Uncertainty

As in most risk assessments, extrapolation of study data to estimate potential risks to human populations from exposure to 1,4-dichlorobenzene has engendered some uncertainty in the results. The uncertainty falls in two major categories, model uncertainty and parameter uncertainty. Model uncertainty "refers to a lack of knowledge needed to determine which is the correct scientific theory on which to base a model," while parameter uncertainty "refers to a lack of knowledge about the values of a model's parameters" (U.S. EPA, 2005a).

Characterization of the full extent of model uncertainty would involve consideration of other models which might better accommodate plausible modes of action. The present assessment used one dose-response model, the multistage model, to characterize the potential risk to human populations from 1,4-dichlorobenzene exposure because of the lack of information supporting another model. In this case there was uncertainty concerning the mode(s) of action. Sufficient information to support a nonlinear low-dose extrapolation was not available, however. Even so, there was some uncertainty regarding whether linear low dose extrapolation provided a reasonable estimate of low dose risk.

Parameter uncertainty can be assessed through confidence intervals and probabilistic analysis. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. Uncertainty in the animal dose-response data can usually be assessed through the ratio of benchmark doses to their lower bounds. For the oral slope factor, this ratio was less than two. For the inhalation unit risk, however, while the model fit passed through the center of the dose-response data for each tumor site, the data were more uncertain than the model fits suggest. As a rough assessment of the uncertainty in these data, multistage models fitted to the female mouse hepatocellular tumors, alternately omitting the low and mid-exposure groups, led to BMCs which varied fivefold and BMCLs which varied fourfold (results not shown), while the ratio between BMCs and BMCLs remained at approximately twofold. There was no information which supported ignoring either of these groups, however. Consequently the recommended unit risk includes all of the exposure groups.

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

6.1. HUMAN HAZARD POTENTIAL

6.1.1. 1,2-Dichlorobenzene

1,2-Dichlorobenzene is used in the production of 3,4-dichloroaniline, a base material for herbicides, and as an insecticide for termites and locust borers. It is also used as a solvent for waxes, gums, resins, tars, rubbers, oils, and asphalts; as a degreasing agent for metals, leather, paper, dry-cleaning, bricks, upholstery, and wool; as an ingredient in metal polishes and paints; and in motor oil additive formulations.

No information is available on health effects of 1,2-dichlorobenzene in humans following oral exposure. The toxicity of 1,2-dichlorobenzene in orally exposed animals was investigated in one chronic and three subchronic studies in rats and mice, and in a developmental toxicity study in rats. The primary target organs in rodents were the liver and kidney. The chronic NTP (1985) mouse study is the most appropriate study for the development of an oral RfD for 1,2-dichlorobenzene based on statistically significant kidney effects observed at the highest dose in male mice. The critical effect was renal tubular regeneration since it was the only nonneoplastic lesion with a statistically significant, dose-related increased incidence.

Information on the toxicity of inhaled 1,2-dichlorobenzene in humans is limited to results of two industrial hygiene surveys, a workplace mortality study, and a series of case reports. The main finding is that occupational exposure caused irritation of the eyes and respiratory passages (Hollingsworth et al., 1958). Data on the toxicity following inhalation exposure in animals are available from a 14-day study of respiratory effects in mice, a multispecies subchronic study, a 2-generation reproduction study in rats, and developmental toxicity studies in rats and rabbits. The 14-day study found nasal olfactory lesions characterized by a complete loss of the olfactory epithelium (Zissu, 1995). This effect is consistent with the respiratory irritation observed in exposed workers, and occurred at concentrations below the lowest subchronic exposure levels that caused systemic effects in the other animal studies. Because the subchronic inhalation studies did not examine the respiratory tract, the critical effect associated with long-term exposures could not be identified.

No information is available on the carcinogenicity of 1,2-dichlorobenzene in humans. Data on cancer in animals are limited to results of one chronic oral bioassay in male and female rats and mice (NTP, 1985). There was no evidence of exposure-related tumorigenic responses in either species. 1,2-Dichlorobenzene could not be assessed for carcinogenicity because of the lack of

human data or evidence of exposure-related carcinogenic responses in rats and mice in bioassays that might not have been adequate tests of carcinogenicity because of uncertainty as to whether the MTD was reached. Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the available carcinogenicity data for 1,2-dichlorobenzene provide *inadequate information to assess carcinogenic potential*.

6.1.2. 1,3-Dichlorobenzene

1,3-Dichlorobenzene is used in the production of herbicides, insecticides, pharmaceuticals and dyes. No information is available on effects of oral or inhalation exposure to 1,3-dichlorobenzene in humans, and no inhalation toxicity studies of 1,3-dichlorobenzene have been performed in animals.

Information on the toxicity of ingested 1,3-dichlorobenzene in animals is limited to findings from one subchronic toxicity study in rats (McCauley et al., 1995) and a developmental toxicity study in rats that has only been reported in abstract form (Ruddick et al., 1983). Based on the subchronic data, the thyroid and pituitary were identified as targets of repeated oral exposures to 1,3-dichlorobenzene in the rat.

No information is available regarding the carcinogenicity of 1,3-dichlorobenzene in humans or animals. In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the lack of human and animal carcinogenicity data for 1,3-dichlorobenzene constitute *inadequate information to assess carcinogenic potential.*

6.1.3. 1,4-Dichlorobenzene

1,4-Dichlorobenzene is used as an air freshener, as a moth repellent in moth balls or crystals, and in other pesticide applications. 1,4-Dichlorobenzene is also used in the manufacture of 2,5-dichloroaniline, pharmaceuticals, and polyphenylene sulfide resins, and in the control of mildew.

Information on the toxicity of 1,4-dichlorobenzene in humans is limited to the results of a workplace health survey and a few case reports. Occupational observations indicate that 1,4-dichlorobenzene is irritating to the eyes and nose. Case reports of people who ingested or inhaled 1,4-dichlorobenzene suggest that the liver, nervous, and hematopoietic systems are targets of toxicity in humans. The limited information on these systemic effects in humans is consistent with findings in exposed animals.

Effects of oral exposure to 1,4-dichlorobenzene in animals were investigated in a number of subchronic, chronic, reproductive and developmental toxicity studies conducted predominantly in rats and mice. Liver and kidney effects represented the best studied and most consistently observed

systemic findings. A limited amount of data indicates that 1,4-dichlorobenzene can affect the hematological system and adrenal and thyroid glands at oral doses equal to or higher than those causing liver and kidney effects. A two-generation reproductive and developmental study in rats (Bornatowicz et al., 1994) found that oral exposure to 1,4-dichlorobenzene caused toxicity in the F_1 and F_2 pups, including decreased birth weight and neonatal survival. Among all the observed effects, the liver was identified as the most sensitive endpoint for oral exposure to 1,4-dichlorobenzene in a beagle dog study conducted by Monsanto Company (1996). In this study, dogs (5 per sex per dose group) were administered 1,4-dichlorobenzene (99.9% pure) in gelatin capsules 5 days/week at initial dose levels of 0, 10, 50, or 150 mg/kg-day (adjusted doses: 0, 7, 36, or 54 mg/kg-day) for 1 year (Monsanto Company, 1996).

The inhalation toxicity of 1,4-dichlorobenzene in animals was evaluated in several studies involving subchronic, chronic, gestational, and multigenerational exposures, mainly in rats. In addition to the presence of effects on the nasal region of the respiratory tract, the findings showed a general pattern of increased liver weight at exposure levels below those inducing overt toxicity. Liver weight was increased in rats exposed to 500 ppm of 1,4-dichlorobenzene for 76 weeks (ICI, 1980), and rats exposed to $\geq$66 ppm for 15–17 weeks in a two-generation reproductive study (Tyl and Neeper-Bradley, 1989); however, these increases in liver weight in the absence of concomitant enzymatic and histopathologic changes were not considered to be adverse. The irritant effect of 1,4-dichlorobenzene on nasal tissues was confirmed in a chronic bioassay (Aiso et al., 2005b; JBRC, 1995). This study identified a NOAEL of 20 ppm and a LOAEL of 75 ppm for dose-related eosinophilic changes to the olfactory epithelium in female rats and for dose-related mineralization of the testis in male mice as the most sensitive effects following chronic inhalation exposure.

Oral cancer bioassays were conducted in male and female rats and mice that were chronically exposed to 1,4-dichlorobenzene (NTP, 1987). The rat study found no increased tumor incidence in females, but an increase in the incidence of renal tubular adenomas or adenocarcinomas in males. These renal tumors were associated with male rat-specific hyaline droplet (α_{2u}-globulin) nephropathy, and are not considered relevant to carcinogenicity in humans. The mouse study (NTP, 1987) showed an increased incidence of hepatocellular neoplasms in both sexes; NTP concluded that there was clear evidence of carcinogenicity in this species following oral exposure. An increase in liver tumors in male and female mice, but not in either sex of rats, was also reported in a lifetime inhalation study from the Japanese literature (Aiso et al., 2005b; JBRC, 1995). Other inhalation bioassays of 1,4-dichlorobenzene did not find tumor increases in exposed rats or mice, but these were not adequate studies because they failed to reach the MTD, had less-than-lifetime exposure durations, and short observation periods. The kidney tumors in rats following oral exposure are not relevant to humans because the mechanism is specific to male rats

(α_{2u}-globulin); the mechanistic basis of the mouse liver tumors has not been adequately defined, but is believed to involve a MOA involving sustained cell proliferation and/or decreased apoptosis.

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 1,4-dichlorobenzene is considered *likely to be carcinogenic to humans* by both the oral and inhalation routes.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

6.2.1.1. *1,2-Dichlorobenzene*

A BMD approach was used to derive a RfD of 0.03 mg/kg-day for 1,2-dichlorobenzene based on data for tubular regeneration in the kidneys of male mice from the 103-week NTP (1985) bioassay. A $BMDL_{10}$ of 29.8 mg/kg-day served as the point of departure for the RfD. The RfD was derived by dividing the $BMDL_{10}$ by a total UF of 1000—10 for interspecies extrapolation, 10 for interindividual variability, and 10 for database deficiencies.

6.2.1.2. *1,3-Dichlorobenzene*

The BMD analysis was performed using data for reduced follicular colloidal density of the thyroid of male rats from the 90-day bioassay by McCauley et al. (1995) (data presented in Section B.2, Table B-3). All dichotomous models in the EPA BMDS (version 1.3.2; U.S. EPA, 2004a) were fitted to these incidence data using a BMR of 10% extra risk. The χ^2 goodness-of-fit statistics for all models indicated poor statistical fits ($p<0.1$). Data from the highest dose group (588 mg/kg-day) were dropped to see if an improved model fit could be achieved. The χ^2 goodness-of-fit statistic for all dichotomous models indicated inadequate statistical fits ($p<0.1$) using the control and first three dose groups. Data from the two highest dose groups (147 and 588 mg/kg-day) were dropped to see if adequate model fits could be achieved (i.e., models were run using data for the control, 9 mg/kg-day, and 37 mg/kg-day dose groups only). The resulting dose associated with a BMR of 10% extra risk was well below the range of experimental doses. As such, BMD modeling provided little resolution of the dose-response curve in the region of the benchmark response. Accordingly, for thyroid data from the McCauley et al. (1995) study, the conclusion was reached that the data are not suitable for BMD modeling.

Consideration was also given to the use of the NOAEL/LOAEL method for the derivation of an oral RfD for 1,3-dichlorobenzene. For this derivation the LOAEL of 9 mg/kg-day from the McCauley et al. (1995) study would be divided by a composite UF of 30,000 involving five areas of uncertainty (3 for interspecies variability, 10 for interindividual variability, 10 for extrapolation from subchronic to chronic exposure, 10 for database deficiencies, and 10 for extrapolation from

LOAEL to NOAEL). Consistent with the recommendations of the RfD/RfC Technical Panel (U.S. EPA, 2002), an RfD was not derived in light of this composite UF. The RfD/RfC Technical Panel concluded that, in cases where maximum uncertainty exists in four or more areas of extrapolation, it is unlikely that the database is sufficient to derive a reference value, and further recommended that no reference value for any particular chemical substance be derived if the composite UF is greater than 3,000.

In summary, data were considered inadequate for derivation of a RfD for 1,3-dichlorobenzene.

6.2.1.3. *1,4-Dichlorobenzene*

An RfD of 0.03 mg/kg-day was based on a $BMDL_{10}$ of 9.06 mg/kg-day for liver lesions (diffuse hepatocellular hypertrophy) in a 1-year chronic toxicity study in dogs exposed to 1,4-dichlorobenzene (Monsanto Company, 1996). The BMDL was calculated using a BMR of 10% extra risk. The RfD was derived by dividing the $BMDL_{10}$ by a total UF of 300—10 for interspecies variability, 10 for interindividual variability, and 3 for database deficiencies.

6.2.2. Noncancer/Inhalation

6.2.2.1. *1,2-Dichlorobenzene*

An RfC was not calculated for 1,2-dichlorobenzene because of inadequate data on effects of long-term exposure. A 14-day study (Zissu, 1995) showed that the upper respiratory tract was a sensitive target for inhalation exposure to 1,2-dichlorobenzene, as serious nasal olfactory lesions occurred in mice at concentrations below the lowest exposure levels that caused systemic effects in subchronic studies. The available subchronic inhalation studies did not evaluate the respiratory tract, indicating that a critical effect for long-term exposures to 1,2-dichlorobenzene cannot be identified. In the absence of an identifiable critical effect, derivation of an RfC for 1,2-dichlorobenzene is precluded.

6.2.2.2. *1,3-Dichlorobenzene*

No information is available on the systemic, reproductive, or developmental toxicity of inhaled 1,3-dichlorobenzene in humans or animals, indicating that the existing inhalation database is inadequate to support the derivation of an RfC for this isomer. It is not feasible to derive an RfC from oral data on 1,3-dichlorobenzene because little is known about the mechanism responsible for long-term oral toxicity of 1,3-dichlorobenzene. The available evidence suggests that hepatic metabolism to a reactive intermediate may be of considerable importance in toxicity, and since the extent of hepatic metabolism is likely to vary dramatically between oral and inhalation exposure, a

route-to-route extrapolation from the oral data is precluded. Derivation of an RfC for 1,3-dichlorobenzene by analogy to 1,2- or 1,4-dichlorobenzene is not feasible because data are inadequate for the derivation of an RfC for 1,2-dichlorobenzene, and available oral data strongly suggest that 1,4-dichlorobenzene is less toxic than either of the other two isomers, and that target sites may vary between the isomers.

6.2.2.3. *1,4-Dichlorobenzene*

An RfC of 0.08 mg/m^3 was derived, based on a $BMCL_{10\ (HEC)}$ of 2.52 mg/m^3 for increases in eosinophilic changes in the olfactory epithelium of female rats (Aiso et al., 2005b; JBRC, 1995). The BMCL was calculated using a using a 10% increase in the incidence of the olfactory lesion as the BMR. The RfC was derived by dividing the $BMCL_{10\ (HEC)}$ of 2.52 mg/m^3 by a total UF of 30—3 for interspecies variability and 10 for interindividual variability.

6.2.3. Cancer/Oral and Inhalation

6.2.3.1. *1,2-Dichlorobenzene*

There are no quantitative assessments of oral or inhalation cancer risk for 1,2-dichlorobenzene. Available chronic bioassay data did not demonstrate carcinogenicity at the oral doses tested (see Section 4.2.1.1), and no chronic inhalation bioassay data were available.

6.2.3.2. *1,3-Dichlorobenzene*

No data are available concerning the carcinogenicity of 1,3-dichlorobenzene, precluding quantitative assessment of oral or inhalation cancer risks for this isomer.

6.2.3.3. *1,4-Dichlorobenzene*

Consistent with U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, a linear approach to dose-response assessment was applied for agents, such as 1,4-dichlorobenzene, where the MOA is uncertain. According to these guidelines, "when the weight-of-evidence evaluation of all available data is insufficient to establish the MOA for a tumor site, and when scientifically plausible based on the available data, linear extrapolation is used as a default approach, ..." As discussed in Section 4.4.1, available evidence indicates that the mechanism leading to the formation of liver tumors in mice following 1,4-dichlorobenzene ingestion is based on sustained mitogenic stimulation and proliferation of hepatocytes, possibly in response to threshold cytotoxicity. The evidence is incomplete, however, as the mitogenic effects of 1,4-dichlorobenzene are not sustained throughout long-term exposure, and similar mitogenic effects are found in the livers of rats, which do not develop liver tumors following 1,4-dichlorobenzene exposure. For the derivation of a

quantitative estimate of cancer risk from1,4-dichlorobenzene exposure, it is therefore appropriate to perform linear extrapolation to determine the cancer slope factor.

Data showing an increased incidence of hepatocellular adenomas and carcinomas in mice from the two-year oral NTP (1987) bioassay were used for the oral cancer dose-response analysis. A multistage model with linear extrapolation from the point of departure was used to derive an oral slope factor of 2×10^{-2} $(mg/kg\text{-}day)^{-1}$.

In a two-year bioassay in mice and rats (Aiso et al., 2005b; JBRC, 1995), inhalation exposure of mice resulted in hepatocellular tumors (males and females), histiocytic sarcomas (males), and bronchoalveolar adenomas/carcinomas (females). A multistage model with linear extrapolation from the point of departure was used to derive an inhalation unit risk of 4×10^{-3} $(mg/m^3)^{-1}$ based on data for hepatocellular carcinomas in male mice and hepatocellular adenomas and carcinomas combined in female mice.

7. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2002) Documentation of the Threshold Limit Values and Biological Exposure Indices. 7th edition, supplement. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Aiso, S; et al. (2005a) Thirteen-week inhalation toxicity of p-dichlorobenzene in mice and rats. J Occup Health 47:249-260.

Aiso, S; et al. (2005b) Carcinogenicity and chronic toxicity in mice and rats exposed by inhalation to para-dichlorobenzene for two years. J Vet Med Sci 67:1019-1029.

Andersen, BN. (1985) Species variation in the tyrosine sulfation of mammalian gastrins. Gen Comp Endocrinol 58:44-50.

Anderson, D; Hodge, MCE. (1976) Paradichlorobenzene: dominant lethal study in the mouse. Central Toxicology Laboratory, Imperial Chemical Industries Limited, Alderly Park, Macclesfield, Cheshire, UK; ICI Report No. CTL/P/296.

Anderson, BE; Zeiger, E; Shelby, MD; et al. (1990) Chromosome aberration and sister chromatid exchange test results with 42 chemicals. Environ Mol Mutagen 16:55-137.

Azouz, WM; Parke, DV; Williams, RT. (1954) The metabolism of dichlorobenzenes. Process Biochem. 57:xii.

Azouz, WM; Parke, DV; Williams, RT. (1955) Studies in detoxication. 62. The metabolism of halogenobenzenes; ortho- and para-dichlorobenzenes. Biochem J 59: 410-415.

Barter, JA; Sherman, JH. (1999) An evaluation of the carcinogenic hazard of 1,4-dichlorobenzene based on internationally recognized criteria. Reg Toxicol Pharmacol 29:64-79.

Bio/dynamics. (1989) An inhalation two-generation reproduction study in rats with orthodichlorobenzene. Chemical Manufacturers Association, Washington, DC; Project No. 87-3157.

Bogaards, JJP; Van Ommen, B; Wolf, CR; et al. (1995) Human cytochrome P450 in the oxidation of chlorinated benzenes. Toxicol Appl Pharmacol 132:44-52.

Bomhard, E; Luckhaus, G; Voigt, W-H; et al. (1988) Induction of light hydrocarbon nephropathy by *p*-dichlorobenzene. Arch Toxicol 61:433-439.

Bomhard, E; Schmidt, U; Loeser, E. (1998) Time course of enzyme induction in liver and kidneys and absorption, distribution and elimination of 1,4-dichlorobenzene in rats. Toxicology 131:73-91.

Bornatowicz, N; Antes, A; Winker, N; et al. (1994) 2-Generationen-fertilitätsstudie mit 1,4-dichlorobenzol an ratten. wien klin wochenschr 106:345-353. (German article and English translation).

Bristol, DW; Crist, HL; Lewis, RG; et al. (1982) Chemical analysis of human blood for assessment of environmental exposure to semivolatile organochlorine chemical contaminants. J Anal Toxicol 6:269-275.

Budavari, S; ed. (1989) The Merck Index - an encyclopedia of chemicals, drugs, and biologicals. 11th edition. Whitehouse Station, NJ: Merck and Co., Inc.

Budavari, S; O'Neil, MJ; Smith, A; et al.; eds. (2001) The Merck Index - an encyclopedia of chemicals, drugs, and biologicals. 13th edition. Whitehouse Station, NJ: Merck and Co., Inc.; pp. 3081-3082.

Campbell, DM; Davidson, RJL. (1970) Toxic haemolytic anaemia in pregnancy due to a pica for paradichlorobenzene. J Obstet Gynecol 77:657-659.

Canonero, R; Campart, GB; Mattoli, F; et al. (1997) Testing of p-dichlorobenzene and hexachlorobenzene for their ability to induce DNA damage and micronucleus formation in primary cultures of rat and human hepatocytes. Mutagenesis. 12:35-39.

Carbonell, E; Puig, M; Xamena, N; et al. (1991) Sister-chromatid exchanges (SCE) induced by *p*-dichlorobenzene in cultured human lymphocytes. Mutat Res 263:57-59.

Carlson, GP. (1977) Chlorinated benzene induction of hepatic porphyria. Experientia 33:1627-1629.

Charbonneau, M; Strasser, J; Lock, EA; et al. (1989) Involvement of reversible binding to $\alpha_{2\mu}$-globulin in 1,4-dichlorobenzene-induced nephrotoxicity. Toxicol Appl Pharmacol 99:122-132.

Chiou, CT; Rutherford, DW; Manes, M. (1993) Sorption of N_2 and EGME vapors on some soils, clays, and minerals oxides and determination of sample surface areas by use of sorption data. Environ Sci Technol 27:1587-1594.

Connor, TH; Theiss, JC; Hanna, HA; et al. (1985) Genotoxicity of organic chemicals frequently found in the air of mobile homes. Toxicol Lett 25:33-40.

Cotter, LH. (1953) Paradichlorobenzene poisoning from insecticides. NY State J Med 53:1690-1692.

DeMarini, DM; Brooks, HG. (1992) Induction of prophage lambda by chlorinated organics: Detection of some single-species/single-site carcinogens. Environ Mol Mutagen 19:98-111.

den Besten, C; Ellenbroek, M; van der Ree, MA; et al. (1992) The involvement of primary and secondary metabolism in the covalent binding of 1,2- and 1,4-dichlorobenzenes. Chem Biol Interact 84:259-275.

Dietrich, DR; Swenberg, JA. (1991) NCI-Black-Reiter (NBR) male rats fail to develop renal disease following exposure to agents that induce α-2u-globulin ($\alpha_{2\mu}$) nephropathy. Fund Appl Toxicol 16:749-762.

Dow Chemical Company. (1981) Initial submission: orthodichlorobenzene. Inhalation teratology probe study in rats and rabbits (final report) with cover letter dated 10/25/91. Submitted under TSCA Section 8E; NTIS No. OTS0535228.

Dow Chemical Company. (1982) Paradichlorobenzene: Inhalation teratology probe study in rabbits. Submitted under TSCA 8D; NTIS No. OTS0206148.

Droz, PO; Wu, MM; Cumberland, WG; et al. (1989) Variability in biological monitoring of solvent exposure. I. Development of a population physiological model. Br J Ind Med 46:447-460. As cited in Hissink et al., 1997c.

Eldridge, SR; Goldsworthy, TL; Popp, JA; et al. (1992) Mitogenic stimulation of hepatocellular proliferation in rodents following 1,4-dichlorobenzene administration. Carcinogenesis 13:409-415.

Elkins, HB. (1950) The Chemistry of Industrial Toxicology. John Wiley and Sons, Inc., New York, NY. p. 147, 205-206, 221-223. As cited in Hollingsworth et al. (1958).

Fischer, R; Smith, PF; Sipes, IG; et al. (1990) Toxicity of chlorobenzenes in cultured rat liver slices. In Vitro Toxicol 3:181-194.

Fischer, RL; Hasal, SJ; Sipes, IG; et al. (1995) Comparative metabolism and toxicity of dichlorobenzenes in Sprague-Dawley, Fischer 344, and human liver slices. Hum Exp Toxicol 14:414-421.

Gargas, ML; Andersen, ME; Clewell, JH. (1986) A physiologically based simulation approach for determining metabolic constants from gas uptake data. Toxicol Appl Pharmacol 86:341-352. As cited in Hissink et al., 1997c.

Gershon, MD; Nunez, EA. (1988) The thyroid gland. In: L. Weis; ed. Cell and tissue biology: a textbook of histology. 6th edition. Baltimore, MD: Urban and Schwarzenberg; pp. 1009-1020.

Ghittori, S; Imbriani, M; Pezzagno, G; et al. (1985) Urinary elimination of p-Dichlorobenzene (p-DCB) and weighted exposure concentration. G Ital Med Lav 7:59-63.

Giavini, E; Broccia, ML; Prati, M; et al. (1986) Teratologic evaluation of *p*-dichlorobenzene in the rat. Bull Environ Contam Toxicol 37:164-168.

Girard, MMR; Tolot, F; Martin, P; et al. (1969) Hèmopathies graves et exposition à des dérivés chlorés du benzéne (á propos de 7 cas). Le Journal de Médecine de Lyon 50:771-773. As cited in IARC, 1982.

Gustafson, DL; Coulson, AL; Feng, L; et al. (1998). Use of medium-term liver focus bioassay to assess the hepatocarcinogenicity of 1,2,4,5-tetrachlorobenzene and 1,4-dichlorobenzene. Cancer Lett 129:39-44.

Gustafson, DL; Long, ME; Thomas, RS; et al. (2000) Comparative hepatocarcinogenicity of hexachlorobenzene, pentachlorobenzene, 1,2,4,5,-tetrachlorobenzene, and 1,4-dichlorobenzene: application of a medium-term liver focus bioassay and molecular and cellular indices. Toxicol Sci 53:245-252.

Hallowell, M. (1959) Acute haemolytic anaemia following the ingestion of para-dichlorobenzene. Arch Dis Child 34:74-75.

Hansch, C; Leo, A; Hoekman, D. (1995) Hydrophobic, electronic, and steric constants. In: Heller, SR; ed. Exploring QSAR. Vol 2. ACS Professional Reference Book. Washington, DC: American Chemical Society; p.17.

Haseman, JK. (1985) Issues in carcinogenicity testing: dose selection. Fundam Appl Toxicol 5:66-78.

Hasmall, SC; Roberts, RA. (1997) Hepatic ploidy, nuclearity, and distribution of DNA synthesis: a comparison of nongenotoxic hepatocarcinogenic liver mitogens. Toxicol Appl Pharmacol 144:287-293.

Hasmall, SC; Pyrah, ITG; Soames, AR; et al. (1997) Expression of the immediate-early genes, c-*fos*, c-*jun*, and c-*myc*: a comparison in rats of nongenotoxic hepatocarcinogens with noncarcinogenic liver mitogens. Fundam Appl Toxicol 40:129-137.

Hawkins, DR; Chasseaud, LF; Woodhouse, RN; et al. (1980) The distribution of excretion and biotransformation of p-dichloro[^{14}C]benzene in rats after repeated inhalation, oral and subcutaneous doses. Xenobiotica 10:81-95.

Hayes, WC; Hanley, TR; Gushow, TS; et al. (1985) Teratogenic potential of inhaled dichlorobenzenes in rats and rabbits. Fundam Appl Toxicol 5:190-202.

Hill, RH; Ashley, DL; Head, SL; et al. (1995) p-Dichlorobenzene exposure among 1000 adults in the United States. Arch Environ Health 50:277-280.

Hissink, AM; Van Ommen, B; Van Bladeren, PJ. (1996a) Dose-dependent kinetics and metabolism of 1,2-dichlorobenzene in rat: effect of pretreatment with phenobarbital. Xenobiotica 26:89-105.

Hissink, AM; Van Ommen, B; Bogaards, JJ; et al. (1996b) Hepatic epoxide concentrations during biotransformation of 1,2- and 1,4-dichlorobenzene. In: Snyder, R; Sipes, IG; Jollow, GJ; et al.; eds. Biological reactive intermediates. V. Basic mechanistic research in toxicology and human risk assessment. New York, NY: Plenum Press; pp. 129-133.

Hissink, AM; Oudshoorn, MJ; Van Ommen, B; et al. (1996c) Differences in cytochrome P450-mediated biotransformation of 1,2-dichlorobenzene by rat and man: implications for human risk assessment. Chem Res Toxicol 9:1249-1256.

Hissink, AM; Dunnewijk, R; Van Ommen, B; et al. (1997a) Kinetics and metabolism of 1,4-dichlorobenzene in male Wistar rats: no evidence for quinone metabolites. Chem Biol Interact 103:17-33.

Hissink, AM; Oudshoorn, MJ; Van Ommen, B; et al. (1997b) Species and strain differences in the hepatic cytochrome P450-mediated biotransformation of 1,4-dichlorobenzene. Toxicol Appl Pharmacol 145:1-9.

Hissink, AM; Van Ommen, B; Kruse, J; et al. (1997c) A physiologically based pharmacokinetic (PB-PK) model for linked to two possible parameters of toxicity. Toxicol Appl Pharmacol 145:301-310.

Hodge, MC; Palmer, S; Wilson, J; et al. (1977) Paradichlorobenzene: teratogenicity study in rats. Imperial Central Toxicology Laboratory, Imperial Chemical Industries Limited, Alderly Park, Macclesfield, Cheshire, UK; ICI Report No. CRL/P/340. Summarized by ATSDR (1998), Dow Chemical Company (1982) and Hayes et al. (1985).

Hollingsworth, RL; Rowe, VK; Oyen, F; et al. (1956) Toxicity of paradichlorobenzene. AMA Arch Ind Health 14:138-147.

Hollingsworth, RL; Rowe, VK; Oyen, F; et al. (1958) Toxicity of o-dichlorobenzene. Arch Ind Health 17:180-187.

HSDB (Hazardous Substances Data Bank). (2002) National Library of Medicine, Bethesda, MD. Available from: <http://toxnet.nlm.nih.gov>.

IARC (International Agency for Research on Cancer). (1982) IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. Vol. 29. Some industrial chemicals and dyestuffs. Lyon, France: International Agency for Research on Cancer, pp. 213-238.

IARC (International Agency for Research on Cancer). (1999) IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. Vol. 73. Some chemicals that cause tumors of the kidney or urinary bladder in rodents and some other substances. Lyon, France: International Agency for Research on Cancer, pp. 223-276.

Imperial Chemical Industries Limited. (1980) Para-dichlorobenzene: long term inhalation study in the rat. Central Toxicology Laboratory, Imperial Chemical Industries Limited, Alderly Park, Macclesfield, Cheshire, UK; Report No. CTL/P/447.

James, NH; Soames, AR; Roberts, RA. (1998) Suppression of hepatocyte apoptosis and induction of DNA synthesis by the rat and mouse hepatocarcinogen diethylhexylphthalate (DEHP) and the mouse hepatocarcinogen 1,4-dichlorobenzene (DCB). Arch Toxicol 72:784-790.

Jan, J. (1983) Chlorobenzene residues in human fat and milk. Bull Environ Contam Toxicol 30:595-599.

Japan Bioassay Research Center. (1995) Toxicology and carcinogenesis studies of p-dichlorobenzene in F344/DuCrj rats and Crj:BDF1 mice (two-year inhalation studies). Submitted to the U.S. Environmental Protection Agency by the Japan Bioassay Research Center.

Kato, Y; Kimura, R. (1997) Role of 3,4-dichlorophenyl methyl sulfone, a metabolite of *o*-dichlorobenzene, in the changes in hepatic microsomal drug-metabolizing enzymes caused by *o*-dichlorobenzene administration in rats. Toxicol Appl Pharmacol 145:277-284.

Kato, Y; Kogure, T; Sato, et al. (1986) Evidence that methylsulfonyl metabolites of m-dichlorobenzene are causative substances of induction of hepatic microsomal drug-metabolizing enzymes by the parent compound in rats. Toxicol Appl Pharmacol 82:505-511.

Kato, Y; Kogure, T; Sato, et al. (1988a) Contribution of methylsulfonyl metabolites of m-dichlorobenzene to the heme metabolic enzyme induction by the parent compound in rat liver. Toxicol Appl Pharmacol 96:550-559.

Kato, Y; Kogure, T; Sato, M; et al. (1988b) Effects of chlorobenzenes and their methyl sulfone metabolites on microsomal enzymes associated with drug metabolism in rat liver. J Pharm Dyn 11:758-762.

Kimura, R; Hyashi, T; Sato, M; et al. (1979) Identification of sulfur-containing metabolites of p-dichlorobenzene and disposition in rats. J Pharmacodyn 2:237-244.

Kimura, R; Sano, H; Itagaki, K; et al. (1984) Identification of sulfur-containing metabolites of m-dichlorobenzene and their disposition and relationship with glutathione in rats. J Pharmacobiodyn 7:234-245.

Kimura, R; Kawai, M; Kato, Y; et al. (1985) Role of 3,5-dichlorophenyl methyl sulfone, a metabolite of m-dichlorobenzene, in the induction of hepatic microsomal drub-metabolizing enzymes by m-dichlorobenzene in rats. Toxicol Appl Pharmacol 78:300-309.

Kimura, R; Ohishi, N; Kato, Y; et al. (1992) Identification of biliary metabolites of m-dichlorobenzene in rats. Drug Metab Dispos 20:161-171.

Klos, C; Dekant, W. (1994) Comparative metabolism of the renal carcinogen 1,4-dichlorobenzene in rat: identification and quantitation of novel metabolites. Xenobiotica 24:965-976.

Krishnaswamy, S; Duan, SX; von Moltke, LL; et al. (2003) Serotonin (5-hydroxytryptamine) glucuronidation in vitro: assay development, human liver microsome activities and species differences. Xenobiotica 33:169-180.

Kumagai, S; Matsunaga, I. (1995) Identification of urinary metabolites of human subjects to *o*-dichlorobenzene. Int Arch Occup Environ Health 67:207-209.

Kumagai, S; Matsunaga, I. (1997) Quantitative determination of urinary metabolites of o-dichlorobenzene using a gas chromatograph. Ind Health 35:399-403.

Lake, BG; Cunninghame, ME; Price, RJ. (1997) Comparison of the hepatic and renal effects of 1,4-dichlorobenzene in the rat and mouse. Fundam Appl Toxicol 39:67-75.

Larsen, GL; Bakke, JE; Huwe, JK. (1990) Methylsulphone metabolites of *m*-dichlorobenzene as ligands for α_{2u}-globulin in rat kidney and urine. Xenobiotica 20:7-17.

Lattanzi, G; Bartoli, S; Bonora, B; et al. (1989) The different genotoxicity of p-dichlorobenzene in mouse and rat: measurement of the in vivo and in vitro covalent interaction with nucleic acids. Tumori 75:305-310.

Lewis, RJ, Sr; ed. (1997) Hawley's condensed chemical dictionary, 13th edition. New York, NY: John Wiley & Sons, Inc.; p. 362.

Lide, DR; ed. (2000) CRC handbook of chemistry and physics. 81st edition. Boca Raton, FL: CRC Press LLC; pp. 3-39.

Loeb, W.F. and Quimby, F.W. (ed). (1999) Clinical Chemistry of Laboratory Animals. 2nd ed. Chapter 11, Lipids and Lipoproteins. Taylor and Francis, p. 216.

Loeser, E; Litchfield, MH. (1983) Review of recent toxicology studies on *p*-dichlorobenzene. Food Chem Toxicol 21:825-832.

Loveday, KS; Anderson, BE; Resnick, MA; et al. (1990) Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro V: Results with 46 chemicals. Environ Mol Mutagen 16:272-303.

Makita, Y. (2005) Effects of perinatal combined exposure to 1,4-dichlorobenzene and 1,1-dichloro-2, 2-bis (p-chlorophenyl) ethylene on rat male offspring. Basic Clin Pharmacol Toxicol 96:361-365.

McCauley, PT; Robinson, M; Daniel, FB; et al. (1995) Toxicity studies of 1,3-dichlorobenzene in Sprague-Dawley rats. Drug Chem Toxicol 18:201-221.

McGregor, DB; Brown, A; Cattanach, P; et al. (1988) Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. Environ Mol Mutagen 12:85-154.

Mes, J; Davies, DJ; Turton, D; et al. (1986) Levels and trends of chlorinated hydrocarbon contaminants in the breast milk of Canadian women. Food Addit Contam 3:313-322.

Miyagawa, M; Takasawa, H; Sugiyama, A; et al. (1995) The in vivo-in vitro replicative DNA synthesis (RDS) test with hepatocytes prepared from male $B6C3F_1$ mice as an early prediction assay for putative nongenotoxic (Ames-negative) mouse hepatocarcinogens. Mutat Res 343:157-183.

Miyai, I; Hirono, N; Fujita, M; et al. (1988) Reversible ataxia following chronic exposure to paradichlorobenzene. J Neurosurg Psychiat 51:453-454.

Mizutani, T; Nakahori, Y; Yamamoto, K. (1994) *p*-Dichlorobenzene hepatotoxicity in mice depleted of glutathione by treatment with buthionine sulfoximine. Toxicology 94:57-67.

Mohtashamipur, E; Triebel, R; Straeter, H; et al. (1987) The bone marrow clastogenicity of eight halogenated benzenes in male NMRI mice. Mutagenesis 2:111-113.

Monsanto Company. (1996) One year study of p-dichlorobenzene administered orally via capsule to beagle dogs. In: Naylor, MW; Stout, LD; eds. Monsanto Company Environmental Health Laboratory. MRID No. 439888-02. ML-94-210. Available from EPA. Write to FOI, EPA, Washington, DC 20460.

Morita, T; Asano, N; Awogi, T; et al. (1997) Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (Groups 1, 2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS-MMS. Mutat Res 389:3-122.

Muller, M. (2002) 1,4-Dichlorobenzene-induced liver tumors in the mouse: evaluation of the role of chlorohydroquinones. Reviews on Environ Health 17:279-290.

Myhr, BC; Caspary, WJ. (1991) Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded compounds in the National Toxicology Program. Environ Mol Mutagen 18:51-83.

Nakamura, S; Oda, Y; Shimada, T; Oki, I; et al. (1987) SOS-inducing activity of chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: examination with 151 chemicals. Mutat Res 192:239-246.

Nedelcheva, V; Gut, I; Souček, P; et al. (1998) Cytochrome P450 catalyzed oxidation of monochlorobenzene, 1,2- and 1,4-dichlorobenzene in rat, mouse, and human liver microsomes. Chem Biol Interact 115:53-70.

NIOSH (National Institute for Occupational Safety and Health). (1997) NIOSH pocket guide to chemical hazards. U.S. Government Printing Office, Washington, DC: U.S. Government Printing Office; p. 96. DHHS (NIOSH) Publication No. 97-140.

NRC (National Research Council). (1983) Risk assessment in the Federal Government: managing the process. Washington, DC: National Academy Press.

NTP (National Toxicology Program). (1985) Toxicology and carcinogenesis studies of 1,2-dichlorobenzene (o-dichlorobenzene) (CAS No. 95-50-1) in F344/N rats and B6C3F_1 mice (gavage studies). Public Health Service, U.S. Department of Health and Human Services; NTP TR 255; NIH Publ. No. 86-2511. Available from: National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP. (1987) Toxicology and carcinogenesis studies of 1,4-dichlorobenzene (CAS No. 106-46-7) in F344/N Rats and B6C3F_1 mice (gavage studies). Public Health Service, U.S. Department of Health and Human Services; NTP TR 319; NIH Publ. No. 87-2575. Available from: National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Osada, T; Nagashima, I; Tsuno, N; et al. (2000) Prognostic significance of glutamine synthetase expression in unifocal advanced hepatocellular carcinoma. J Hepatol 33:247-253.

Pagnotto, LD; Walkley, JE. (1965) Urinary dichlorophenol as an index of para-dichlorobenzene exposure. Am Ind Hyg Assoc J 26:137-142.

Paolini, M; Pozzetti, L; Silingardi, P; et al. (1998) Isolation of a novel metabolizing system enriched in phase-II enzymes for short-term genotoxicity bioassays. Mutat Res 413:205-217.

Perocco, P; Bolognesi, S; Alberghini, W. (1983) Toxic activity of seventeen industrial solvents and halogenated compounds on human lymphocytes cultured in vitro. Toxicol Lett 16:69-85.

Potter, DW; Tran, T. (1993) Apparent rates of glutathione turnover in rat tissues. Toxicol Appl Pharmacol 120:186-192.

Reygagne, A; Garnier, R; Chataigner, D; et al. (1992) Encéphalopathie due á l'inhalation volontaire répétée de paradichlorobenzéne. Presse Médicale 21:267.

Riley, R; Chart, I; Gaskell, B; et al. (1980) Paradichlorobenzene: Long term inhalation study in the mouse. Report No. CTL/P/448. Imperial Chemical Industries Limited, Central Toxicology Laboratory, Alderly Park, Macclesfield, Cheshire, UK. As cited in Loeser and Litchfield, 1983.

Robbiano, L; Carrozzino, R; Porta Puglia, C; et al. (1999) Correlation between induction of DNA fragmentation and micronuclei formation in kidney cells from rats and humans and tissue-specific carcinogenic activity. Toxicol Appl Pharmacol 161:153-159.

Robinson, M; Bercz, JP; Ringhand, HP; et al. (1991) Ten- and ninety-day toxicity studies of 1,2-dichlorobenzene administered by oral gavage to Sprague-Dawley rats. Drug Chem Toxicol 14:83-112.

Rossberg, M; Aktiengesellschaft, H; Lendle, W; et al. (2002) Chlorinated hydrocarbons. In: Ullmann's encyclopedia of industrial chemistry. Weinheim, Germany: Wiley-VCH-Verlag.

Ruddick, JA; Black, WD; Villeneuve, DC; et al. (1983) A teratological evaluation following oral administration of trichloro- and dichlorobenzene isomers to the rat. Teratology 27:73A-74A.

Schmidt, U; Löser, E. (1977) Studies of the absorption, distribution and elimination of 1,4 dichlorobenzene after once-only administration and in the feeding test with rats. Bayer AG Toxicology Institute; Report No. 6874. Submitted under TSCA Section 8D; EPA Document No. 878210308; NTIS No. OTS0206248.

Sherman, JH; Nair, RS; Steinmetz, KL; et al. (1998) Evaluation of unscheduled DNA synthesis (UDS) and replicative DNA synthesis (RDS) following treatment of rats and mice with p-dichlorobenzene. Teratog Carcinog Mutagen 18:309-318.

Shimizu, M; Yasui, Y; Matsumoto, N. (1983) Structural specificity of aromatic compounds with special reference to mutagenic activity in *Salmonella typhimurium* - a series of chloro- or fluoro-nitrobenzene derivatives. Mutat Res 116:217-238.

Sipes, G., McQueen, C.A., and Gandolfi, A.J. (ed). (1997) Comprehensive Toxicology, Volume 6 Cardiovascular Toxicity. New York: Elsevier Science LTD, p. 112.

Spirtas, R; Stewart, PA; Lee, JS; et al. (1991) Retrospective cohort mortality study of workers at an aircraft maintenance facility. I. Epidemiological results. Br J Ind Med 48:515-530.

Staudinger, J; Roberts, PV. (1996) A critical review of Henry's Law Constants for environmental applications. Crit Rev Environ Sci Technol 26:205-297.

Stine, ER; Gunawardhana, L; Sipes, IG. (1991) The acute hepatotoxicity of the isomers of dichlorobenzene in Fischer-344 and Sprague-Dawley rats: isomer-specific and strain-specific differential toxicity. Toxicol Appl Pharmacol 109:472-481.

Suzuki, A; Nagai, H; Hiratsuka, H; et al. (1991) Effects of 1,4-dichlorobenzene on antibody production in guinea pigs. Oyo Yakuri 42:197-208. (English abstract.)

Tegethoff, K; Herbold, BA; Bomhard, EM. (2000) Investigations on the mutagenicity of 1,4-dichlorobenzene and its main metabolite 2,5-dichlorophenol in vivo and in vitro. Mutat Res 470:161-167.

Tyl, RW; Neeper-Bradley, TL. (1989) Two-generation reproduction study of inhaled paradichlorobenzene in Sprague-Dawley (CD) rats. Sponsored by The Chemical Manufacturers Association, Chlorobenzenes Program, Washington, DC; Bushy Run Research Center Project Report 51-593.

Umemura, T; Takada, K; Nakaji, Y; et al. (1989) Comparison of the toxicity of p-dichlorobenzene (p-DCB) administered to male F344 rats orally or by the inhalation route. Sci Rep Res Inst Tohoku Univ 36:1-9.

Umemura, T; Takada, K; Ogawa, Y; et al. (1990) Sex difference in inhalation toxicity of *p*-dichlorobenzene (*p*-DCB) in rats. Toxicol Lett 52:209-214.

Umemura, T; Tokumo, K; Williams, GM. (1992) Cell proliferation in the kidneys and livers of rats and mice by short term exposure to the carcinogen p-dichlorobenzene. Arch Toxicol 66:503-507.

Umemura, T; Saito, M; Takagi, A; et al. (1996) Isomer-specific acute toxicity and cell proliferation in livers of $B6C3F_1$ mice exposed to dichlorobenzene. Toxicol Appl Pharmacol 137:268-274.

Umemura, T; Takada, K; Shulz, C; et al. (1998) Cell proliferation in the livers of male mice and rats exposed to the carcinogen *p*-dichlorobenzene: Evidence for thresholds. Drug Chem Toxicol 21:57-66.

Umemura, T; Saito, M; Kodama, Y; et al. (2000) Lack of oxidative DNA damage or initiation of carcinogenesis in the kidneys of male F344 rats given subchronic exposure to *p*-dichlorobenzene (*p*DCB) at a carcinogenic dose. Arch Toxicol 73:54-59.

U.S. EPA (Environmental Protection Agency). (1981) An exposure and risk assessment for dichlorobenzenes. Office of Water, Regulations and Standards, Washington, DC; EPA/440/4-81/019.

U.S. EPA. (1986a) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014-34025.

U.S. EPA. (1986b) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006-34012.

U.S. EPA. (1987) Health effects assessment for dichlorobenzenes. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH; EPA 600/8-88/028. Available from: National Technical Information Service, Springfield, VA; PB88-179387.

U.S. EPA. (1988) Recommendations for and documentation of biological values for use in risk assessment. EPA 600/6-87/008. Available from: National Technical Information Service, Springfield, VA; PB88-179874/AS.

U.S. EPA. (1991a) Guidelines for developmental toxicity risk assessment. Federal Register 56(234):63798-63826.

U.S. EPA. (1991b) $Alpha_{2\mu}$-globulin: association with chemically induced renal toxicity and neoplasia in the male rat. Risk Assessment Forum, Washington, DC; EPA/625/3-91/019F.

U.S. EPA. (1992) Draft report: a cross-species scaling factor for carcinogen risk assessment based on equivalence of $mg/kg^{3/4}$/day. Fed Reg 57(109):24152-24173.

U.S. EPA. (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity studies. Federal Register 59(206):53799.

U.S. EPA. (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. EPA/600/8-90/066F. Available from: National Technical Information Service, Springfield, VA; PB2000-500023, and <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1995) Use of the benchmark dose approach in health risk assessment. U.S. Environmental Protection Agency. EPA/630/R-94/007. Available from: National Technical Information Service (NTIS), Springfield, VA; PB95-213765, and <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1996) Guidelines for reproductive toxicity risk assessment. Federal Register 61(212):56274-56322.

U.S. EPA. (1998a) Guidelines for neurotoxicity risk assessment. Federal Register 63(93):26926-26954.

U.S. EPA. (1998b) Science policy council handbook: peer review. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-98-001. Available from: National Technical Information Service, Springfield, VA; PB98-140726, and <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1998c) Assessment of thyroid follicular cell tumors. U.S. Environmental Protection Agency, Washington, DC. EPA/630/R-97/002.

U.S. EPA. (2000a) Science policy council handbook: peer review. 2nd edition. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-00-001. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000b) Science policy council handbook: risk characterization. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-00-002. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA (2000c) Benchmark dose technical guidance document [external review draft]. EPA/630/R-00/001. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000d) Supplemental guidance for conducting for health risk assessment of chemical mixtures. EPA/630/R-00/002. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2002) A review of the reference dose and reference concentration processes. Risk Assessment Forum, Washington, DC; EPA/630/P-02/0002F. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2004a) Benchmark dose software (version 1.3.2). Available online at http://cfpub1.epa.gov/ncea/cfm/bnchmrk/dwnldu.cfm?ActType=default

U.S. EPA. (2004b) An examination of EPA risk assessment principles and practices. Risk Assessment Task Force, Washington, DC. EPA/100/B-04/001.

U.S. EPA. (2005a) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC. EPA/630/P-03/001B. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, Washington, DC. EPA/630/R-03/003F. Available from: <http://www.epa.gov/iris.backgr-d.htm>.

Valentovic, MA; Ball, JG; Anestis, D; et al. (1993) Modification of P450 activity and its effect on 1,2-dichlorobenzene toxicity in Fischer 344 rats. Toxicology 79:169-180.

Verschueren, K. (2001) Handbook of environmental data on organic chemicals. 4th edition. Vol. 1-2. New York, NY: John Wiley & Sons; pp. 725-736.

Waters, MD; Sandhu, SS; Simmon, VF; et al. (1982) Study of pesticide genotoxicity. Basic Life Sci 21:275-326.

Weiss, G. (1986) Hazardous chemicals handbook. 1986. Park Ridge, NJ.: Noyes Data Corporation; pp. 353-355.

Williams, GM; Mori, H; McQueen, CA. (1989) Structure-activity relationships in the rat hepatocyte DNA-repair test for 300 chemicals. Mutat Res 221:263-286.

Yoshida, T; Andoh, K; Kosaka, H; et al. (2002a) Inhalation toxicokinetics of p-dichlorobenzene and daily absorption and internal accumulation in chronic low-level exposure to humans. Arch Toxicol 76:306-315.

Yoshida, T; Andoh, K; Fukuhara, M. (2002b) Urinary 2,5-dichlorophenol as biological index for p-dichlorobenzene exposure in the general population. Arch Environ Contam Toxicol 43:481-485.

Younis, HS; Hoglen, NC; Kuester, RK; et al. (2000) 1,2-Dichlorobenzene-mediated hepatocellular oxidative stress in Fischer-344 and Sprague-Dawley rats. Toxicol Appl Pharmacol 163:141-148.

Younis, HS; Parrish, AR; Sipes, IG. (2003) The role of hepatocellular oxidative stress in Kupffer cell activation during 1,2-dichlorobenzene-induced hepatotoxicity. Toxicol Sci 76:201-211.

Zenser, L-P; Lang, A; Knecht, U. (1997) N-acetyl-S-(dichlorophenyl) cysteines as suitable biomarkers for the monitoring of occupational exposure to 1,2-dichlorobenzene. Int Arch Occup Environ Health 69:252-254.

Zissu, D. (1995) Histopathologic changes in the respiratory tract of mice exposed to ten families of airborne chemicals. J Appl Toxicol 15:207-213.

APPENDIX A
Summary of External Peer Review and Public Comments and Disposition

The Toxicological Review and IRIS Summary for Dichlorobenzenes have undergone both internal peer review by scientists within EPA and a more formal external peer review by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1998b, 2000a). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows. EPA also received scientific comments from the public. These comments and EPA's response are included in a separate section of this appendix.

I. External Peer Review Comments

A. Reference Dose (RfD)

1,2-Dichlorobenzene

(1) **Comment:** Three reviewers observed that the RfD was based on a combination of data from the 13-week (range-finding) and two-year chronic NTP (1985) studies, and recommended that results from the chronic study (male mouse kidney) be given preference over the subchronic study as being more reliable and providing higher confidence. Two reviewers disagreed with the statement that "the lack of a LOAEL in the 102-week study precludes analyzing the chronic data using BMD analysis" and with identification of both the mid- and high-dose from the chronic NTP study as NOAELs. One reviewer was comfortable with use of the 13-week NOAEL.

Response: The revised assessment is based on kidney effects found in male mice following chronic exposure (NTP, 1985). Since kidney effects were observed in the chronic NTP study at the highest dose of 85.7 mg/kg-day, the lowest dose of 42.9 mg/kg-day is considered the NOAEL. BMD modeling was performed using data for renal tubular regeneration in male mice and yielded a $BMDL_{10}$ of 29.8 mg/kg-day. The revised RfD is based on the $BMDL_{10}$ as the point of departure.

Discussion of the results of the NTP subchronic study, which identified liver toxicity as the critical effect, has been revised so that the basis for the point of departure (i.e., the chronic NTP bioassay) is unambiguous.

(2) **Comment:** One reviewer noted that RfD values should be represented as one significant figure.

Response: The RfD has been changed to one significant figure.

(3) **Comment:** One reviewer indicated the PBPK models summarized in Section 3 of the Toxicological Review should be evaluated further to determine whether it is appropriate to use a model for extrapolation from animals to humans to determine an HED.

Response: Although rat PBPK models for 1,2-dichlorobenzene have been summarized in the Toxicological Review, it is not considered appropriate to use the models for determining the point of departure for the following reasons:

1) The human model has very little calibration data. The only parameter not estimated or scaled directly from the rat model was the Vmax for human liver microsomes, which was measured *in vitro*.

2) The authors of the PBPK models did not present data to directly correlate their model runs with a toxic endpoint. They presented predictions of blood dichlorobenzene levels, covalently-bound metabolites, and GSH levels in the liver.

Accordingly, it was determined that the models for rats are still in the early stages of development, and the human model has not been adequately calibrated against a system more complex than isolated hepatic microsomes. The reasons for not using the PBPK model were expanded in the Toxicological Review.

1,3-Dichlorobenzene

(1) **Comment:** One reviewer suggested that a pathologist be consulted to determine whether the thyroid and pituitary effects observed in the 1,3-dichlorobenzene principal study should be

considered adverse. Two reviewers considered the effects on the thyroid and pituitary to be appropriate critical effects.

Response: The text in Section 5.1.2.1 has been revised to support the thyroid as the critical target organ. Dr. Douglas Wolf, Pathologist (National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. EPA), was asked to critically review the principal study. His comments regarding the thyroid and pituitary effects are provided below:

> *The changes in thyroid histology are likely biologically relevant and are supported by the other studies with like compounds (van den Berg, Chem Bio Interact 76:63-75, 1990; den Besten, Toxicol Appl Pharmacol 111:69-81, 1991; NTP TR 319, 1987; Elcombe, Environ Health Perspect 110:363-375, 2002). The small increases in cholesterol reported in these studies are consistent with hypothyroxinemia. The pituitary vacuolization is unlikely to be an adverse event, in addition there is no evidence in these studies of significant perturbations in circulating levels of sex hormones and so the pituitary cellular changes are highly unlikely to be castration cells but rather a general response to some stimulus (i.e., decreased T_4) to stimulate production of TSH and therefore an adaptive response. The discussion of hypermetabolism in this paper is purely speculative and should not be considered in this assessment. In fact, typically hypothyroxinemia results in lowered metabolism.*
>
> *A likely mechanistic interpretation of these events is induction of metabolizing enzymes in the liver resulting in more rapid degradation of T_4. Because of the short half-life of T_4 in the rat and little residual thyroglobulin in the thyroid, changes in the rat thyroid can appear rapidly and become significant.*
>
> *The changes in colloid do indicate a response to treatment, but without hormone levels it is difficult to say whether these animals are adequately responding or if their ability to respond has been overtaxed. I do not believe using colloid depletion alone as a BMR is justified; one would need to correlate that with follicular hypertrophy and possibly follicular cell hyperplasia. Some evidence of hormone changes would be highly supportive.*
>
> *Also, rats are markedly more sensitive than adult humans to the effects of chemicals that affect thyroid hormone production and the pituitary should not be used in these studies as there is no evidence that the change described is adverse,*

neither is it related to sex hormone disruption, 10x over interprets the interspecies differences.

With regard to adaptive vs adverse responses in the thyroid, that is really a perspective decision. A snapshot in time when one looks at the fixed thyroid gland and sees decreased colloid and even follicular hypertrophy, that change in and of itself is adaptive, in that the gland is doing what it is supposed to do when there is a perceived physiologic need for more T4 to be made and mobilized to the circulation. However, when this process occurs for long periods of time, at high doses, or a critical life stage events then an adverse health outcome can result. From a risk assessment point of view the colloid depletion and hypertrophy, or the decreased T_4 (any one or all of these) could be considered precursor effects. Therefore, protecting for these effects will prevent everything downstream. It is considered that with some xenobiotics they increase T_4 metabolism in the liver, which sets up the cyclic process that results in increased TSH and (in the rat) thyroid tumors. But decreased T_4, or hypothyroxinemia, in and of itself can be a concern in, for example, pregnant dams (or women).

Based on the consultation provided by Dr. Wolf, the 1,3-dichlorobenzene assessment was revised to consider the thyroid only as the critical target organ. EPA acknowledges that, in the absence of other supporting data (i.e., follicular hypertrophy, follicular cell hyperplasia, or evidence of hormone changes), it is uncertain whether changes in the thyroid represent an adaptive change or indication of toxicity. Because, as Dr. Wolf points out, prolonged colloid depletion can results in adverse health outcomes, this effect was considered a potential precursor event.

Subsequent quantitative analysis using both BMD and traditional LOAEL/NOAEL methods revealed that the thyroid data set was not suitable for dose-response modeling and did not support derivation of an RfD.

(2) **Comment:** Two reviewers commented on the poor fit of the BMD curve for thyroid data (reduced follicular colloidal density). These reviewers suggested that the highest dose in the 1,3-dichlorobenzene study be dropped to obtain a better model fit of the data for thyroid effects.

Response: BMD modeling using EPA's BMDS (version 1.3.2; U.S. EPA, 2000c) and data from all dose levels from the McCauley et al. (1995) study did not provide an adequate fit of the data (based on the goodness of fit criterion, χ^2 *p*-value > 0.1). The highest dose group (588 mg/kg-day) was dropped to determine if the model fit could be improved; adequate model fits were not achieved

with this modified data set. Data from the two highest dose groups (147 and 588 mg/kg-day) were then dropped to see if adequate model fits could be achieved. Some of the models in BMDS provided adequate fits of this reduced data set; however, the dose associated with the BMR of 10% extra risk for reduced thyroid follicular colloidal density was well below the range of experimental doses.

As discussed in EPA's BMD guidance (U.S. EPA, 2000c), the aim of BMD modeling is to model the dose-response data for an adverse effect in the observable range and then select a BMD at the low end of the observable range to use as a point of departure for deriving the RfD. In the situation presented by the McCauley et al. (1995) thyroid data, BMD modeling provides little resolution of the dose-response curve in the region of the BMR. Accordingly, for thyroid data from the McCauley et al. (1995) study, the conclusion was reached that the data are not suitable for BMD modeling.

(3) **Comment:** Two reviewers commented that based on differences in thyroid physiology between humans and rats, a UF of 3 may be appropriate for interspecies extrapolation (rather than the current default of 10). A third reviewer considered a full factor of 10 appropriate.

Response: The *Assessment of Thyroid Follicular Cell Tumors* document (U.S. EPA, 1998c) discusses the fact that rodents do not have a high-affinity binding protein, thyroxin-binding globulin, that is present in humans. This leads to an accelerated production of thyroid hormones and a shorter half-life for thyroid hormones in rats compared to humans. The shorter half-life and reduced binding protein make rodents more sensitive to thyroid altering chemicals. This information has been added to the Toxicological Review. Based on this information the interspecies UF was changed to 3. Ultimately, however, an RfD for 1,3-dichlorobenzene was not derived because the database for this isomer was considered insufficient to support reference value derivation.

(4) **Comment:** Two reviewers recommended that a UF of 10 be applied for lack of supporting database (i.e., a single developmental study available in abstract form only and no other studies available for the 1,3-isomer). One reviewer considered the current database UF of 3 to be adequate.

Response: The report *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) states that if the RfD/RfC is based on animal data, a factor of 3 is often applied if either a developmental toxicity study or a two-generation reproduction study is missing, or a factor of 10 applied if both are missing. In the case of 1,3-dichlorobenzene, a developmental study is

available, but in abstract form only and detailed study findings are not presented. A two-generation reproduction study is not available for the 1,3-dichlorobenzene isomer. Therefore, a database UF of 10 was considered appropriate for database deficiencies. Ultimately, however, an RfD for 1,3-dichlorobenzene was not derived because the database for this isomer as a whole was considered insufficient to support reference value derivation.

(5) **Comment:** One reviewer observed that because there is limited opportunity for marked inter-individual variation in metabolism (only one chlorine is placed vicinal to the most favored p-hydroxylation and the other vicinal position is vacant) and because the compound is likely to be highly metabolized, inter-individual differences in toxicokinetics are expected to be minimal. Accordingly, the reviewer suggested that the UF for inter-individual differences was too conservative and that a UF of 3 would be sufficient.

Response: In the absence of chemical-specific information demonstrating the range of metabolism of 1,3-dichlorobenzene in humans, a full default UF for intra-species extrapolation was considered appropriate. Ultimately, however, an RfD for 1,3-dichlorobenzene was not derived because the database for this isomer was considered insufficient to support reference value derivation.

1,4-Dichlorobenzene

(1) **Comment:** One reviewer did not consider it appropriate to derive an RfD from a BMDL (using data for multifocal chronic inflammation in the Monsanto dog study) that is a factor of 30 below the NOAEL of 7 mg/kg-day, given the minimal effects seen at this NOAEL.

Response: The critical effect was changed to diffuse hepatocellular hypertrophy. Using combined incidence data from male and female dogs and output from the log probit model in EPA's BMDS, the BMD_{10} and $BMDL_{10}$ were 24 and 9 mg/kg-day, respectively. These values are within the range of the LOAEL (36 mg/kg-day) and NOAEL (7 mg/kg-day) from this study.

(2) **Comment:** Two reviewers commented that the general health of the animals in the beagle dog study is questionable in light of the gastrointestinal (GI) effects observed in all animals. One reviewer suggested that this issue be evaluated further, as the liver effects and pulmonary effects seen following exposure to 1,4-dichlorobenzene may have been a secondary response to the GI complications.

Response: An in-depth review of the Data Evaluation Record (DER) prepared by the Office of Pesticide Programs and the Monsanto Company (1996) study itself revealed no GI effects in the treated or untreated dogs. In addition, Dr. John Cicmanec, DVM (National Risk Management Research Laboratory, ORD, U.S. EPA) was consulted regarding the evaluation of health status of the animals and his comments are presented below:

> *Based on the fact that EHL designated a study number of #94093 indicates that it was performed after 1994. A commercial animal supplier who breeds and raises their own dogs would have been used for a study performed in this time period. With this being the case it is very unlikely that the dogs would have had intestinal parasites. I believe that it is more likely that the test material, 1,4-dichlorobenzene, caused the lesions in the digestive tract and these changes should be considered compound-related.*

Because no GI lesions were reported in either the DER or the study, Monsanto Company (1996) was retained as the principal study.

(3) **Comment:** One reviewer suggested that BMD modeling be applied to the serum enzyme changes observed in the chronic beagle dog study for 1,4-dichlorobenzene.

Response: In general, EPA does not use changes in serum enzyme levels as the basis for a reference value. The preference is to use as the basis for the point of departure a treatment-related histopathologic change or some other finding associated with toxicity. Accordingly, serum enzyme level changes were not subject to BMD analysis.

(4) **Comment:** One reviewer commented that a BMR of 10% for continuous end-points (e.g., organ weight) is fundamentally different from that for binary end-points and this difference should be clarified.

Response: In deriving RfD and RfC values, the BMR was selected based on available BMD guidance (U.S. EPA, 2000c).

For quantal data, an extra risk of 10% has been used as the BMR. The 10% response is at or near the limit of sensitivity in most cancer bioassays and in some noncancer bioassays as well. For continuous data, EPA's BMD guidance states that if there is a minimal level of change in the endpoint that is generally considered to be biologically significant (for example, a change in average adult body weight of 10%, or the doubling of average level for some liver enzyme), then that

amount of change can be used to define the BMR. In the absence of any other idea of what level of response to consider adverse, a change in the mean equal to one control standard deviation from the control mean can be used. This gives an excess risk of approximately 10% for the proportion of individuals below the 2nd percentile or above the 98th percentile of controls for normally distributed effects. In the dichlorobenzene assessment, all endpoints analyzed using BMD methods were quantal, and an extra risk of 10% was selected as the BMR.

(5) **Comment:** One reviewer observed that the statistical results in Table 5-3 (Summary of Liver Histopathology Incidence) and the statistics reported in the table in Appendix B.3 (BMD Modeling of Incidence Data for Liver Lesions) were incorrect. This reviewer also commented that only statistically significant changes should be modeled.

Response: The statistical results in Table 5-3 were corrected. The endpoint used to determine the point of departure (hepatocellular hypertrophy) was statistically significantly elevated in male dogs. The tables in Appendix B.3 were modified to present the goodness of fit *p*-value for χ^2 rather than the χ^2 value.

(6) **Comment:** Several reviewers stated that information in the open literature is sufficient to indicate that dogs are more sensitive to 1,4-dichlorobenzene exposures than other mammals, including humans. Accordingly, the reviewers recommended that the interspecies UF be reduced from 10 to either 1 or 3.

Response: The supporting information provided by one reviewer was considered as the basis for reducing the default UF from 10 to 3. The rationale for an interspecies UF of 3 relates to the lower rates of glucuronidation and sulfation in the dog as compared to humans and rodents. Because the toxicokinetics of 1,4-dichlorobenzene in humans and dogs is incompletely characterized (e.g., comparative information on phase 1 biotransformation reactions is not available), however, interspecies differences in toxicokinetics cannot be fully predicted. Accordingly, the default UF of 10 was retained.

(7) **Comment:** One reviewer commented that, in general, the 1,4-dichlorobenzene database should be considered complete, although some uncertainties (e.g., sensitivity of young animals) might suggest a database UF of 3.

Response: A database UF of 3 was applied in deriving the RfD to account for uncertainty associated with developmental and reproductive toxicity data for ingested 1,4-dichlorobenzene (see Section 5.1.3.3).

B. Reference Concentration (RfC)

1,2-Dichlorobenzene

Reviewers generally concurred with the conclusion reached in the Toxicological Review that data for 1,2-dichlorobenzene were inadequate to derive an RfC.

1,3-Dichlorobenzene

Reviewers generally concurred with the conclusion reached in the Toxicological Review that data for 1,3-dichlorobenzene were inadequate to derive an RfC.

1,4-Dichlorobenzene

(1) **Comment:** One reviewer commented that a more detailed justification for the use of an extra risk of 5% as the BMR rather than 10% should be included in the Toxicological Review.

Response: The critical endpoint used in the external review draft assessment was decreased pup survival in a two-generation study. A 5% decrease was selected as the BMR because of the severity of the effect. The principal study and critical effects were subsequently changed to the JBRC study (Aiso et al, 2005b; JBRC, 1995) and to incidence of eosinophilic changes of the olfactory epithelium in female rats and mineralization of the testes in male mice. A 10% change in incidence was used as the BMR, consistent with EPA BMD guidance (U.S. EPA, 2000c).

(2) **Comment:** Two reviewers observed that a new inhalation study by the JBRC is available, and that this study should be considered in the derivation of the RfC.

Response: The JBRC study (Aiso et al., 2005b; JBRC, 1995) was evaluated and selected as the principal study for deriving the RfC for 1,4-dichlorobenzene.

(3) **Comment:** One reviewer observed that the database lacks information on respiratory effects of 1,4-dichlorobenzene in animals compared to effects seen in humans and questioned whether the interspecies UF of 3 was sufficient to address these uncertainties.

Response: The RfC methodology (U.S. EPA, 1994b) recommends a UF of 3 for animal to human extrapolation based on the blood:air partition coefficient (for category 3 gases). As explained in the Toxicological Review, because the human blood:air partition coefficient is unavailable for 1,4-dichlorobenzene, a default of 1 was used in deriving the HEC, which accounts for the toxicokinetic portion of animal to human extrapolation. The UF of 3 accounts for the pharmacodynamic portion of uncertainty in interspecies extrapolation. No changes were made to the UF_A.

C. Cancer Characterization

1,2-Dichlorobenzene

(1) **Comment:** Three reviewers commented that conclusions regarding the WOE descriptor should address the adequacy of the carcinogenicity testing for 1,2-dichlorobenzene and whether or not the MTD was reached in the chronic NTP study. On balance, three reviewers thought the MTD was probably reached and that 1,2-dichlorobenzene should be considered not likely to be carcinogenic to humans.

Response: Section 4 discusses the adequacy of carcinogenicity testing for 1,2-dichlorobenzene (specifically Sections 4.2.1.1 and 4.6.1). The available studies provide no evidence for compound-related neoplastic lesions in either rats or mice at the tested doses. In the chronic NTP study (1985), with the exception of tubular regeneration in male mice at the highest dose, no compound-related lesions were observed in either the rats or the mice. There were no significant effects on body weight or survival in rats and mice, with the exception of high-dose male rats, where survival was significantly shorter than control. NTP (1985) states, however, that gavage error may have contributed to deaths in high-dose males, and thus the lower survival of high-dose male rats does not necessarily mean that the MTD was exceeded. Section 4.6.1 discusses the nature

of the available carcinogenicity data and limitations in the data introduced by failure to clearly establish that the MTD was reached. Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a WOE descriptor of "not likely to be carcinogenic to humans" generally requires extensive human experience that demonstrates lack of carcinogenic effect, or, in the absence of human data, animal evidence that demonstrates lack of carcinogenic effects in both sexes in well-designed and well-conducted studies in at least two appropriate animal species. Given the unresolved uncertainty concerning the MTD, the WOE descriptor (data inadequate for an evaluation of human carcinogenic potential) is considered appropriate.

1,3-Dichlorobenzene

Reviewers generally concurred with the conclusions in the Toxicological Review that data are inadequate for an assessment of human carcinogenic potential.

1,4-Dichlorobenzene

(1) **Comment:** Two reviewers suggested that discussion of the role of α_{2u}-globulin nephropathy in relation to the renal tumors in male rats should be further elaborated in the Toxicological Review.

Response: Discussion of the role of α_{2u}-globulin in the development of kidney tumors in male rats, described in Section 4.6.3, was expanded.

(2) **Comment:** One reviewer recommended that differences in metabolism of 1,4-dichlorobenzene in humans, rats and mice be discussed further in the Toxicological Review.

Response: Differences in metabolism of 1,4-dichlorobenzene in mice, rats and humans were summarized in Section 3.3 to the extent that such information was available in the published and unpublished literature.

(3) **Comment:** Two reviewers commented that a new inhalation study (JBRC, 1995) is available for consideration in the carcinogenicity assessment of the 1,4-isomer.

Response: The inhalation carcinogenicity results from the 1995 JBRC study (subsequently published by Aiso et al. (2005b)) were included in the Toxicological Review and data from that study were used for the carcinogenicity assessment of 1,4-dichlorobenzene.

(4) **Comment:** One reviewer commented that the cancer WOE statement, as presented, does not state whether it applies to both routes of exposure, or just the oral route. The reviewer suggested that this be clarified.

Response: The statement was revised to clarify that the WOE designation applies to both the oral and inhalation exposure routes.

(5) **Comment:** One reviewer commented that the cancer WOE characterization should be changed to "Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential," while another reviewer stated that the WOE characterization should be suggestive at low doses and likely at high doses.

Response: The 2005 *Guidelines for Carcinogen Risk Assessment* state that the descriptor, "Likely to Be Carcinogenic to Humans," covers a broad spectrum, including the situations where "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans" or "a rare animal tumor response in a single experiment that is assumed to be relevant to humans." In the case of 1,4-dichlorobenzene, there are no human studies of its carcinogenic potential. Animal studies, however, show carcinogenic response in more than one sex, strain, site, and exposure route. NTP (1987) found hepatocellular adenoma, hepatocellular carcinoma and combined hepatocellular adenoma/carcinoma occurred with positive dose-response trends in both male and female mice, with tumor incidences in low-dose males and high-dose groups of both sexes statistically significantly higher than the control groups. In addition, the high-dose male mice demonstrated four cases of hepatoblastoma, a rare type of hepatocellular carcinoma. Mice exposed to 1,4-dichlorobenzene by inhalation displayed dose-related increases in hepatocellular tumors (males and females), histiocytic sarcomas (males), and bronchoalveolar adenomas/carcinomas (females), as well as hepatoblastomas, also a rare tumor in this institute's historical controls (Aiso et al., 2005b; JBRC, 1995). Based on the development of a rare tumor as well as the adequate data demonstrating carcinogenicity in animals following exposure to 1,4-dichlorobenzene, the cancer characterization of "Likely to be Carcinogenic to Humans" was retained, consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

(6) **Comment:** Two reviewers commented that based on the 1999 draft revised cancer guidelines both a linear and nonlinear extrapolation for 1,4-dichlorobenzene should be presented, if accompanied by sufficient documentation of the proposed key events.

Response: According to the 2005 *Guidelines for Carcinogen Risk Assessment*, "When the weight-of-evidence evaluation of all available data is insufficient to establish the MOA for a tumor site and when scientifically plausible based on the available data, linear extrapolation is used as a default approach, because linear extrapolation generally is considered to be a health-protective approach." Accordingly, because a cancer MOA for 1,4-dichlorobenzene has not been conclusively established, only a default linear extrapolation approach was presented in the Toxicological Review.

D. General Comments

(1) **Comment:** One reviewer suggested that selection of the most appropriate model for BMD modeling based on the smallest AIC is an ad hoc method, that alternative methods should be considered, and that AIC values that differ minimally should be considered essentially the same value.

Response: All of the BMD models available in the software package were applied to the data. Models that passed the goodness-of-fit test with a χ^2 *p*-value >0.1 were retained as potentially useful models. Of all models that passed the goodness-of-fit criterion, the model with the lowest AIC was selected as the best fitting model, based on criteria established in the BMD guidance document (U.S. EPA, 2000c).

(2) **Comment:** One reviewer commented that BMD modeling was applied inconsistently in the assessments for the three isomers. The reviewer further observed the assessments for 1,2- and 1,3-dichlorobenzene were consistent with BMD guidance; however, for 1,4-dichlorobenzene, the model with the lowest BMDL was selected as the basis for RfD derivation rather than the model with the lowest AIC.

Response: The three isomer assessments were revised so that BMD modeling was applied consistently across all isomer assessments and consistent with EPA BMD guidance (U.S. EPA, 2000c).

(3) **Comment:** Two reviewers commented that study summaries should include the number of animals per group where that information is available.

Response: Animal numbers, where reported, were included in the study descriptions.

(4) **Comment:** One reviewer suggested that where unpublished studies are used as the basis for key decisions, the Toxicological Review should indicate whether the studies were conducted according to good laboratory practice (GLP) guidelines and current EPA test protocols.

Response: Citations for all unpublished studies (e.g., Office of Pesticide Program DERs) were added to the reference section. Adherence to GLP guidelines was documented in the Toxicological Review as appropriate.

(5) **Comment:** One reviewer suggested that data on neurological effects observed in humans exposed to 1,4-dichlorobenzene via inhalation be compared with animal studies to determine whether these effects occur only at high exposures.

Response: Dose-response information on 1,4-dichlorobenzene neurotoxicity in humans is insufficient to support a comparison with neurotoxicity data in experimental animals.

(6) **Comment:** One reviewer commented that increased liver weight accompanied by hepatocyte hypertrophy, reported in the two-generation inhalation toxicity study of 1,2-dichlorobenzene (Bio/dynamics, 1989), should be considered adverse rather than adaptive.

Response: Where no other indication of liver toxicity was observed (as in the Bio/dynamics [1989] study), increased liver weight and hepatocellular hypertrophy were generally considered to be an adaptive response. Where other liver lesions were present (e.g., Monsanto Company [1996] study in dogs), hypertrophy was considered potentially adverse.

(7) **Comment:** One reviewer suggested that a table summarizing the NOAELs and LOAELs for inhalation studies on the three dichlorobenzene isomers would be useful.

Response: Tables 4-11 and 4-12, which summarize the inhalation studies for the 1,2- and 1,4-isomers (including NOAELs and LOAELs), were added to the Toxicological Review. No inhalation studies of the 1,3-isomer were identified.

(8) **Comment:** One reviewer commented that consideration of age-related enzyme development and consequences for tissue dose should be included if possible. If such data are unavailable, the Toxicological Review should document that data are insufficient to conduct an analysis of age-specific susceptibility.

Response: Data for dichlorobenzenes are insufficient to document age-related changes in enzyme development and the consequences on toxicity to children and other age groups. The lack of appropriate data to address this issue was noted in Section 4.7.1.

(9) **Comment:** One reviewer commented that since thyroid effects were associated with exposure to all three isomers, a more detailed discussion of thyroid effects following dichlorobenzene exposure should be included in the Toxicological Review.

Response: The association between dichlorobenzene exposure and thyroid toxicity is discussed in Sections 4 and 5 of the Toxicological Review. The available literature suggests that thyroid effect are predominantly associated with the 1,3-isomer in the single subchronic toxicity study of this isomer; however, the thyroid is not the most sensitive endpoint for the other two isomers. Additional discussion of the thyroid beyond that presented in Sections 4 and 5 was not considered necessary.

(10) **Comment:** One reviewer commented that Figures 3.2 and 3.3 should be evaluated for accuracy. For example, the formation of the 1,2-epoxide is absent in Figure 3.3.

Response: The figures (3-2 and 3-3) provide an overview of the metabolic pathways for 1,3- and 1,4-dichlorobenzene most relevant to the derivation of human health toxicity values. Detailed metabolic information may not be captured in these figures. Identification of the 1,2-epoxide intermediate for 1,4-dichlorobenzene was added to Figure 3-3.

(11) **Comment:** One reviewer stated that interspecies differences in metabolism of 1,4-dichlorobenzene were not sufficiently discussed.

Response: A discussion of interspecies differences in metabolism of 1,4-dichlorobenzene was added to Section 3.3.3. While it is possible that the differences in toxicity, relative to rodents, observed in the few available studies of rabbits and dogs are the result of differences in metabolism, data on the metabolism of 1,4-dichlorobenzene in these species are not available.

(12) **Comment:** One reviewer suggested the addition of doses for the initiation/promotion assays in the document.

Responses: The doses for the initiation/promotion assays were added to the text.

(13) **Comment:** One reviewer commented that while evidence for genotoxicity of 1,4-dichlorobenzene is minimal, the implications of the more evenly divided data in micronucleus formation should be discussed.

Response: Discussion of the body of genotoxicity results is provided in Sections 4.6.1, 4.6.2, and 4.6.3. of the Toxicological Review, including the positive and negative findings from micronucleus assays.

(14) **Comment:** One reviewer suggested that information on colony size in the mouse lymphoma assays should be added to the 1,2- and 1,4-dichlorobenzene genotoxicity sections. The reviewer stated that the sex of animals used in the in vivo assays also should be included in the genotoxicity section.

Response: Information on colony size in mouse lymphoma assays was added to the genotoxicity sections for 1,2-dichlorobenzene and 1,4-dichlorobenzene. The sex of the animals used in the in vivo genotoxicity assays was included in the genotoxicity sections.

(15) **Comment:** One reviewer commented that the effects of 1,4-dichlorobenzene and its metabolites on hepatocyte proliferation, inhibition of hepatocyte apoptosis and effects on gap junction communication should be presented.

Response: A more thorough discussion of the effects of 1,4-dichlorobenzene on molecular endpoints (e.g., inhibition of apoptosis, effects on gap junctions, mitogenesis) and on the process of cell division was added to the Toxicological Review (Section 4.4.1.2.2).

(16) **Comment:** One reviewer suggested that an updated literature search be conducted on MOA for dichlorobenzenes.

Response: An updated literature search was conducted and no significant new studies were identified that would change the values and information presented in this assessment.

II. Public Comments

The U.S. EPA received two sets of public comments. These comments pertained to the mode of carcinogenic action (MOA) for 1,4-dichlorobenzene, and whether the MOA supported the use of a linear or nonlinear approach for the carcinogenicity assessment of 1,4-dichlorobenzene. Specific comments were as follows:

(1) **Comment:** One reviewer commented that the WOE is convincing that 1,4-dichlorobenzene does not induce cancer via a DNA reactive, mutagenic or genotoxic mechanism. A second reviewer concluded that the evidence for an interaction of 1,4-dichlorobenzene with DNA is inconclusive. Given the consistent evidence for cell proliferation, this reviewer considered a nonlinear model to be appropriate for quantitative carcinogenic risk assessment.

Response: For reasons discussed in the response to external peer reviewer comments, only a linear extrapolation approach was presented in the Toxicological Review.

(2) **Comment:** Two reviewers observed that the α_{2u}-globulin pathway is likely responsible for the induction of rat kidney tumors by 1,4-dichlorobenzene and that an RfD safety factor (nonlinear) approach is the more appropriate risk extrapolation model.

Response: As discussed in Section 4.6.3 of the Toxicological Review, the evidence suggests that male rats kidney tumors induced by 1,4-dichlorobenzene are produced by a sequence of events initiated by the binding of 1,4-dichlorobenzene with the male rat-specific protein α_{2u}-globulin. It is generally accepted that this MOA is specific to the male rat and not relevant to humans. Therefore, male rat kidney tumors were not modeled in this assessment.

(3) **Comment:** Two reviewers concluded that evidence is strong that 1,4-dichlorobenzene induces liver tumors via a nongenotoxic mitogenic MOA and that this MOA is consistent with an RfD safety factor (nonlinear) model. This reviewer also considered the appropriate descriptor to be "suggestive evidence of carcinogenic potential."

Response: As discussed in Section 4.6.3 of the Toxicological Review, the MOA for liver tumors is not fully understood. While evidence exists suggesting that the mechanism leading to mouse liver formation is nongenotoxic and based on sustained mitogenic stimulation and hepatocyte proliferation, evidence for this MOA is incomplete. Accordingly, as discussed in response to external peer reviewer comments, only a linear extrapolation approach is included in the Toxicological Review.

As discussed in response to external peer reviewer comments, under the 2005 *Guidelines for Carcinogenic Risk Assessment*, cancer bioassay findings support a WOE descriptor of "likely to be carcinogenic to humans."

References quoted by Dr. Wolf:

den Besten, C; Vet, JJ; Besselink, HT; et al. (1991) The liver, kidney, and thyroid toxicity of chlorinated benzenes. Toxicol Appl Pharmacol 111(1):69-81.

Elcombe, CR; Odum, J; Foster, JR; et al. (2002) Prediction of rodent nongenotoxic carcinogenesis: evaluation of biochemical and tissue changes in rodents following exposure to nine nongenotoxic NTP carcinogens. Environ Health Perspect 110(4):363-375.

NTP (National Toxicology Program). (1987) Toxicology and carcinogenesis studies of 1,4-dichlorobenzene (CAS No. 106-46-7) in F344/N Rats and B6C3F$_1$ mice (gavage studies). Public Health Service, U.S. Department of Health and Human Services; NTP TR 319; NIH Publ. No. 87-2575. Available from: National Institute of Environmental Health Sciences, Research Triangle Park, NC.

van den Berg, KJ. (1990) Interaction of chlorinated phenols with thyroxine binding sites of human transthyretin, albumin and thyroid binding globulin. Chem Biol Interact 76(1):63-75.

APPENDIX B
Documentation of BMD Modeling

B.1. BMD MODELING OF INCIDENCE DATA FOR TUBULAR REGENERATION IN THE KIDNEYS OF MALE MICE ORALLY EXPOSED TO 1,2-DICHLOROBENZENE FOR 103 WEEKS

Incidence data for tubular regeneration in the male mouse kidney from the 103-week oral NTP (1985) bioassay are summarized in Table B-1.

Table B-1. Incidence of tubular regeneration in the kidneys of male mice orally exposed to 1,2-dichlorobenzene for 103 weeks

	Duration-adjusted dose (mg/kg-day)[a]		
	0	**42.9**	**82.7**
Male mouse	8/48	12/50	17/49[b]

[a] Gavage doses of 0, 60 or 120 mg/kg, 5 day/week, were multiplied by 5/7 to derive the duration-adjusted doses.
[b] Significantly (p<0.05) different from control; Fisher's Exact Test performed by EPA.

Source: NTP, 1985.

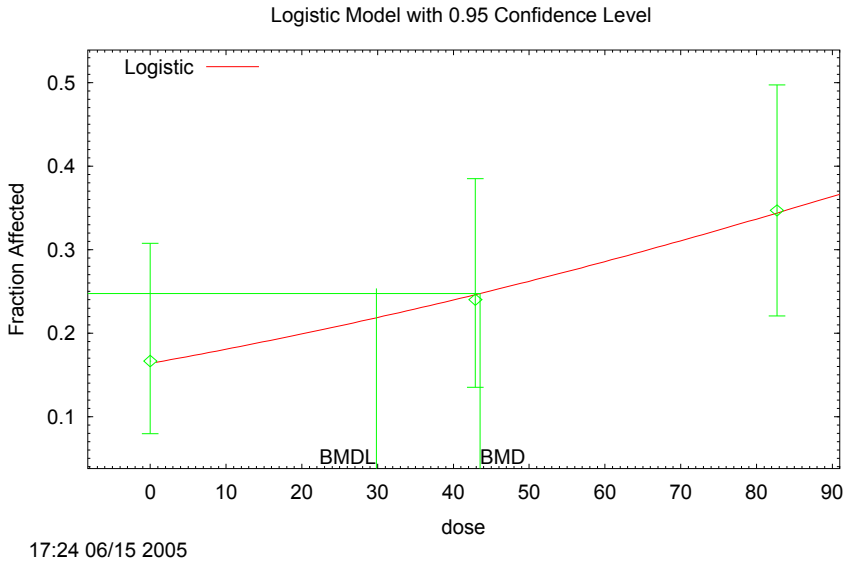
All dichotomous models in EPA'S BMDS (version 1.3.2; U.S. EPA, 2000c) were considered in modeling these data. The gamma, log-logistic, and Weibull models were not applied to this data set because the number of parameters in the models equaled the number of treatment groups and did not allow sufficient degrees of freedom. The BMD modeling results for the remaining models are summarized in Table B-2.

Table B-2. BMD modeling of incidence data for tubular regeneration in the kidneys of male mice exposed to 1,2-dichlorobenzene for 103 weeks

Model[a]	GOFP[b]	AIC[c]	BMD_{10}[d] (mg/kg-day)	$BMDL_{10}$[e] (mg/kg-day)
Logistic	0.9031	165.639	43.5	29.8
Log probit	0.8824	165.646	49.9	32.5
Probit	0.8791	165.647	42.6	28.6
Multistage (degree of polynomial =1)	0.7377	165.737	37.5	20.6
Quantal quadratic	0.7809	165.701	54.4	39.4

[a] The output file for the selected model is provided in this appendix.
[b] GOFP = Goodness-of-fit *p*-value for χ^2.
[c] AIC = Akaike Information Criterion.
[d] BMD_{10} = BMD calculated by BMDS associated with a 10% extra risk.
[e] $BMDL_{10}$ = 95% Lower confidence limit on the BMD_{10} as calculated by BMDS.

Source: NTP, 1985.




```
=====================================================================
        Logistic Model .  Revision: 2.1 .    .  Date: 2000/02/26 03:38:20 .
        Input Data File: G:\IRIS_CHEMICALS\DCBS\BMD\12-RFD\12DCB_MOUSE_KIDNEY_TUB_REGEN.(d)
        Gnuplot Plotting File:  G:\IRIS_CHEMICALS\DCBS\BMD\12-RFD\12DCB_MOUSE_KIDNEY_TUB_REGEN.plt
                                        Wed Jun 15 17:24:08 2005
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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


   Dependent variable = KidneyIncidence
   Independent variable = Dose(mg/kg/d)
   Slope parameter is not restricted

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


                  Default Initial Parameter Values
                     background =           0   Specified
                      intercept =    -1.57806
                          slope =   0.0113771


           Asymptotic Correlation Matrix of Parameter Estimates

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

              intercept        slope

 intercept            1        -0.83

     slope        -0.83            1


                          Parameter Estimates

       Variable           Estimate             Std. Err.
```

intercept	-1.63108	0.346638
slope	0.0119059	0.00590574

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-80.812			
Fitted model	-80.8194	0.0148669	1	0.903
Reduced model	-82.9368	4.24967	2	0.1195

AIC: 165.639

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1637	7.857	8	48	0.05588
42.9000	0.2460	12.298	12	50	-0.09774
82.7000	0.3438	16.846	17	49	0.04644

Chi-square = 0.01 DF = 1 P-value = 0.9031

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 43.5154

BMDL = 29.8208

B.2. BMD MODELING OF INCIDENCE DATA FOR THYROID LESIONS IN RATS ORALLY EXPOSED TO 1,3-DICHLOROBENZENE FOR 13 WEEKS

Incidence data for reduced follicular colloidal density in the male rat thyroid are summarized in Table B-3.

All dichotomous models in EPA's BMDS (version 1.3.2; U.S. EPA, 2000c) were fitted to these incidence data. The χ^2 goodness-of-fit statistics for all of these models indicated poor fits ($p<0.1$).

Data from the highest dose group (588 mg/kg-day) were dropped to see if an improved model fit could be achieved. The χ^2 goodness-of-fit statistic for all dichotomous models indicated inadequate statistical fits ($p<0.1$) using the control and first three dose groups.

Data from the two highest dose groups (147 and 588 mg/kg-day) were dropped to see if adequate model fits could be achieved (i.e., models were run using data for the control, 9 mg/kg-day, and 37 mg/kg-day dose groups only). The gamma, log-logistic, log-probit, and Weibull models could not be applied to this data set because the number of parameters in the models equaled the number of treatment groups and did not allow sufficient degrees of freedom. Model results for the remaining models are summarized in Table B-4. Although adequate models fits were obtained with these models (i.e., χ^2 goodness-of-fit p-value > 0.1), the dose associated with a BMR of 10% extra risk for reduced thyroid follicular colloidal density was well below the range of experimental doses. The model output and graph for the probit model is provided as an example of the model fit and dose at the BMR.

Table B-3. Incidence of reduced follicular colloidal density of the thyroid in male rats orally exposed to 1,3-dichlorobenzene for 90 days

Lesion	Dose (mg/kg-day)				
	0	9	37	147	588
Thyroid, reduced follicular colloidal density	2/10	8/10[a]	10/10[a]	8/9[a]	8/8[a]

[a] Significantly ($p<0.05$) different from control; Fisher's Exact Test performed by Syracuse Research Corporation.

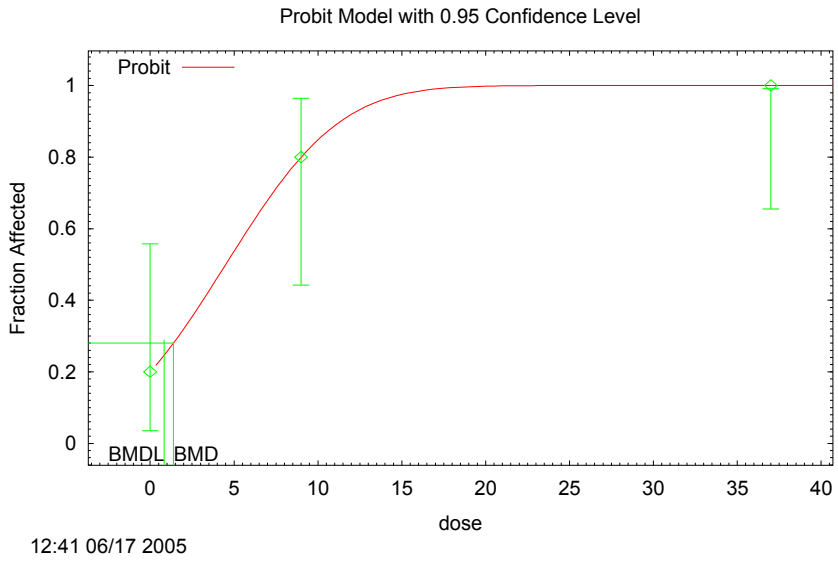
Source: McCauley et al., 1995.

Table B-4. BMD modeling of incidence data for reduced follicular colloidal density of the thyroid in male rats exposed to 1,3-dichlorobenzene

Model[a]	GOFP[b]	AIC[c]	BMD_{10}[d] (mg/kg-day)	$BMDL_{10}$[e] (mg/kg-day)
Probit	0.9999	24.0161	1.38	0.833
Quantal quadratic	1.0000	24.0161	2.48	1.76
Logistic	0.9831	24.017	1.43	0.785
Multistage (degree of polynomial = 1)	0.8705	24.0654	0.665	0.341

[a] Model results are those for models fit to data for the control, 9 mg/kg-day, and 37 mg/kg-day groups only.
[b] GOFP = Goodness-of-fit *p*-value for χ^2.
[c] AIC = Akaike Information Criterion.
[d] BMD_{10} = BMD calculated by BMDS associated with a 10% extra risk.
[e] $BMDL_{10}$ = 95% Lower confidence limit on the BMD_{10} as calculated by BMDS.

Source: McCauley et al., 1995.




```
================================================================
        Probit Model .  Revision: 2.1 .    .  Date: 2000/02/26 03:38:53 .
        Input Data File: G:\IRIS_CHEMICALS\DCBS\BMD\13-RFD\13DCB_RFD_THYROID_LESION_MINUS2DOSE.(d)
        Gnuplot Plotting File:
G:\IRIS_CHEMICALS\DCBS\BMD\13-RFD\13DCB_RFD_THYROID_LESION_MINUS2DOSE.plt
                                          Fri Jun 17 12:41:48 2005
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = ThyroidLesions
   Independent variable = Dose(mg/kg/d)
   Slope parameter is not restricted

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


                  Default Initial (and Specified) Parameter Values
                     background =           0   Specified
                      intercept =   -0.377062
                          slope =   0.0667914


           Asymptotic Correlation Matrix of Parameter Estimates

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

              intercept        slope

 intercept            1       -0.71

     slope        -0.71           1
```

Parameter Estimates

Variable	Estimate	Std. Err.
intercept	-0.841621	0.451816
slope	0.187027	0.0709961

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-10.008			
Fitted model	-10.008	1.2144e-008	1	0.9999
Reduced model	-19.0954	18.1748	2	0.0001131

AIC: 24.0161

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2000	2.000	2	10	2.617e-007
9.0000	0.8000	8.000	8	10	-1.896e-006
37.0000	1.0000	10.000	10	10	7.791e-005

Chi-square = 0.00 DF = 1 P-value = 0.9999

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.38365

BMDL = 0.832934

B.3. BMD MODELING OF INCIDENCE OF DIFFUSE HEPATOCELLULAR HYPERTROPHY IN MALE AND FEMALE BEAGLE DOGS EXPOSED ORALLY TO 1,4-DICHLOROBENZENE (MONSANTO COMPANY, 1996)

Incidence data for diffuse hepatocellular hypertrophy in male and female beagle dogs from the one-year Monsanto Company (1996) study are summarized in Table B-5. Because the incidence was similar in male and female dogs and because there was no evidence of sex-related differences in response of the liver to 1,4-dichlorobenzene toxicity, the male and female data were combined for BMD analysis.

Table B-5. Summary of incidence of diffuse hepatocellular hypertrophy in male and female beagle dogs exposed to 1,4-dichlorobenzene in gelatin capsules

	Dose (mg/kg-day)			
	0	**7**	**36**	**54**
Male dog	0/5	0/5	3/5	5/5
Female dog	0/5	0/5	2/5	4/5
Male and female dog combined	0/10	0/10	5/10	9/10

Source: Monsanto Company, 1996.

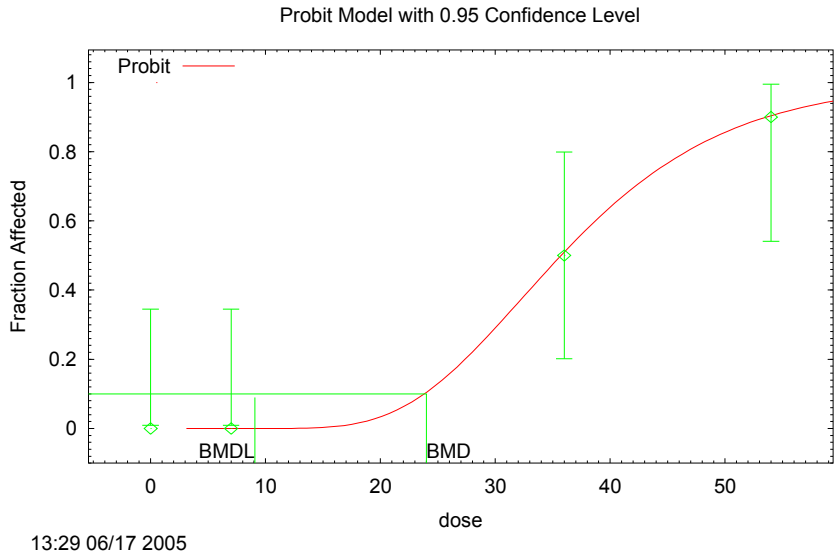
All dichotomous models in the EPA's BMDS (version 1.3.2; U.S. EPA, 2000c) were fit to the incidence data for diffuse hepatocellular hypertrophy in male and female beagle dogs (combined) using a BMR of 10% extra risk. Model results are summarized in Table B-6.

Table B-6. BMD modeling of incidence data for diffuse hepatocellular hypertrophy in male and female beagle dogs (combined) exposed to 1,4-dichlorobenzene

Model[a]	GOFP[b]	AIC[c]	BMD_{10}[d] (mg/kg-day)	$BMDL_{10}$[e] (mg/kg-day)
Log probit	1.0000	24.3646	24.0	9.06
Gamma	0.9997	24.3659	22.6	8.41
Log logistic	0.9993	24.3673	24.1	9.32
Quantal quadratic	0.8704	23.4114	12.9	10.2
Weibull	0.9742	24.4561	20.0	7.89
Multistage (degree of polynomial = 3)	0.9970	22.4661	19.3	6.45
Probit	0.9164	24.612	21.7	11.8
Quantal linear	0.3090	27.8142	4.30	2.77
Logistic	0.8545	24.8204	22.6	12.8

[a] The output file for the selected model is provided in this appendix.
[b] GOFP = Goodness-of-fit *p*-value for χ^2.
[c] AIC = Akaike Information Criterion.
[d] BMD_{10} = BMD calculated by BMDS associated with a 10% extra risk.
[e] $BMDL_{10}$ = 95% lower confidence limit on the BMD_{10} as calculated by BMDS.

Source: Monsanto Company, 1996.




```
=====================================================================
          Probit Model .  Revision: 2.1 .    .  Date: 2000/02/26 03:38:53 .
          Input Data File: C:\BMDS\UNSAVED1.(d)
          Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                                    Fri Jun 17 13:29:18 2005
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function

   Dependent variable = LiverInc
   Independent variable = Dose(mg/kg-d)
   Slope parameter is restricted as slope >= 1

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


   User has chosen the log transformed model


                  Default Initial (and Specified) Parameter Values
                     background =            0
                      intercept =     -4.36683
                          slope =      1.33335


           Asymptotic Correlation Matrix of Parameter Estimates

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

              intercept        slope

 intercept            1           -1

     slope           -1            1
```

Parameter Estimates

Variable	Estimate	Std. Err.
background	0	NA
intercept	-11.3265	6.16589
slope	3.16073	1.65293

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-10.1823			
Fitted model	-10.1823	2.26683e-006	2	1
Reduced model	-25.8979	31.4311	3	<.0001

AIC: 24.3646

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	10	0
7.0000	0.0000	0.000	0	10	-0.001065
36.0000	0.5000	5.000	5	10	1.378e-005
54.0000	0.9000	9.000	9	10	-1.442e-005

Chi-square = 0.00 DF = 2 P-value = 1.0000

Benchmark Dose Computation

Specified effect =	0.1	0.05
Risk Type =	Extra risk	
Confidence level =	0.95	
BMD =	24.0001	24.1
BMDL =	9.06092	6.68

B.4. BMD MODELING OF EOSINOPHILIC CHANGES TO THE OLFACTORY EPITHELIUM IN FEMALE RATS

Incidence data for eosinophilic changes in the olfactory epithelium in female rats from the 2-year JBRC (Aiso et al., 2005b; JBRC, 1995) study are summarized in Table B-7.

Table B-7. Incidence of eosinophilic changes in the olfactory epithelium in female rats

Exposure concentration (ppm)	Duration-adjusted concentration[a] (ppm)	HEC[b]		Incidence[d]
		(ppm)	(mg/m^3)[c]	
0	0	0	0	27/50
19.8	3.5	0.56	3.4	29/50
74.8	13.4	2.1	12.6	39/50
298.4	53.3	8.5	51.1	47/50

[a] The exposure concentrations were duration-adjusted for continuous exposure, using the following formula: $Conc_{[continuous]}$ = Conc x 5 days/7 days x 6 hours/24 hours

[b] For changes in the olfactory epithelium, the HEC was calculated using the rules for a category 1 gas with effects in the extrathoracic region as described by U.S. EPA (1994b), and using reference values found in U.S. EPA (1994b), Tables 4-4 and 4-5 and equation 4-4:

$HEC = \text{Duration-adjusted Concentration} \times RGDR_{ET}$

$RGDR_{ET} = [(V_E/SA_{(ET)})_A/(V_E/SA_{(ET)})_H]$

$= (0.24\ m^3/day/15\ cm^2)/(20\ m^3/day/200\ cm^2)$

$= 0.16$

where:

$RGDR_{ET}$ = Regional gas dose ratio,

V_E = minute volume (mL/min = cm^3/min),

$SA_{(ET)}$ = surface area of the extrathoracic region (cm^2), and

A, H = subscripts denoting laboratory animal and human, respectively.

[c] Conversion factor: 1 ppm = 6.01 mg/m^3.

[d] Incidence of olfactory epithelial lesions of moderate or greater severity in female rats.

Source: Aiso et al., 2005b; JBRC, 1995.

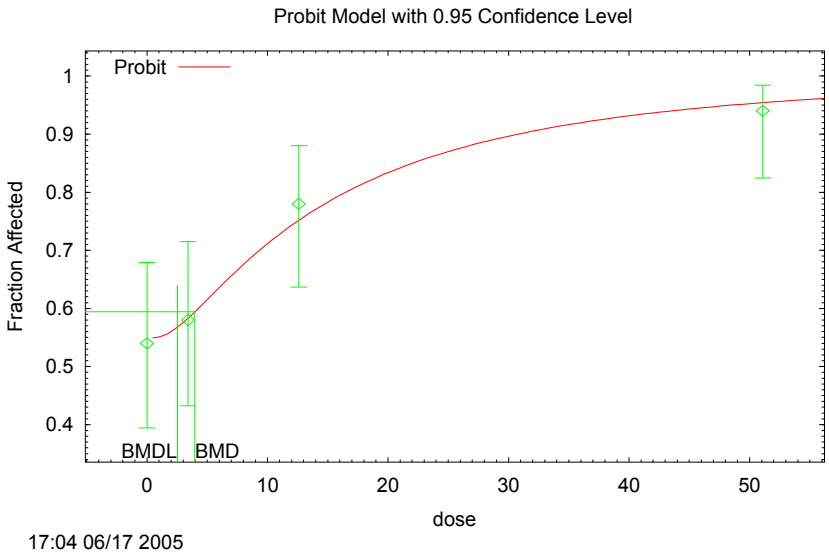
Dose-response modeling was performed using dichotomous models available in EPA's BMDS (version 1.3.2; U.S. EPA, 2000c). A 10% extra risk in incidence of olfactory epithelial lesion was used as the BMR. Model results are summarized in Table B-8.

Table B-8. BMC analysis for eosinophilic changes in the olfactory epithelium in female rats

Model[a]	GOFP[b]	AIC[c]	BMC_{10}[d] (mg/m^3)	$BMCL_{10}$[e] (mg/m^3)
Log probit	0.7928	216.861	3.97	2.52
Weibull	0.6830	217.183	2.41	1.62
Gamma	0.6830	217.183	2.41	1.62
Multistage (degrees of polynomial = 2)	0.6830	217.183	2.41	1.62
Quantal linear	0.6830	217.183	2.41	1.62
Logistic	0.4929	217.857	3.32	2.37
Probit	0.4037	218.286	3.80	2.86
Log logistic	0.7243	218.535	2.59	0.70
Quantal quadratic	0.0908	221.436	11.6	9.1

[a] The output file for the selected model is provided in this appendix.
[b] GOFP = Goodness-of-fit *p*-value for χ^2.
[c] AIC = Akaike Information Criterion.
[d] BMC_{10} = BMC calculated by BMDS associated with a 10% extra risk.
[e] $BMCL_{10}$ = 95% lower confidence limit on the BMC_{10} as calculated by BMDS.

Source: Aiso et al., 2005b; JBRC, 1995.




```
=====================================================================
          Probit Model .  Revision: 2.1 .    .  Date: 2000/02/26 03:38:53 .
          Input Data File: G:\IRIS_CHEMICALS\DCBS\BMD\14-RFC RAT OLF
EPITH\14-RFC_RATF_OLF_EPITH2.(d)
          Gnuplot Plotting File:  G:\IRIS_CHEMICALS\DCBS\BMD\14-RFC RAT OLF
EPITH\14-RFC_RATF_OLF_EPITH2.plt
                                                  Fri Jun 17 17:04:03 2005
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = OlfEpith
   Independent variable = Conc(mg/m3)
   Slope parameter is restricted as slope >= 1

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


   User has chosen the log transformed model


                  Default Initial (and Specified) Parameter Values
                     background =         0.54
                      intercept =     -2.68166
                          slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

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

             background    intercept

background            1        -0.55
```

intercept -0.55 1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0.549321	0.0553922
intercept	-2.66107	0.295939
slope	1	NA

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-106.206			
Fitted model	-106.431	0.450206	2	0.7984
Reduced model	-120.43	28.4495	3	<.0001

AIC: 216.861

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.5493	27.466	27	50	-0.1325
3.4000	0.5833	29.163	29	50	-0.04683
12.6000	0.7518	37.591	39	50	0.4613
51.1000	0.9542	47.711	47	50	-0.4814

Chi-square = 0.46 DF = 2 P-value = 0.7928

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.97299

BMDL = 2.5227

B.5. BMD MODELING OF MINERALIZATION OF THE TESTES OF MALE MICE

Incidence data for mineralization of the testes in male mice from the two-year JBRC study (Aiso et al., 2005b; JBRC, 1995) are summarized in Table B-9.

BMD analysis was performed on the data for mineralization of mouse testis using the dichotomous models in EPA's BMDS (version 1.3.2; U.S. EPA, 2000c) and a BMR of 10% extra risk. None of the models provided an adequate fit of the data (based on the goodness-of-fit statistic, p>0.1, and visual examination of the models in the low-dose region of the curve). Therefore, the data were modeled by dropping the high-exposure (298.3 ppm) group. The BMD model results with the modified data set are summarized in Table B-10.

Table B-9. Incidence of mineralization of the testes in male mice

Exposure concentration (ppm)	Duration-adjusted concentration[a] (ppm)	HEC[b]		Incidence
		(ppm)	(mg/m^3)[c]	
0	0	0	0	27/49
19.9	3.6	3.6	22	35/49
74.8	13.4	13.4	81	42/50
298.3	53.3	53.3	320	41/49

[a] The exposure concentrations were duration-adjusted for continuous exposure, using the following formula: $Conc_{[continuous]}$ = Conc x 5 days/7 days x 6 hours/24 hours.
[b] For changes in the testes of male mice, the HEC was calculated using the equations for a category 3 gas with extrarespiratory effects, as described by U.S. EPA (1994b). The HEC for extrarespiratory effects produced by a category 3 gas is calculated by multiplying the duration-adjusted concentration by the ratio of blood:gas partition coefficients ($H_{b/g}$) in animals and humans (U.S. EPA, 1994b). $H_{b/g}$ values were not available for 1,4-dichlorobenzene in mice and humans. In the absence of data on the blood/gas coefficients, a default value of 1 was used for the ratio of partition coefficients.
[c] Conversion factor: 1 ppm = 6.01 mg/m^3.

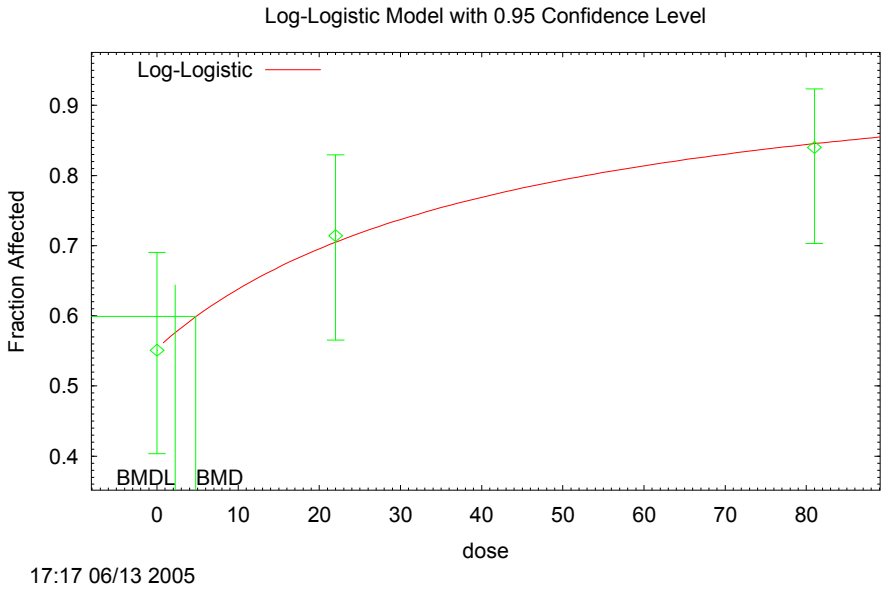
Source: Aiso et al., 2005b; JBRC, 1995.

Table B-10. BMD modeling of mineralization in the testes of male mice

Model[a]	GOFP[b]	AIC[c]	BMC_{10}[d] (mg/m^3)	$BMCL_{10}$[e] (mg/m^3)
Log logistic	0.8556	174.048	4.78	2.26
Gamma	0.5163	174.44	8.06	4.95
Multistage (degree of polynomial = 1)	0.5163	174.44	8.06	4.95
Quantal linear	0.5163	174.44	8.06	4.95
Weibull	0.5163	174.44	8.06	4.95
Logistic	0.4200	174.673	10.0	6.72
Probit	0.3959	174.744	10.6	7.35
Log probit	0.4000	174.74	10.6	7.35
Quantal quadratic	0.1541	176.071	27.4	20.8

[a] Model results are those for models fit to data for the control, low-dose and mid-dose groups only. The output file for the selected model is provided in this appendix.
[b] GOFP = Goodness-of-fit *p*-value for χ^2.
[c] AIC = Akaike Information Criterion.
[d] BMC_{10} = BMC calculated by BMDS associated with a 10% extra risk.
[e] $BMCL_{10}$ = 95% lower confidence limit on the BMC_{10} as calculated by BMDS.

Source: Aiso et al., 2005b; JBRC, 1995.




```
=====================================================================
          Logistic Model .  Revision: 2.1 .    .  Date: 2000/02/26 03:38:20 .
          Input Data File: G:\IRIS_CHEMICALS\DCBS\BMD\14-RFC MOUSE
TESTES\14RFC_MALE_MOUSE_TESTES_MINUSHIGHCONC.(d)
          Gnuplot Plotting File:  G:\IRIS_CHEMICALS\DCBS\BMD\14-RFC MOUSE
TESTES\14RFC_MALE_MOUSE_TESTES_MINUSHIGHCONC.plt
                                                    Mon Jun 13 17:17:05 2005
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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


   Dependent variable = Testes
   Independent variable = Conc(mg/3)
   Slope parameter is restricted as slope >= 1

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


   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =      0.55102
                     intercept =     -3.72211
                         slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

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

             background    intercept

background            1        -0.63
```

intercept -0.63 1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0.554442	0.0688921
intercept	-3.76113	0.516352
slope	1	NA

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.0074			
Fitted model	-85.0239	0.0331336	1	0.8556
Reduced model	-90.0664	10.1181	2	0.006352

AIC: 174.048

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.5544	27.168	27	49	-0.04819
22.0000	0.7053	34.557	35	49	0.1387
81.0000	0.8455	42.275	42	50	-0.1076

Chi-square = 0.03 DF = 1 P-value = 0.8556

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.77746

BMDL = 2.25636

B.6. BMD MODELING FOR HEPATIC TUMORS IN MICE EXPOSED ORALLY TO 1,4-DICHLOROBENZENE

Incidence data for hepatocellular adenomas or carcinomas (combined) in male and female mice from the NTP (1987) bioassay are summarized in Table B-11.

The multistage model in BMDS (version 1.3.2; U.S. EPA, 2000c) was used for all multistage model fits. A BMR of 10% extra risk (female mice) or 50% extra risk (male mice) was selected; see text, Section 5.3.3.1.5, for discussion of the basis for BMR selection. An oral slope factor was calculated by linear extrapolation from the BMCL. The data results of multistage modeling are summarized in Table B-12.

Table B-11. Tumor incidence data used for dose-response assessment for 1,4-dichlorobenzene

Exposure (mg/kg-day)	Male $B6C3F_1$ mouse		Female $B6C3F_1$ mouse	
	HED[a] (mg/kg-day)	Hepatocellular adenoma or carcinoma[b]	HED[a] (mg/kg-day)	Hepatocellular adenoma or carcinoma[b]
0	0	17/46	0	15/48
214	33	22/40	31	10/46
429	66	40/42	63	36/48

[a] HED was calculated using a dose scaling factor of body weight$^{0.75}$. Growth in treated male and female mice was similar to the respective controls; therefore, time-weighted average body weights in the controls (0.040 kg for males and 0.032 kg for females) were used to represent mouse body weights for purposes of dose scaling.
[b] Denominators were adjusted for early mortality.

Source: NTP, 1987.

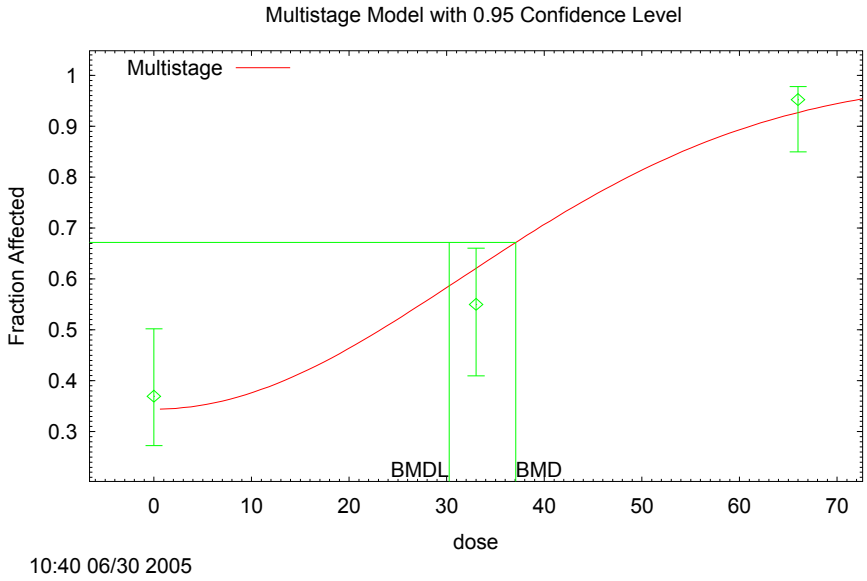
Table B-12. Results of multistage modeling of mouse tumor incidence

	Model degree[a]	GOFP[b]	BMR	BMD[c] (mg/kg-day)	BMDL[d] (mg/kg-day)	BMR/BMDL (mg/kg-day)$^{-1}$
Male hepatocellular adenoma or carcinoma	2	0.2379	50%	37.1	30.3	1.7×10^{-2}
Female hepatocellular adenoma or carcinoma	4	0.1251	10%	35.4	25.0	4.0×10^{-3}

[a] Determined by the lowest degree of the multistage model giving an adequate (p>0.1) model fit as described in U.S. EPA (2000c).
[b] GOFP = Goodness-of-fit p-value for χ^2.
[c] BMD = BMD calculated by BMDS associated with the BMR.
[d] BMDL = 95% lower confidence limit on the BMD as calculated by BMDS.

Source: NTP, 1987.

Male mice, hepatocellular adenomas and carcinomas




```
=====================================================================
        Multistage Model. .  Revision: 2.1 .    .  Date: 2000/08/21 03:38:21 .
        Input Data File: G:\IRIS_CHEMICALS\DCBS\BMD\14_ORALCANCER\14_ORALCANCER_MALEMOUSE_LIVER.(d)
        Gnuplot Plotting File:
G:\IRIS_CHEMICALS\DCBS\BMD\14_ORALCANCER\14_ORALCANCER_MALEMOUSE_LIVER.plt
                                          Thu Jun 30 10:40:13 2005
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = LiverAd+Carc
   Independent variable = Dose(mg/kg-d)

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =     0.273052
                        Beta(1) =            0
                        Beta(2) =  0.000614816


           Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.46
Beta(2)	-0.46	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.343869	0.105067
Beta(1)	0	NA
Beta(2)	0.000504392	0.000129707

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-65.8675			
Fitted model	-66.5805	1.42595	1	0.2324
Reduced model	-85.1743	38.6136	2	<.0001

AIC: 137.161

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.3439	15.818	17	46	0.114
i: 2					
33.0000	0.6212	24.847	22	40	-0.302
i: 3					
66.0000	0.9271	38.938	40	42	0.374

Chi-square = 1.39 DF = 1 P-value = 0.2379

Benchmark Dose Computation

Specified effect = 0.5

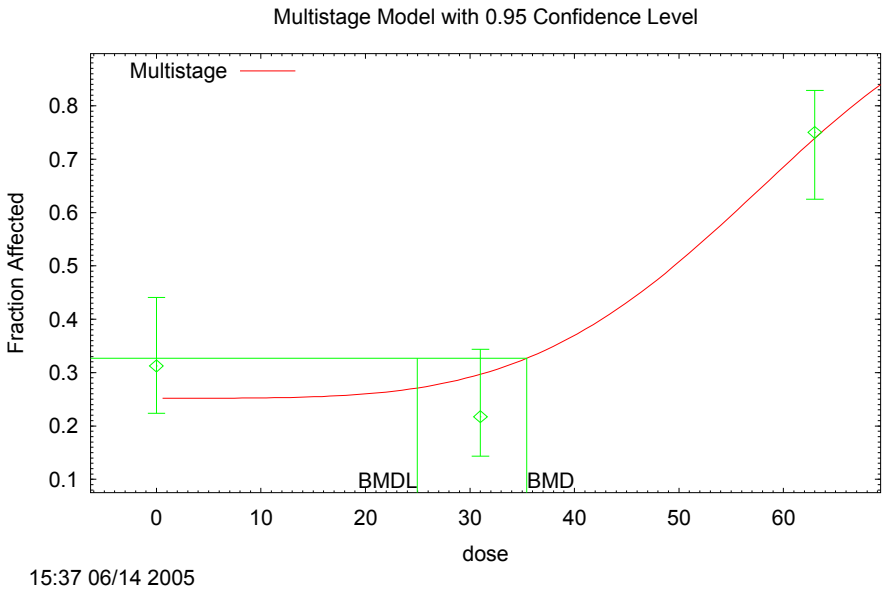
Risk Type = Extra risk

Confidence level = 0.95

BMD = 37.0705

BMDL = 30.2579

Female mice, hepatocellular adenomas and carcinomas




```
=====================================================================
        Multistage Model. .  Revision: 2.1 .    .  Date: 2000/08/21 03:38:21 .
        Input Data File:
G:\IRIS_CHEMICALS\DCBS\BMD\14_ORALCANCER\14_ORALCANCER_FEMALEMOUSE_LIVER.(d)
        Gnuplot Plotting File:
G:\IRIS_CHEMICALS\DCBS\BMD\14_ORALCANCER\14_ORALCANCER_FEMALEMOUSE_LIVER.plt
                                        Tue Jun 14 15:37:09 2005
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Observation # < parameter # for Multistage model.
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3-beta4*dose^4)]

   The parameter betas are restricted to be positive


   Dependent variable = LiverAd+Carc
   Independent variable = Dose(mg/kg-d)

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 5
 Total number of specified parameters = 0
 Degree of polynomial = 4


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =     0.311993
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) =            0
                        Beta(4) = 9.52202e-008
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1) -Beta(2) -Beta(3)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(4)
Background	1	-0.44
Beta(4)	-0.44	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.251826	0.0923417
Beta(1)	0	NA
Beta(2)	0	NA
Beta(3)	0	NA
Beta(4)	6.68308e-008	1.97322e-008

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-80.8892			
Fitted model	-82.0864	2.39443	1	0.1218
Reduced model	-97.0138	32.2491	2	<.0001

AIC: 168.173

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.2518	12.088	15	48	0.322
i: 2					
31.0000	0.2966	13.644	10	46	-0.380
i: 3					
63.0000	0.7389	35.468	36	48	0.057

Chi-square = 2.35 DF = 1 P-value = 0.1251

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 35.4344

BMDL = 24.9571

B.7. BMD MODELING TUMORS IN MICE EXPOSED BY INHALATION TO 1,4-DICHLOROBENZENE

Inhalation exposure of mice to 1,4-dichlorobenzene (Aiso et al., 2005b; JBRC, 1995) resulted in hepatocellular tumors in male and female mice, histiocytic sarcomas in male mice, and bronchoalveolar adenomas/carcinomas in female mice. The incidence data are presented in Table B-13.

Dose conversion for analysis of bronchoalveolar tumors in female mice: the HEC was calculated using the rules for a category 1 gas (U.S. EPA, 1994b), as described in Section 5.3.3.2.3. The resulting HEC values for the female mouse lung tumors were therefore 0, 12.2, 45.6, and 181 ppm, corresponding to 0, 74.8, 275, and 1090 mg/m^3, for the 0, 20, 75, and 300 ppm groups, respectively. The average of the two lower exposures was 28.9 ppm, or 173 mg/m^3.

The multistage model in BMDS (version 1.3.2; U.S. EPA, 2000c) was used for all multistage model fits. The multistage model was fitted to each data set, and a 10% response value (BMC_{10}) and the 95% lower bound on the dose causing a 10% response ($BMCL_{10}$) were calculated for each tumor type. An inhalation unit risk was calculated by linear extrapolation from the $BMCL_{10}$. The data results of multistage modeling are summarized in Table B-14.

Table B-13. Incidence data for tumors in mice exposed by inhalation

Tumor type	Incidence of tumors (%)			
	Control	20 ppm[a]	75 ppm[a]	300 ppm[a]
Male mice				
Hepatocellular adenoma	13/49 (27%)	9/49 (18%)	7/50 (14%)	13/49 (26%)
Hepatocellular carcinoma	12/49[b] (24%)	17/49 (34%)	16/50 (32%)	38/49 (76%)
Hepatocellular adenoma or carcinoma	20/49[b] (40%)	21/49 (42%)	18/50 (36%)	41/49 (82%)
Hepatic histiocytic sarcoma	0/49[b] (0%)	3/49 (6%)	1/50 (2%)	6/49 (12%)
Female mice				
Hepatocellular carcinoma	2/50[b] (4%)	4/50 (8%)	2/49 (4%)	41/50 (82%)
Hepatocellular adenoma	2/50[b] (4%)	10/50 (20%)	6/49 (12%)	20/50 (40%)
Hepatocellular adenoma or carcinoma	4/50[b] (8%)	13/50 (26%)	7/49 (14%)	45/50 (90%)
Bronchoalveolar adenoma or carcinoma	1/50[b] (2%)	4/50 (8%)	2/49 (4%)	7/50 (14%)

[a] Dose conversion for analysis of liver tumors: Administered concentrations of 1,4-dichlorobenzene were duration-adjusted as described in Section 5.2.3.2, resulting in mean continuous exposure levels of 0, 3.6, 13.4, and 53.3 ppm. The HEC was then calculated using the equations for a category 3 gas with extrarespiratory effects (U.S. EPA, 1994b), by multiplying the duration-adjusted concentration by the ratio of blood:gas partition coefficients ($H_{b/g}$) in animals and humans. Because $H_{b/g}$ values were not available for 1,4-dichlorobenzene in mice and humans, a default value of 1 was used for the ratio of partition coefficients. The HEC values for the mouse were therefore 0, 3.6, 13.4, and 53.3 ppm, corresponding to 0, 22, 81, and 320 mg/m^3, for the 0, 20, 75, and 300 ppm groups, respectively (1 ppm = 6.01 mg/m^3).

[b] Significant positive linear trend by Peto test.

Source: Aiso et al., 2005b; JBRC, 1995.

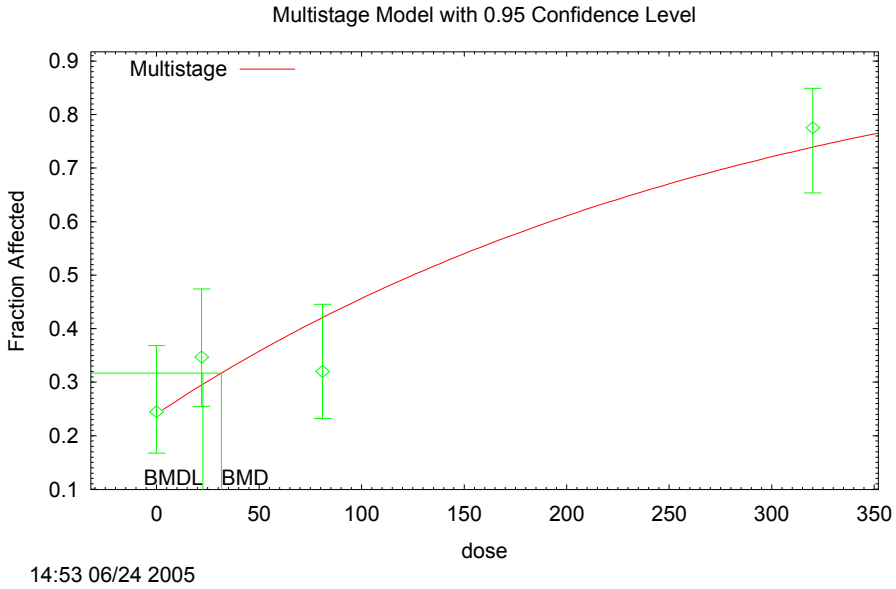
Table B-14. Results of multistage modeling of mouse inhalation tumor data

Tumor type	Polynomial degree[a]	GOFP[b]	BMC_{10}[c] (mg/m^3)	$BMCL_{10}$[d] (mg/m^3)	$0.1/BMCL_{10}$ $(mg/m^3)^{-1}$
Male mice					
Hepatocellular carcinoma	1	0.31	31.6	22.6	4.5×10^{-3}
Hepatocellular adenoma or carcinoma	2	0.46	92.0	39.8	2.5×10^{-3}
Histiocytic sarcoma	1	0.57	207	128	7.8×10^{-4}
Female mice					
Hepatocellular adenoma or carcinoma	2	1.0	41.3	22.9	4.4×10^{-3}
Bronchoalveolar adenoma or carcinoma	2	0.15	1089	524	1.9×10^{-4}

[a] Determined by the lowest degree of the multistage model giving an adequate (p>0.1) model fit as described in U.S. EPA (2000c).
[b] GOFP = Goodness-of-fit p-value for χ^2.
[c] BMC_{10} = BMC calculated by BMDS associated with a 10% extra risk.
[d] $BMCL_{10}$ = 95% lower confidence limit on the BMC_{10} as calculated by BMDS.

Source: Aiso et al., 2005b; JBRC, 1995.

Male mice, hepatocellular carcinomas




```
=====================================================================
      Multistage Model. .  Revision: 2.1 .    .  Date: 2000/08/21 03:38:21 .

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1)]

   The parameter betas are restricted to be positive

   Dependent variable = carc
   Independent variable = hec_mpcm

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                     Background =     0.218119
                        Beta(1) =    0.0037881


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)
Background            1        -0.53

   Beta(1)        -0.53            1

                        Parameter Estimates

      Variable           Estimate            Std. Err.
    Background             0.24087           0.0834058
```

Beta(1)	0.00333519	0.000947461

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-116.345			
Fitted model	-117.905	3.1211	2	0.21
Reduced model	-134.101	35.5116	3	<.0001

AIC: 239.811

Goodness of Fit

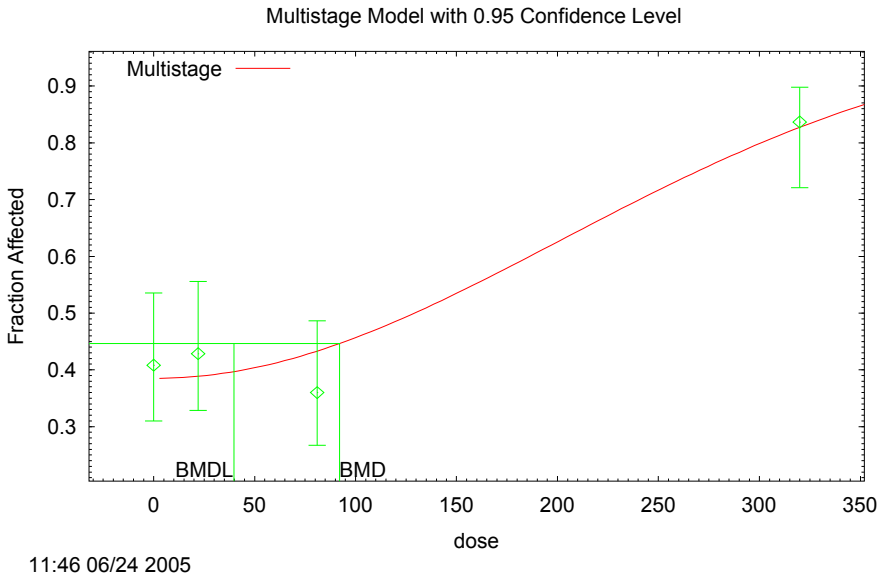
Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.2409	11.803	12	49	0.022
i: 2					
22.0000	0.2946	14.434	17	49	0.252
i: 3					
81.0000	0.4206	21.029	16	50	-0.413
i: 4					
320.0000	0.7389	36.206	38	49	0.190

Chi-square = 3.07 DF = 2 P-value = 0.2158

Benchmark Dose Computation

Specified effect = 0.1	Specified effect = 1e-006
Risk Type = Extra risk	Risk Type = Extra risk
Confidence level = 0.95	Confidence level = 0.95
BMD = 31.5905	BMD = 0.000299833
BMDL = 22.6168	BMDL = 0.000299026

Male mice, hepatocellular adenomas or carcinomas




```
=====================================================================
      Multistage Model. .  Revision: 2.1 .    .  Date: 2000/08/21 03:38:21 .
      Input Data File: G:\_BMDS\UNSAVED1.(d)
      Gnuplot Plotting File:  G:\_BMDS\UNSAVED1.plt
                                          Fri Jun 24 11:46:35 2005
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = hepadcarc
   Independent variable = hec_mpcm

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                     Background =     0.382623
                        Beta(1) =            0
                        Beta(2) =  1.2917e-005

            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Beta(1)
                  have been estimated at a boundary point, or have been specified by the user,
                  and do not appear in the correlation matrix )

              Background      Beta(2)
```

Background	1	-0.35
Beta(2)	-0.35	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.385125	0.0666436
Beta(1)	0	NA
Beta(2)	1.24393e-005	3.55442e-006

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-121.074			
Fitted model	-121.859	1.57161	2	0.4558
Reduced model	-136.527	30.9071	3	<.0001

AIC: 247.719

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.3851	18.871	20	49	0.097
i: 2					
22.0000	0.3888	19.052	21	49	0.167
i: 3					
81.0000	0.4333	21.666	18	50	-0.299
i: 4					
320.0000	0.8280	40.571	41	49	0.061

Chi-square = 1.56 DF = 2 P-value = 0.4592

Benchmark Dose Computation

Specified effect = 0.1

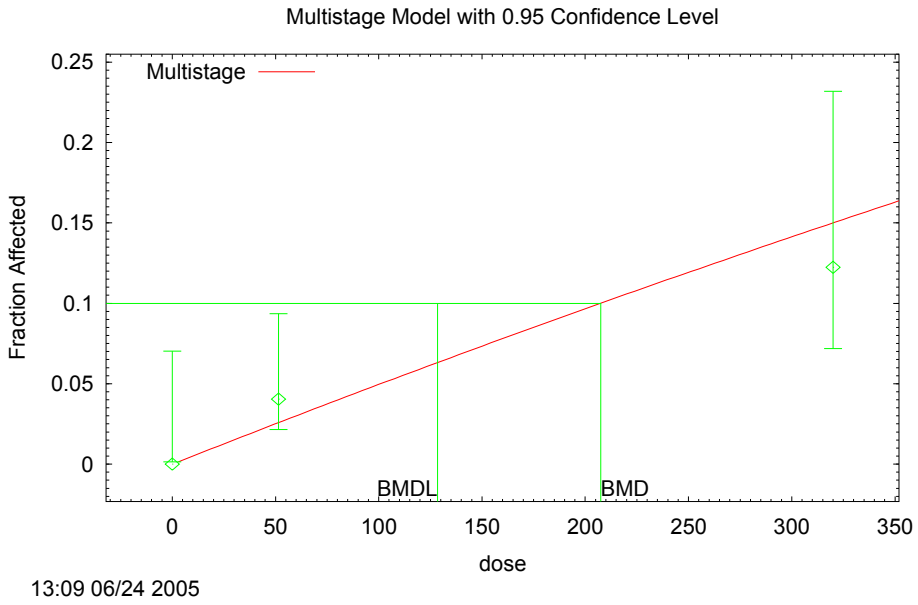
Risk Type = Extra risk

Confidence level = 0.95

BMD = 92.0324

BMDL = 39.8086

Male mice, histiocytic sarcomas




```
=====================================================================
      Multistage Model. .  Revision: 2.1 .    .  Date: 2000/08/21 03:38:21 .
=====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = sarcoma
   Independent variable = hec_mpcm

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =   0.00975994
                        Beta(1) =  0.000383417
                        Beta(2) =            0


            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Background    -Beta(2)
                  have been estimated at a boundary point, or have been specified by the user,
                  and do not appear in the correlation matrix )

                 Beta(1)

   Beta(1)            1
```

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA
Beta(1)	0.000507896	0.00050756
Beta(2)	0	NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus5 has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-34.9704			
Fitted model	-35.4828	1.02475	2	0.5991
Reduced model	-39.548	9.15511	2	0.01028

AIC: 72.9656

Goodness of Fit

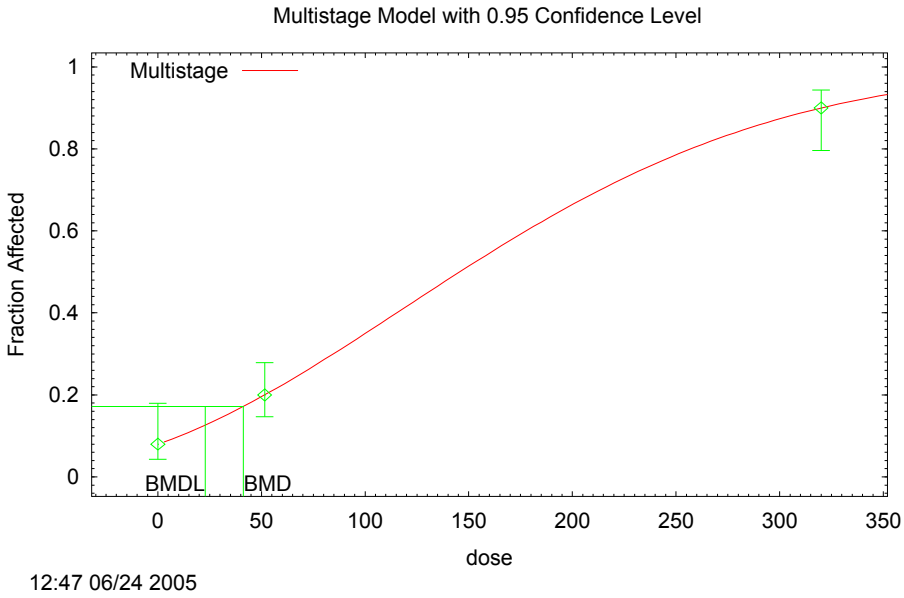
Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.0000	0.000	0	49	0.000
i: 2					
51.5000	0.0258	2.556	4	99	0.580
i: 3					
320.0000	0.1500	7.350	6	49	-0.216

Chi-square = 1.13 DF = 2 P-value = 0.5685

Benchmark Dose Computation

Specified effect = 0.1	Specified effect = 1e-006
Risk Type = Extra risk	Risk Type = Extra risk
Confidence level = 0.95	Confidence level = 0.95
BMD = 207.445	BMD = 0.00196891
BMDL = 128.477	BMDL = 0.00196601

Female mice, hepatocellular adenomas or carcinomas




```
 ======================================================================
      Multistage Model. .  Revision: 2.1 .    .  Date: 2000/08/21 03:38:21 .
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = br_adcarc
   Independent variable = dose

 Total number of observations = 4
 Total number of records with missing values = 1
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =         0.08
                        Beta(1) =   0.00190417
                        Beta(2) = 1.57214e-005

           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)      Beta(2)

Background            1        -0.79         0.68

   Beta(1)        -0.79            1        -0.94

   Beta(2)         0.68        -0.94            1

                         Parameter Estimates
```

Variable	Estimate	Std. Err.
Background	0.08	0.135647
Beta(1)	0.00190417	0.00422057
Beta(2)	1.57214e-005	1.31343e-005

Error in computing chi-square; returning 2

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-80.2329			
Fitted model	-80.2329	0	0	NA
Reduced model	-128.859	97.2528	2	<.0001

AIC: 166.466

Goodness of Fit

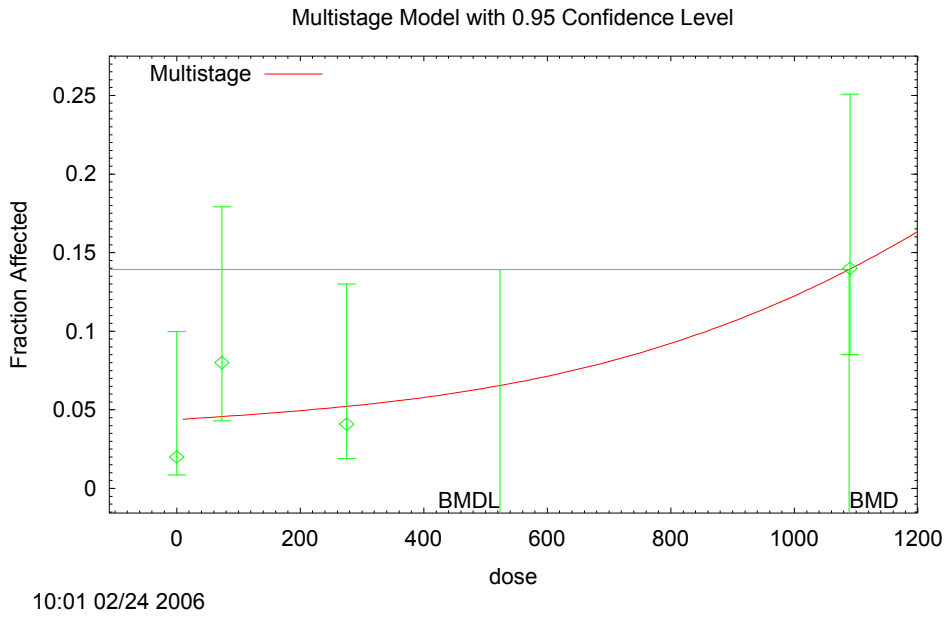
Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.0800	4.000	4	50	0.000
i: 2					
51.5000	0.2000	20.000	20	100	0.000
i: 3					
320.0000	0.9000	45.000	45	50	-0.000

Error in computing chi-square; returning 2
Chi-square = 0.00 DF = 0 P-value = undefined

Benchmark Dose Computation

Specified effect = 0.1	Specified effect = 1e-006
Risk Type = Extra risk	Risk Type = Extra risk
Confidence level = 0.95	Confidence level = 0.95
BMD = 41.2695	BMD = 0.00052516
BMDL = 22.8727	BMDL = 0.000461707

Female mice, bronchoalveolar adenomas or carcinomas




```
=============================================================================
      Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21
=============================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = #LungTumors
   Independent variable = Conc(mg/m3)

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =    0.0451268
                        Beta(1) = 1.56447e-005
                        Beta(2) =           0
                        Beta(3) =   6.752e-011


            Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(1)	Beta(3)
Background	1	-0.76	0.69
Beta(1)	-0.76	1	-0.98
Beta(3)	0.69	-0.98	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.0436788	0.124965
Beta(1)	2.80007e-005	0.000917187
Beta(2)	0	NA
Beta(3)	5.80084e-011	7.09252e-010

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-47.4446			
Fitted model	-48.488	2.08697	1	0.1486
Reduced model	-50.655	6.42094	3	0.09283

AIC: 102.976

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.0437	2.184	1	50	-0.567
i: 2					
73.3000	0.0457	2.283	4	50	0.788
i: 3					
275.0000	0.0522	2.556	2	49	-0.229
i: 4					
1090.0000	0.1396	6.978	7	50	0.004

Chi-square = 2.15 DF = 1 P-value = 0.1424

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1088.79

BMDL = 523.669

Specified effect = 0.1	Specified effect = 1e-006
Risk Type = Extra risk	Risk Type = Extra risk
Confidence level = 0.95	Confidence level = 0.95
BMD = 1088.79	BMD = 0.0357134
BMDL = 523.669	BMDL = 0.00585679