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)

11/04/03

NOTICE

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

U.S. Environmental Protection Agency
Washington D.C.

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FOREWORD

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

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent 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.

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Director has achieved a consensus approval 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 the Regional Offices.

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Summaries of the external peer reviewers' comments *[and public comments, if applicable]* and the disposition of their recommendations are in Appendix A.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is 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 noncancer effects during a lifetime. The inhalation RfC 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). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure 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 presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for dichlorobenzenes 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 may include the following: the *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *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); *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); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in*

Health Risk Assessment (U.S. EPA, 1995); *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999); Science Policy Council Handbook: Peer Review (U.S. EPA, 1998b, 2000a); *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b).

Literature search strategy employed for this compound were based on the CASRN and at least one common name. The following databases were searched for literature published between January 1990 and August 2002: TOXLINE, MEDLINE, BIOSIS/NTIS, RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC/EMICBACK, and DART/ETICBACK. 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 August 2002.

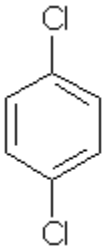
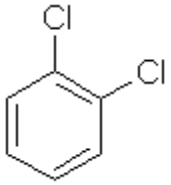
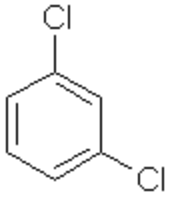
2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

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 chemical identity is shown in Table 2-1. Physical and chemical properties for 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. In a preparation using ferric chloride and sulfur monochloride, 1,4-dichlorobenzene has the highest yield at 75%. 1,2-Dichlorobenzene is produced with a 25% yield, and 1,3-dichlorobenzene is produced with a yield of 0.2%. Production of 1,2-dichlorobenzene in the United States has decreased from 24,700 tons in 1975 to 15,800 tons in 1993. Production of 1,4-dichlorobenzene, however, has increased from 6,800 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).

Dichlorobenzenes are used primarily as reactants in chemical synthesis, as process solvents, and as formulation solvents (U.S. EPA, 1981; 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). 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 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 and pharmaceuticals, polyphenylene sulfide resins, and in the control of mildew (IARC, 1999; U.S. EPA, 1981).

Table 2-1. Chemical Identity of Dichlorobenzene Isomers

Characteristic				Reference
Chemical Name	1,4-Dichlorobenzene	1,2-Dichlorobenzene	1,3-Dichlorobenzene	Lide, 2000
Synonyms	p-Dichlorobenzene; p-Chlorophenyl chloride; PDB; p-Dichlorobenzol	o-Dichlorobenzene; p-Chlorophenyl chloride; PDB; o-Dichlorobenzol	m-Dichlorobenzene; m-Phenylene dichloride; m-DCB; m-Dichlorobenzol	HSDB, 2002
Trade names	Paradi; Persia-Perazol; Paradow; Santochlor Paramoth; Para-zene; Di-chloricide	Chloroben; Cloroben; Dilatin DB; Dowtherm E	No data	HSDB, 2002 Budavari, 1989
Chemical formula	C6H4Cl2	C6H4Cl2	C6H4Cl2	Budavari et al., 2001
Chemical structure				Verschueren, 2001
CAS Registry	106-46-7	95-50-1	541-73-1	Budavari et al., 2001
NIOSH RTECS	CZ4550000	CZ4500000	CZ4499000	HSDB, 2002
EPA Hazardous Waste	U072; D027	U070; F002	U071	HSDB, 2002
EPA Pesticide Chemical Code	061501	059401	No data	HSDB, 2002
OHM/TADS	No data	No data	No data	

CAS = Chemical Abstracts Service; NIOSH = National Institute for Occupational Safety and Health; RTECS = Registry of Toxic Effects of Chemical Substances; EPA = Environmental Protection Agency; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute

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 .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	0.0015 atm-m^3/mol	2.83x10^{-3} atm-m^3/mol	2.7x10^{-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

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

Quantitative data on the extent or rate of absorption of dichlorobenzene isomers in humans following oral, inhalation, or dermal exposure are not available. However, qualitative evidence of absorption in humans comes from reports of the detection of dichlorobenzenes or their metabolites in samples of human breast milk (Jan, 1983, Mes et al., 1986), blood (Bristol et al., 1982; Hill et al., 1995), and urine (Ghittori et al., 1985; Hill et al., 1995; Kumagi and Matsunaga, 1995, 1997; Pagnotto and Walkley, 1965; Zenser et al., 1997). 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 (7.5 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, 1995, 1997; Zenser et al., 1997) or 1,4-dichlorobenzene (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 (Azouz et al., 1955; Bomhard et al., 1998; Hissink et al., 1996a,b, 1997a; Schmidt and Löser 1977). 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., 1996a,b, 1997a).

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 indications of absorption by the respiratory tract have been reported in several studies of rats exposed by inhalation to 1,4-dichlorobenzene (Hawkins et al., 1980; Umemura et al., 1989, 1990). 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 at 3-6 hours after exposure ceased, 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 by the gastrointestinal tract: 1) translocation of parent compounds 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 compounds in fat when metabolism is saturated; and 5) minor distribution of parent compounds 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.

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

Table 3-1. Tissue Concentrations of Radioactivity in Male Wistar Rats at Four Time Points after Oral Administration of 10 mg/kg ^{14}C-Labeled 1,2-Dichlorobenzene in Corn Oil (Source: Hissink et al., 1996a)

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

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). 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 in urine and 6-8% was in feces collected within 72 hours (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 (event number 2 above) 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 excreted bile within about 30 hours of dose administration, whereas in non-cannulated rats, orally administered radioactivity from ^{14}C-labeled 1,2-dichlorobenzene was predominately (75-84%) 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 was in the bile, 40-50% in the urine, and <5% in the feces collected within 24 hours of dose administration (Hissink et al., 1997a).

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

Table 3-2. Tissue Concentrations of Radioactivity (ppm) in Female CFY/Sprague-Dawley Rats During and After Exposure to Up to 10 Consecutive 250-mg/kg Oral Doses of ^{14}C-Labeled 1,4-Dichlorobenzene (Source: Hawkins et al., 1980)

	Fat	Kidney	Liver	Plasma	Lung	Muscle
# 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

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

higher at most sacrifice intervals in rats exposed by inhalation than in 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 relative to inhalation exposure. Further support for this pattern with regard to distribution following oral or inhalation exposure comes from the observation that in male F344 DuCrj rats exposed to 500 ppm 1,4-dichlorobenzene for 24 hours, the highest peak tissue concentrations of parent compound were 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 of ceasing exposure. This observation supports the findings from oral exposure studies that storage of dichlorobenzene isomers in tissues is temporary (i.e., the parent compounds are rapidly eliminated).

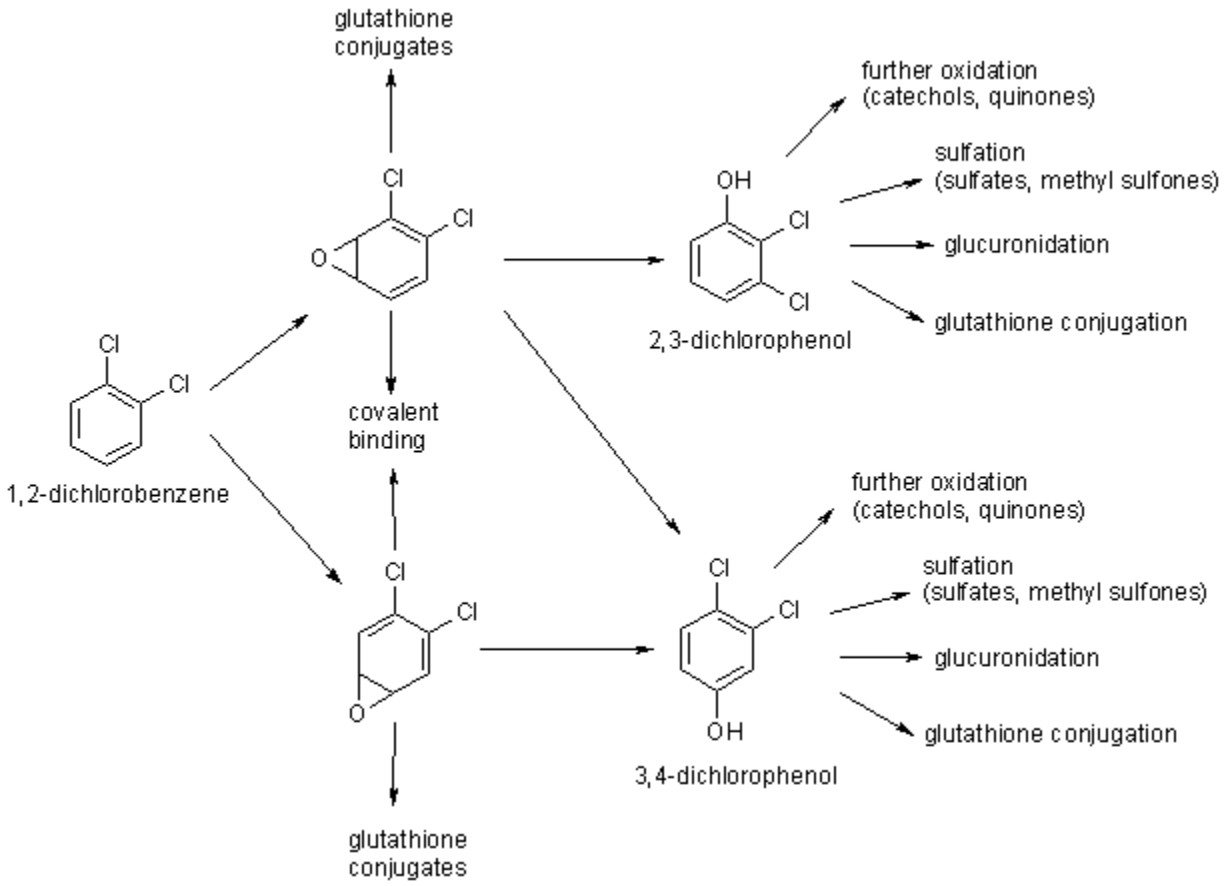
3.3. METABOLISM

Data indicate that the dichlorobenzenes are extensively metabolized, as evidenced by the lack of detectable parent compound in the urine or feces in available studies. A proposed scheme of the major metabolites of each of the dichlorobenzene isomers is presented in Figures 3-1 to 3-3. Metabolism is believed to occur primarily in the liver, and does not appear to be route-dependent (Hissink et al., 1997a).

The initial step in the metabolism of all three isomers is hydroxylation by cytochrome P450 enzymes, most notably cytochrome P450 2E1 (Bogaards et al., 1995; Hissink et al., 1996b,c; Nedelcheva et al., 1998). While all three isomers are metabolized mainly by P450 2E1, metabolism of the 1,4-isomer appears to occur to a lesser magnitude (Nedelcheva et al., 1998). Oxidation of the aromatic ring is believed to lead to epoxide formation, which is believed to be the source of the considerable levels (9-50%, depending on study conditions and dichlorobenzene isomer) of covalent binding demonstrated in *in vitro* studies of dichlorobenzene metabolism (den Besten et al., 1992). The epoxide may also react directly with glutathione to form a glutathione conjugate, or may be converted to one or more dichlorophenol metabolites (Hissink et al., 1996c).

Following oxidation by cytochrome P450, first to epoxide intermediates and then mainly to dichlorophenols, extensive secondary metabolism occurs. Evidence for this consists both of detection of considerable levels of secondary metabolites in the urine of exposed animals, as well as only small amounts of detectable urinary dichlorophenols (Hawkins et al., 1980; Hissink et al., 1996b).

Conjugation to glucuronic acid is believed to be of considerable importance, particularly for the 1,4-isomer. Studies in animals have demonstrated that 22-36% of 1,4-dichlorobenzene (Azouz et al., 1954; Hawkins et al., 1980; Hissink et al., 1996b, 1997a) is eliminated in the urine as the glucuronide conjugate. Reports concerning the extent of glucuronidation of 1,2-dichlorobenzene vary widely, with studies ranging from reporting virtually no



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

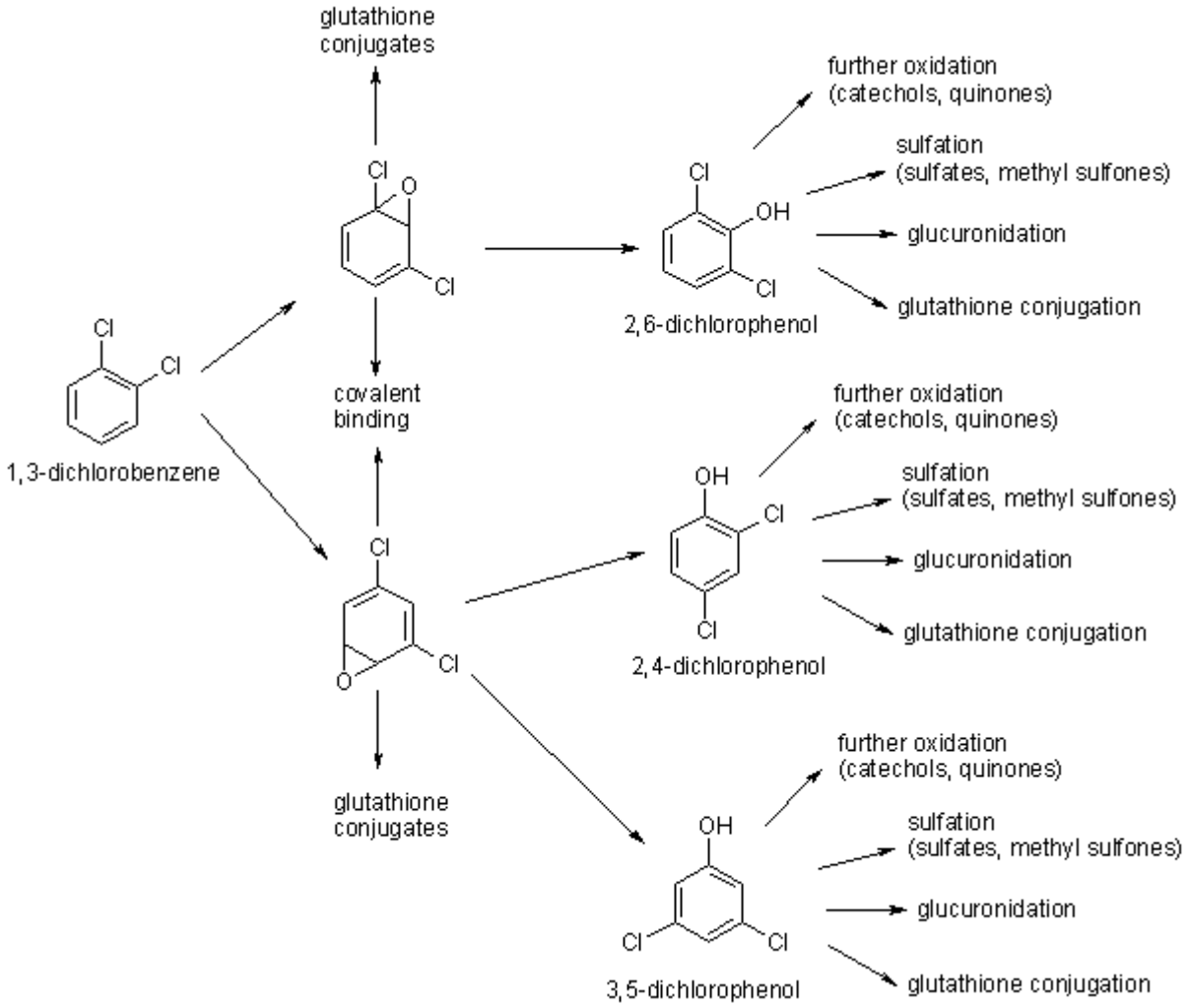


Figure 3-2. Metabolism of 1,3-Dichlorobenzene

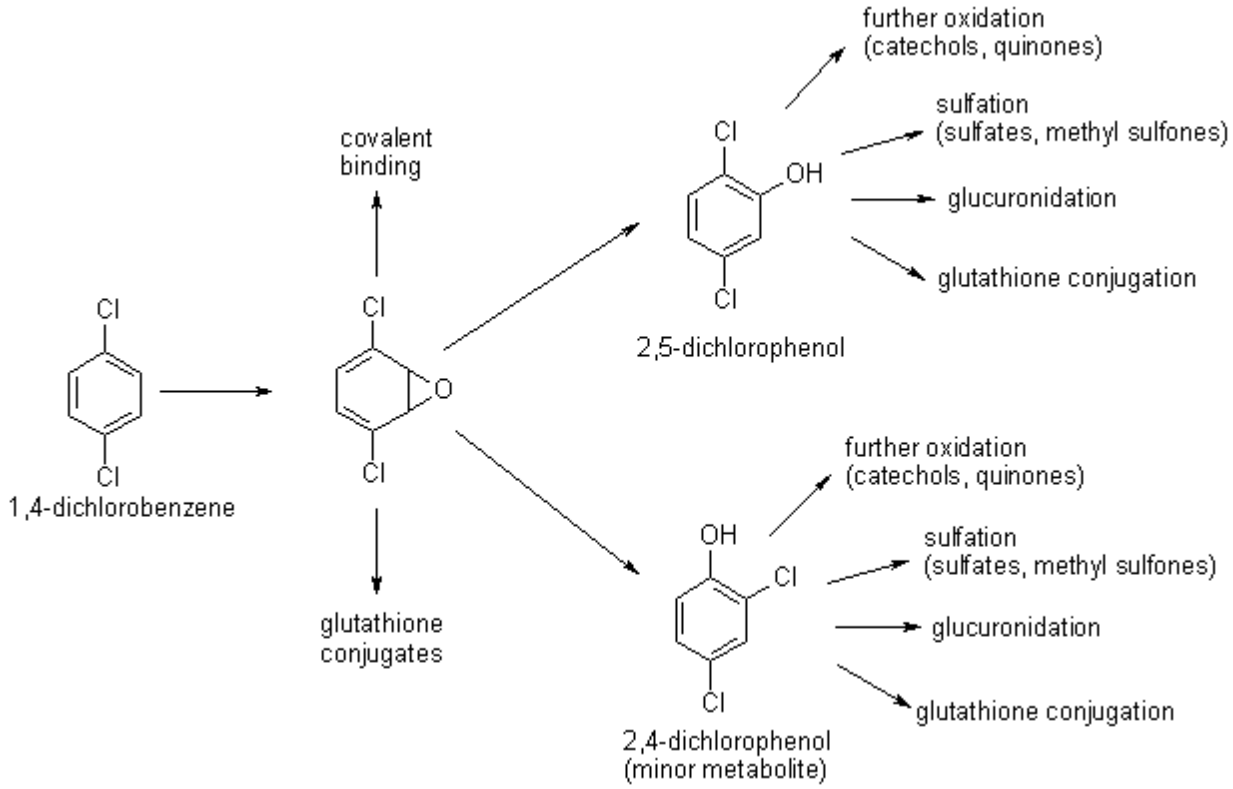


Figure 3-3. Metabolism of 1,4-Dichlorobenzene

glucuronidation in a study in rats (Hissink et al., 1996b) to those 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, intraspecies 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 (Azouz et al., 1954; Hissink et al., 1996b) and 27-65% of a single oral dose of 1,4-dichlorobenzene (Azouz et al., 1954; Hawkins et al., 1980, Hissink et al., 1996b, 1997a).

In vitro studies have also identified conjugation to glutathione, with subsequent metabolism to the n-acetyl cysteine and mercapturic acid, as a potential metabolic pathway. However, the *in vivo* relevance of this pathway appears to vary considerably from study to study, and between the isomers of dichlorobenzene; 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 glutathione following a single administration in rats accounted for approximately 60% of the dose (Hissink et al., 1996b). In rabbits, the mercapturic acid consisted of less than 10% of the urinary metabolites (Azouz et al., 1954). Glutathione conjugation appears to be of minimal importance for 1,4-dichlorobenzene, with only small, if any, detectable levels of the mercapturic acid in the urine of exposed animals (Azouz et al., 1954; Hissink et al., 1996b, 1997a).

A minor pathway of toxicological significance involves the formation of methyl sulfone metabolites. Following oxidation by cytochrome 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 re-absorbed and transported back to the liver. While these represent a proportionally small percentage of the total metabolites, they are extremely potent inducers of cytochrome P450 enzymes (Kato et al., 1986, 1988a,b; Kato and Kimura, 1997; Kimura et al., 1985; Larsen et al., 1990), with even small levels of methyl sulfones resulting in considerable hepatic enzyme induction.

3.4. ELIMINATION AND EXCRETION

As discussed previously in Sections 3.1 and 3.2 , 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 principally as metabolites in the urine. Excretion via exhaled breath or feces represent minor pathways. The studies show that neither parent compounds nor metabolites persist in fat or other tissues (see Tables 3-1 and 3-2).

As discussed in Sections 3.1, levels of parent compounds 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 (Ghittori et al., 1985; Kumagai and Matsunaga, 1995,

1997; Zenser et al., 1997; 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 workshift 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) does not currently recommend biological exposure indices for workplace exposure to dichlorobenzene isomers.

3.5. PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS

A physiologically-based pharmacokinetic (PBPK) model has been developed for 1,2-dichlorobenzene in rats and humans (Hissink et al., 1997b). 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. (1997b) have four compartments connected by blood flows: rapidly perfused tissues including the lung, kidneys and spleen; slowly perfused tissues comprising muscle and skin; fat; and the 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 accounted for 1,2-dichlorobenzene metabolism and reduced glutathione (GSH) synthesis, turnover, and consumption.

Physiologic 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 of the parameters to data for rats exposed to 5, 50, or 250 mg/kg 1,2-dichlorobenzene (Table 3-3).

Metabolism in the model is described as the initial, P450-mediated, saturable formation of an epoxide, followed by epoxide transformation via three competing pathways that are assumed to independently follow pseudo first-order kinetics (i.e., they are non-saturable): 1) conversion into dichlorophenol; 2) covalent binding to cellular macromolecules; and 3) conjugation with GSH. Michaelis-Menten constants, Vmax and Km, for the saturable cytochrome-P450 oxidation of 1,2-dichlorobenzene were initially estimated (in units of nmol/min-mg protein) from *in vitro* experiments with rat and human liver microsomes (Table 3-3). Scaling for use in the models assumed respective rat and human values of 45 and 77 mg microsomal protein per gram liver. 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

Table 3-3. Parameters in PBPK Models for 1,2-Dichlorobenzene Developed by Hissink et al. (1997b)

Parameter	Rat	Human
Physiologic parameters (as per Gargas et al., 1986)		
Body weight (kg)	0.258	70
Percentages of body weight		
Liver	4	3.14
Fat	7	23.1
Rapidly perfused	5	2.66
Slowly perfused	75	62.1
Flows (L/hour)		
[QC or QP= 15L/hour (body weight)$^{0.74}$]		
Cardiac output (QC)	5.50	348.0
Alveolar ventilation (QP)	5.50	348.0
Percentages of cardiac output		
Liver	25	25
Fat	9	9
Rapidly perfused	51	51
Slowly perfused	15	15
Partition coefficients [calculated by methods of Droz et al. (1989) based on water:air, oil:air, and blood:air partition coefficients]		
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		
1,2-Dichlorobenzene oxidation		
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 (estimated by fitting parameters to data for rats at indicated dose levels)		
Ka (hour^{-1})		
5 mg/kg	0.5	–
50 mg/kg	0.18	–
250 mg/kg	0.06	0.06

was about 4-fold 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 resultant constants for dichlorophenol formation and GSH conjugation were 300 $hour^{-1}$ and 650 $hour^{-1}$, respectively (Table 3-3). *In vitro* data 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).

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., 1997b). 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 values by a factor of 10 did not increase the predicted human hepatic concentrations, 24 hours after dosing, to a value above about 240 μM. Thus, the models predicted that equivalent administered doses in rats and humans would produce rat hepatic concentrations of covalently bound metabolites that are at least 6-fold higher in rats than humans.

The models were also used to predict hepatic concentrations of GSH (expressed as a percentage of an assumed baseline concentration of 6.5 mM) following an oral dose of 250 mg/kg 1,2-dichlorobenzene (Hissink et al., 1997b). The rat model predicted that maximum depletion of GSH (about 70% depletion) occurred at 15 hours after dosing with 250 mg/kg. 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 100% depletion) occurred at 10 hours after dosing. Thus, the models predicted that humans may be more susceptible to 1,2-dichlorobenzene depletion of hepatic GSH levels than are rats. Hissink et al. (1997b) noted that if depletion of GSH is the only factor involved in acute 1,2-dichlorobenzene hepatotoxicity, the models predict that humans may be more susceptible than rats at the same administered dose levels. Whereas if covalent binding of reactive metabolites is the critical factor, humans may be less susceptible to 1,2-dichlorobenzene acute hepatotoxicity than rats.

4. HAZARD IDENTIFICATION

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

4.1.1. Oral Exposure

Information on the toxicity of ingested dichlorobenzenes is limited to 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). Traces of 2,5-dichloroquinol 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, although 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 count, total and differential white blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, blood urea nitrogen, sedimentation rate, or urinalysis) were attributable to exposure. Additional information on the medical examinations was not provided. Hollingsworth et al. (1958) noted that his researchers detected 1,2-dichlorobenzene odor at a concentration of 50 ppm without eye or nasal irritation during repeated vapor inhalation experiments on animals. An earlier source (Elkins, 1950) reported that occupational exposure to 100 ppm of 1,2-dichlorobenzene caused irritation of the eyes and respiratory passages.

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 and 3832 of the subjects were determined to be alive and deceased, respectively. Determination of standardized mortality ratios (SMRs)

showed that mortality in the entire cohort was slightly reduced from all causes of death (SMR=9 [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 number for the Utah population. The only causes of death assessed by 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 intermittently or continually occupationally exposed 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 can cause 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 are considered to be fairly good warning properties for excessive exposures to 1,4-dichlorobenzene, but the industrial experience indicated that it is possible for people to become sufficiently acclimated 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 (red blood cell count, total and differential white blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, blood urea nitrogen, 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 are 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 from her bedroom, bedding, and clothing via liberal use of 1,4-dichlorobenzene as an insect repellant for 6 years (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 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 (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 $\geq$135 mg/kg-day. Additional effects were found at 270 mg/kg-day that included slight to moderate cloudy swelling in 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 weight in the absence of histopathological 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 blood urea nitrogen (BUN)], urinalysis (6 indices), ophthalmic condition, and selected organ weights (brain, liver, spleen, lungs, thymus, kidneys, adrenal glands, heart, and testes or ovaries). Histological 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 each 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). The only observed alterations in food consumption were increased total food consumption in the females rats at 400 mg/kg-day group during weeks 11-13. Statistically significant changes in organ weights included dose-related increases in absolute and relative liver weights in both sexes at $\geq$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 $\geq$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). Histopathological alterations were only observed 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, histological examinations were not performed in the low- and middle-dose groups, and were limited to one half of each 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. Thus, the increase in liver weight without clear evidence of tissue damage or increase in liver associated enzymes is considered to be an adaptive response to 1,2-dichlorobenzene exposure. The 400 mg/kg-day dose is a LOAEL based on hepatic degeneration, hypertrophy and necrosis. A NOAEL was not identified because the 100 mg/kg-day rats were not examined for pathology.

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 in doses of 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). 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 histological 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 histopathological 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 ($p<0.05$, Fisher Exact test conducted for this assessment) increased at 179 mg/kg-day (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 weight was significantly increased at $\geq$89.3 mg/kg-day in both sexes and slight decreases in serum triglycerides (357 mg/kg-day; males, 179 mg/kg-day; females) and serum protein (179-357 mg/kg-day; males, 21.4-357 mg/kg-day; females) were observed which may reflect hepatic effects of the chemical at these doses. Changes in other serum chemistry indices included increases in cholesterol and total protein that were generally slight, particularly at lower dose levels. Serum cholesterol was significantly ($p<0.05$) increased in males at $\geq$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 $\geq$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 $\geq$21.4 mg/kg-day (7.8, 4.7, 6.3, 6.3 and 17.2%) and males at $\geq$179 mg/kg-day (-1.4%, 1.4%, 0, 7.1 and 7.1%). Blood urea nitrogen 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, hematocrit, erythrocyte counts, and mean corpuscular volume 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), and slight changes in serum cholesterol. 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 (0, 42.9, or 85.7 mg/kg-day). Evaluations included clinical signs, body weight, and 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. 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 appears to be mainly from 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 was either reached or exceeded. Mean body weight was slightly reduced ($\approx$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 non-neoplastic lesions in the liver, kidneys, or any other tissues, indicating that 42.9 mg/kg-day and 85.7 mg/kg-day were the chronic NOAELs in rats.

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 ($p<0.05$) increased 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 the Fisher 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 (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 histological 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 histopathological 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 GGPT 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 2-fold 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 indicate that the NOAEL and LOAEL are 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 (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 is unclear whether an MTD was achieved. The only exposure-related nonneoplastic lesion was a significantly ($p<0.05$, Fisher Exact test performed for this study evaluation) increased incidence 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. The toxicological significance of the tubular regeneration is unclear because no degenerative or necrotic lesions were observed in the kidneys of the male mice, no regeneration or other renal lesions were found in female mice, and the kidney was not identified as a target at

higher doses in the subchronic mouse studies. NTP (1985) did not assess the toxicological significance or discuss any other aspect of the renal tubular regeneration. Therefore, 85.7 mg/kg-day is considered a NOAEL for 1,2-dichlorobenzene in the chronic mouse study.

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 than 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 the 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/bronchilar 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, hematocrit, mean corpuscular volume, 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. Histological examinations were performed on all tissues that were examined grossly in all high-dose rats and one-half of control rats, as well as in the 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 same 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. As discussed below, the histological and serum chemistry evaluations indicated that the thyroid, pituitary, and liver were sensitive targets at exposure levels as low as 9 mg/kg-day.

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 the Fisher Exact test and a criterion of significance of $p \leq 0.05$. Histological 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). Pituitary effects included significantly increased incidences of cytoplasmic vacuolization in the pars distalis in 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, and 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). The pituitary lesion was reported to be similar to "castration cells" found in gonadectomized rats and considered to be an indicator of gonadal deficiency. No compound-related pituitary lesions were observed in female rats. In possibly related changes, serum cholesterol was significantly ($p \leq 0.05$) increased in males at ≥9 mg/kg-day and females at ≥37 mg/kg-day in a dose-related manner, and serum calcium was significantly increased in both sexes at ≥37 mg/kg-day. The investigators suggested that these serum chemistry changes might reflect a disruption of hormonal feedback mechanisms, or target organ effects on the pituitary, hypothalamus, and/or other endocrine organs.

Pathological changes in the liver were found at doses of 1,3-dichlorobenzene higher than 9 mg/kg-day (McCauley et al., 1995). Hepatic effects occurred in both sexes at 147 and 588 mg/kg-day, including significant ($p \leq 0.05$) increases in relative liver weight (51 and 85% increases in males and 32 and 74% increases in females compared to controls) and incidences of liver lesions. Absolute organ weights were not reported. Liver lesions were characterized by inflammation, hepatocellular alterations (characterized by spherical, brightly eosinophilic

homogeneous inclusions), and hepatocellular necrosis. Liver lesions that were significantly ($p \leq 0.05$) increased 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 7/9) 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). 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 pituitary-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 histopathological 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 toxic effects in rats at all tested dose levels, indicating that the LOAEL is 9 mg/kg-day and a NOAEL is 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 dosages of 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 delta-aminolevulinic acid; body weight and liver histology 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 other significant increase in liver weight was in the 50 mg/kg-day group after 120 days. Small (10-24%), but statistically significant ($p < 0.05$), increases 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 transience 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 delta-aminolevulinic acid and porphobilinogen were not increased. The

available information therefore indicates that there was a low potential for porphyria and that there are 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 in dosages of 10, 100, or 500 mg/kg, 5 days/week (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 histological 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 olive oil-gum arabic emulsion by gavage in dosages of 0, 18.8, 188, or 376 mg/kg, 5 days/week for 138 doses in 192 days (0, 13.5, 135, or 270 mg/kg-day). Organ weight, 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 are not considered adverse due to lack of any accompanying histopathological 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 (358 mg/kg-day), and in 5 rabbits that were treated with 1000 mg/kg for 92 doses in 219 days (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 reported) were used for comparative purposes. Clinical signs, body weight, hematology, histology, and presence of cataracts were evaluated. Effects included weight loss, definite to marked tremors, weakness, and slight liver histopathology (cloudy swelling, very few areas of focal, caseous necrosis) at $\geq$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 at reduced dosages because a no-effect level was not 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 dosages in the first 13-week rat study were 0, 300, 600, 900, 1200, or 1500 mg/kg (0, 214, 429, 643, 857, or 1071 mg/kg-day) (NTP, 1987). Comprehensive histological exams were performed in the control and three highest dose groups; at lower dosages, 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 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 number 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 blood urea nitrogen levels at ≥643 mg/kg-day.

Serum chemistry changes included significantly increased alkaline phosphatase 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). 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 the higher doses. Slight, but statistically significant, decreases in erythrocyte count, hematocrit, 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 is 214 mg/kg-day, based on changes in liver-associated serum indices and red blood cell parameters.

The dosages in the second 13-week rat study were 0, 37.5, 75, 150, 300, or 600 mg/kg (0, 27, 54, 107, 214, or 429 mg/kg-day) (NTP, 1987). This study was performed because renal lesions occurred at all dosages in males in the first 13-week study. Comprehensive histological examinations were performed in the control and three highest dose groups; at lower dosages,

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 histopathological 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-moderate; 214 mg/kg-day: 3/10, moderate; 429 mg/kg-day: 9/10, moderate).

A chronic beagle dog study evaluated the systemic effects of 1,4-DCB in male and female beagle dogs 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 (adjusted doses; 0, 7, 36, 107 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 (71 mg/kg-day) during the third week of exposure for males and further reduced to 75 mg/kg-day (54 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 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 undetected. 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 thyroids/parathyroids. Histopathological examinations were conducted on tissues obtained from the adrenals, aorta, brain, caecum, 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, thyroids, 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). Increases in absolute and/or relative adrenal (absolute weight; 125 and 130% control in males; 135 and 141% controls in females; relative weight; 143 and 158% control in males; 138 and 153% control in females) and thyroid (absolute weight; 118 and 123% control in males; 139 and 132% control in females; relative weights; 133 and 149% control in males; 143 and 141% control in females) weights were observed in both sexes at the two highest doses and were considered possible treatment related effects, although no histopathological lesions were found to explain the increase in the adrenals and thyroid (Monsanto Company, 1996).

Histopathological examination revealed several liver lesions only in the dosed groups and were considered either direct or indirect/adaptive effects to 1,4-DCB and were consistent with gross necropsy findings, organ weight data and clinical results. Liver lesions of mild to moderately severe nature were observed in all mid and high dose male and female dogs. Hepatocellular hypertrophy, multifocal to diffuse with increasing dose level were statistically significant ($p \leq 0.01$, Fisher's exact test, one-tailed) in all male and female dogs at mid and high doses and 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 female dog. Hepatic portal inflammation was noticed only in the mid and high dose groups in males, while no clear dose-response pattern was observed in the females. 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 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 levels in the females. The authors concluded that the lesion could be associated to the test chemical at the mid and high dose in the females where it was accompanied by increased kidney weights and grossly observed renal discoloration (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-DCB exposure (Monsanto Company, 1996). At the 6 month sampling period, hematological parameters included a reduction in basophils at the high dose level and an increase in platelet counts at the mid and high doses in female dogs. Number of RBCs were significantly reduced in both sexes at the high dose level, while HCT was lowered in the high dose males. At the terminal sampling period, numbers of large unstained 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. Alkaline phosphatase, ALT, AST, and GGT were elevated in both sexes. 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 effects were observed in the urine of males or females at any dose level.

In the chronic NTP (1987) study in F344/N rats, groups of 50 males and 50 females were treated with 1,4-dichlorobenzene (>99% pure) in corn oil by gavage, 5 days/week for 103 weeks. The dosages in this study were 0, 150, or 300 mg/kg (0, 107, or 214 mg/kg-day) in males and 0,

300, or 600 mg/kg (0, 214, or 429 mg/kg-day) in females. Evaluations consisted of 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 induced in the kidneys of the male rats. Incidences of nonneoplastic renal lesions in male rats were increased at $\geq$107 mg/kg-day and included epithelial hyperplasia of the renal pelvis (1/50, 30/50, 31/50 in the control to high dose groups), mineralization of the collecting tubules in the renal medulla (4/50, 46/50, 47/50), and focal hyperplasia of renal tubular epithelium (0/50, 1/50, 9/50). Incidences of nephropathy were similar in the control and treated male groups, although the severity of this lesion was increased in the treated males. In females, increased nephropathy was the only renal lesion that was treatment-related (21/49, 32/50, 41/49). The nephropathy in the female rats was characterized by the occurrence of several interrelated changes, including degeneration and regeneration of the tubular epithelium, tubular dilatation with attenuation and atrophy of the epithelium, granular casts in tubules, thickening of basement membranes, and minimal accumulation of interstitial collagen, but no kidney tumors. Other lesions included hyperplasia of the parathyroid gland, which was increased in male rats (4/42, 13/42, 20/38). NTP concluded that the parathyroid hyperplasia is likely secondary to renal effects (i.e., is probably related to a decrease in functional renal mass, a subsequent alteration in serum phosphate and calcium excretion by the kidney, and stimulation of the parathyroid gland to release parathyroid hormone). The male rat-specific hyaline droplet ($\alpha_{2\mu}$-globulin) nephropathy syndrome likely contributed to the kidney effects observed in the males. 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 increased incidences of tubular cell adenocarcinoma (1/50, 3/50, 7/50) and combined tubular cell adenoma or 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 ($\alpha_{2\mu}$-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 dosages in the first 13-week mouse study were 0, 600, 900, 1000, 1500, or 1800 mg/kg (0, 429, 643, 714, 1071, or 1286 mg/kg-day) (NTP, 1987). Comprehensive histological exams were performed in the control and two highest dose groups; at lower dosages, 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 and not clearly affected in females. Mortality apparently related to chemical exposure (no gavage error deaths reported) was found in both sexes at ≥1071 mg/kg-day (3-9 deaths per group). 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 to brain weight ratio 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 is 429 mg/kg-day based on hepatocellular degeneration in both sexes, and decreased white blood cell count in males.

The dosages in the second 13-week mouse study were 0, 84.4, 168.8, 337.5, 675, or 900 mg/kg (0, 60, 121, 241, 482, or 643 mg/kg-day) (NTP, 1987). This study was performed because liver lesions occurred in both sexes at all dosages in the first 13-week study. Comprehensive histological exams were performed in the control and two highest dose groups; at lower dosages, 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 are 241 and 482 mg/kg-day.

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 (0, 214, or 429 mg/kg-day) doses of 1,4-dichlorobenzene (>99% pure) in corn oil by gavage, 5 days/week for 103 weeks. Evaluations consisted of body weight, clinical signs, necropsy, and histology in all animals. Body weight and survival were comparable in the control and treated mice. Nonneoplastic lesions and tumors in the liver were prominent effects of exposure in both sexes, as summarized in Table 4-1. 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. Additionally observed in the high-dose male mice were four cases of hepatoblastoma, an extremely rare type of hepatocellular carcinoma. 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 controls and 0/1784 in untreated controls) 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 4-1. Liver Lesions in the NTP (1987) Two-year Gavage Study of 1,4-Dichlorobenzene in $B6C3F_1$ Mice

	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.

Other histopathological 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 capsule focal hyperplasia (11/47, 21/48, 28/49). The combined incidence of adrenal gland pheochromocytomas or malignant pheochromocytomas in male mice occurred with a significant positive trend (0/47, 2/48, 4/49), but the incidence rates are lower than the historical 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). The incidence of alveolar/bronchiolar carcinomas was slightly increased 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 non-neoplastic lesions in the liver, kidneys, thyroid, adrenals, and lymph nodes in both dose groups, this study identifies 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) (Bomhard et al., 1988; Eldridge et al., 1992; Gustafson et al., 1998; Lake et al., 1997; Umemura et al., 1998, 2000). 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 support conclusive evidence that 1,4-dichlorobenzene induces renal tubular tumors in male rats by a non-DNA-reactive mechanism, through a male rat-specific $\alpha_{2\mu}$-globulin-related response. Other findings contribute to evidence indicating that the mechanism leading to the formation of mouse liver tumors by 1,4-dichlorobenzene may be non-genotoxic and 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 replicating fraction was significantly increased in the proximal convoluted tubules, but not the proximal straight tubules or distal tubules, of the exposed rats. 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. Histological 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 Fischer 344 CDF rats were treated with 1,4-dichlorobenzene in corn oil by gavage in daily dosages of 0, 75, 150, 300, or 600 mg/kg (Bomhard et al., 1988). Five animals of each sex and dosage 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, hematocrit, 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 increased from 20% at 75 mg/kg-day to 40% at 600 mg/kg-day in males and increased 23% in females at 600 mg/kg-day. Other effects observed in male rats included significantly increased urinary excretion of lactate dehydrogenase (LDH) (day 9-week 12) and protein (weeks 4-12) at ≥75 mg/kg-day, and increased beta-N-acetylglucosaminidase (NAG) excretion (week 12) at 600 mg/kg-day. Urinary LDH, total protein and NAG values 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 histological signs of renal damage were observed only in males. Renal histopathological 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 longer parts of tubules at ≥300 mg/kg-day. The female rats showed no comparable renal histopathology. The renal effects in male rats are a consequence of male rat specific $\alpha_{2\mu}$-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 (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 (214 or 429 mg/kg-day) of compound for 1-13 weeks. Study 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 cytochrome P450 content, and 7-pentoxyresorufin *O*-dependentylase activity (a marker for induction of cytochrome 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 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.

Hepatic effects in the rats included significantly increased liver weight 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 cytochrome 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 7-pentoxyresorufin *O*-dependentylase 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 hepatic 7-ethoxyresorufin *O*-deethylase and erythromycin *N*-demethylase 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 the rats included increased kidney weight at ≥107 mg/kg-day for 4 and 13 weeks, and increased P_1/P_2 renal proximal tubule cell labeling indices 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 can be derived from this stud y based on centrilobular hepatocellular hypertrophy in rats. A NOAEL was not identified because histopathology was not performed at the lower doses.

Hepatic effects in the mice included significantly increased liver weight and 7-pentoxyresorufin *O*-dependentylase 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 cytochrome P450 at 429 mg/kg-day for 1-13 weeks; increased 7-ethoxyresorufin *O*-deethylase, erythromycin *N*-demethylase and microsomal protein at 429 mg/kg-day for 1 week; and marked centrilobular hypertrophy at 429 mg/kg-day for 13 weeks. Renal effects in the mice included increased P_1/P_2 renal proximal tubule cell labeling indices 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 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 (0, 214, or 429 mg/kg-day) (mice) or 0 or 600 mg/kg (0 or 429 mg/kg-day) (rats) (Eldridge et al., 1992). Study endpoints included body weight, absolute liver weight, hepatocyte BrdU labeling index, plasma enzyme activities (ALT, AST, LDH, and SDH), and liver histology. Significant increases in hepatocyte labeling index were only observed in male and in 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 the rats at 6 weeks and was not observed at 12 weeks, and there were no significant increases in the mice after 6 or 13 weeks. Absolute liver weight was 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 histopathological 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 is a LOAEL for hypertrophic liver lesions and 214 mg/kg-day is a NOAEL because none of the reported changes are 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 (0, 107, 214, or 429 mg/kg-day) (mice) or 0, 75, 150, or 300 mg/kg (0, 54, 107, or 214 mg/kg-day) (rats) (Umemura et al., 1998). Study 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 weight was significantly increased after 1 and 4 weeks in the mice at ≥429 mg/kg-day and rats at ≥107 mg/kg-day. The CFR was increased after 1 week in the mice at ≥214 mg/kg-day and rats at ≥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 the mice exposed to ≥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 are 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 injection of 0.9% saline (12 animals) or 200 mg/kg of nitroso-diethylamine (NDEA) 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 NDEA in corn oil. All animals were partially hepatectomized 1 week after the start of 1,4-dichlorobenzene exposure. The study was ended 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-dichlorobenzene-related increased incidences of hepatic foci were found, suggesting that the compound is 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/cu.m, 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 histopathological 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 histological 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 take 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 histological 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 is a target for inhalation exposures 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*

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) of 1,4-dichlorobenzene (≥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 were 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). Additionally, 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. Clinical signs, organ weights, gross pathology, and histopathology were examined following all of the exposures. Additional study endpoints reported for the 96, 158, and 173 ppm groups included final body weight and relative organ weights (lungs, heart, liver, kidneys, spleen, 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 appear to have been limited to the 96 ppm exposures. Relative liver weight was significantly ($p \leq 0.05$) increased 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 weight was 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, unkempt appearance, unconsciousness, and a few deaths) and histopathological 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 of two rabbits). No additional relevant information on the design and results of this study was reported. The NOAEL and LOAEL are 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 of 1,4-dichlorobenzene were conducted in rats and mice (Imperial Chemical Industries Limited, 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 of 1,4-dichlorobenzene for 5 hours/day, 5 days/week for up to 76 weeks (Imperial Chemical Industries Limited, 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, hematocrit, MCHC, packed cell volume, platelet count, 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 (Imperial Chemical Industries Limited, 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 and were unaffected by exposure in the females. The urinalysis values were not statistically significantly different than the controls, but were based on a small numbers 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 weight was 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 and unaffected in females. There was no clear histological 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 is identified based on the 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 maximum tolerated dose, 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 appear to be the same as in the Imperial Chemical Industries Limited (1980) rat inhalation study summarized above. There was no histological 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 TSCATS).

The translation of an incomplete summary of a Japanese inhalation carcinogenicity study of 1,4-dichlorobenzene in rats and mice is available (Chlorobenzene Producers Association, 1997). Groups of 50 male and 50 female F344/DuCrj rats and 50 male and 50 female Crj:BDF_1 mice were exposed to 0, 20, 75, or 300 ppm of 1,4-dichlorobenzene, 5 days/week for 104 weeks. Incidences of liver tumors in male and female mice and lung tumors in female mice were increased as summarized in Table 4-2. The available summary of this study provides no additional information on the experimental design or results.

Table 4-2. Liver and Lung Tumors in Two-year Mouse Inhalation Study of 1,4-Dichlorobenzene (Chlorobenzene Producers Association, 1997)

Lesion	0 ppm	20 ppm	75 ppm	300 ppm
Number of male mice examined	49	49	50	50
Hepatoma	12	17	16	38
Hepatic histiocytoma carcinoma	0	3	1	6
Number of female mice examined	50	50	49	50
Hepatoma	2	4	2	41
Hepatocellular adenoma	2	10	6	20
Lung bronchiole/alveolar epithelial carcinoma	1	1	1	4

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 abstract (English) and data 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 IgE, IgG, and IgM antibody production. Determinations of IgE antibody titers (passive cutaneous anaphylaxis test) and IgG and IgM antibody titers (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 intravenously injected 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 is 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 as an abstract with inadequately reported methods and results. 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 weights, deciduoma, skeletal and visceral changes, and histopathology. No teratological effects were reported. No other information regarding developmental or maternal toxicity was noted. Based on the limited available information, 200 mg/kg-day is 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 as an abstract with inadequately reported methods and results. 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 teratological effects were reported. No other information regarding developmental or maternal toxicity was noted. Based on the limited available information, 200 mg/kg-day is 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 by daily 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). No reason was provided for the different pre-mating exposure durations in the F_0 males and females. Exposure in 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 exposures 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 killed 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 is not indicated if additional organs were weighed. Histopathological examinations were limited to selected adult tissues (liver, kidneys, spleen, vagina, cervix, uterus, ovaries, mammary gland, testes, epididymides, penis, prostate, seminal vesicles, and spermatic cord) from F_0 and F_1 animals that had no living young, died prematurely, or were killed 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 occurred at $\geq$90 mg/kg that included statistically significant (method of analysis and p values not reported) reduced 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). Significant reductions in body weight were also observed at 270 mg/kg-day in F_1 pups at postnatal days 7, 14, and 21, as well as at 270 mg/kg-day in F_2 pups at birth and 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 and F_2 pups at $\geq$90 mg/kg-day (33, 467, and 1033% higher than controls at 30, 90, and 270 mg/kg-day). 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 occurred 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 $\geq$90 mg/kg-day (3.3, 7.4, and 22.3% less than controls at 30, 90, and 270 mg/kg-day). 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 $\geq$90 mg/kg-day, including dry and scaly skin until approximately postnatal day 7 (0, 0, $\approx$70 and 100% of the

litters at 0, 30, 90, and 270 mg/kg-day), and tail constriction that appeared between days 4 and 21 in all or nearly all litters (percentages not reported) and occasionally led to loss of parts of the tail (Bornatowicz et al., 1994). 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 $\geq$90 mg/kg-day, and changes in absolute and/or relative organ weights in kidneys (increased) and spleen (reduced) in F_1 males at 270 mg/kg-day. There were no effects on organ weights in females of either generation. The only histopathological finding attributed to exposure was unspecified kidney damage in both generations (effect levels, possible male specificity, and other information not reported). This study identifies a NOAEL and LOAEL of 30 and 90 mg/kg-day 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 mated 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 gestation days 6-15 (Giavini et al., 1986). Sacrifices were performed on gestation 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 $\geq$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 and dose-related at $\geq$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 $\geq$750 mg/kg-day, although there was no change in incidences of affected litters. Mean fetal body weight was significantly reduced (8.1%) at 1000 mg/kg-day. No other exposure-related fetal effects were observed. This study identifies a NOAEL and LOAEL of 250 and 500 mg/kg-day for developmental toxicity based on skeletal variations. These doses are also a NOAEL and LOAEL for maternal toxicity based on decreased body weight gain.

Another oral developmental toxicity study of 1,4-dichlorobenzene is available as an abstract with inadequately reported methods and results. 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 teratological effects were reported. No other information regarding developmental or maternal toxicity was noted. Based on the limited available information, 200 mg/kg-day is a NOAEL for maternal and developmental toxicity of 1,4-dichlorobenzene in rats.

4.3.2. Inhalation Exposure

4.3.2.1. *1,2-Dichlorobenzene*

A 2-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 m^3, respectively) (Bio/dynamics, 1989). F_0 adults were exposed for 6 hours/day, 7 days/week for a 10-week pre-mating period and during mating. Following mating, F_0 males were exposed 6 hours/day, 7 days/week until sacrifice at 3 to 4 weeks post-mating. Bred 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 a 11 week pre-mating period, mating, gestation, and lactation. Although the respiratory tract was not examined, a comprehensive range of toxicological responses were evaluated including mortality, clinical signs, body weights, food consumption, organ weights, reproductive parameters, gross necropsy of selected tissues, and histological examination (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 weights (measured on lactation days 0, 4, 14, 21, and 28), sex ratio, gross necropsy (all tissues), and histological 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 is 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 pre-mating period, increased absolute (males) and relative (both sexes) kidney weight, and increased absolute and relative (both sexes) liver weights. Histopathological examination revealed hypertrophy of central lobular hepatocytes in adult F_0 and F_1 rats of both sexes exposed to 150 and 394 ppm. Histopathological lesions of the kidney at these exposure levels featured dilated renal tubular lumen with intraluminal granular casts, predominantly at the corticomedullary junction. Adult F_0 and F_1 males from all exposure groups had intracytoplasmic granules/droplets in the proximal convoluted tubular epithelium of the kidney; the severity of this condition increased as exposure level increased. 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 ($\alpha_{2\mu}$-globulin) nephropathy. The NOAEL and LOAEL for systemic toxicity are 50 and 150 ppm based on decreased body weight; the increases in liver weight are not considered adverse in the absence of degenerative histopathological changes.

The inhalation developmental toxicity of 1,2-dichlorobenzene has been investigated in rats and rabbits. A probe 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 probe 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 days 6-15 (rats) or 6-18 (rabbits) of gestation, and sacrificed on the day following the last exposures (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 non-pregnant 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 $\geq$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 non-significant, 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 mated female Fischer 344 rats and 28-30 inseminated 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 gestation day 21(rats) or 29 (rabbits) (Hayes et al., 1985). Maternal 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 non-pregnant 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 gestation days 6-8, 12-15, and 6-20 at $\geq$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 increased 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 $\geq$100 ppm. The lowest concentration, 100 ppm, is 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 is a NOAEL for developmental effects in this species. The NOAEL and LOAEL for developmental toxicity in rats are 200 and 400 ppm 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 successfully mate in the first 10 days of the mating period were paired with the satellite females for 10 days. Main study females that did not successfully mate during the first 10 days of the mating period were paired with proven males for the remaining 11 days of the mating period. Exposures of the main study F_0 females were continued throughout the mating period and the first 19 days of gestation, discontinued from gestation day 20 through postnatal day 4, and then resumed until sacrifice at weaning on postnatal day 28. Exposures of the satellite F_0 females were continued through mating until sacrifice on gestation day 15. Exposures of the F_0 males continued until sacrificed 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. Histological 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 α2μ 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 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, although 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 $\alpha_{2\mu}$-globulin accumulation and increased kidney weights at ≥66 ppm and other characteristic histological 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 pre-breed exposure periods 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. The liver weight effects were more pronounced in males than females. Mean relative liver weights in the 66, 211, and 538 ppm adult male groups were 4.8, 13.9, and 52.1% higher than controls in the F_0 generation (sacrificed at week 15) 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 histological changes were induced at the lower exposure concentrations. The increases in liver weight and hepatocellular hypertrophy are 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, unkempt appearance, urine stains, salivation, and nasal and ocular discharges); these effects only sporadically occurred 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 LOAEL of 538 ppm are identified 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 methanesulfonate 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 ≥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 weight, and external, visceral, and skeletal abnormalities. There were no exposure-related indications of maternal toxicity, embryotoxicity, fetotoxicity, or teratogenicity, indicating that 500 ppm is a NOAEL for these endpoints. No additional relevant information was provided in the available study summary.

A probe 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) in 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 non-pregnant 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 inseminated 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 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 non-pregnant 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 gestation days 6-8 and a slight, non-significant increase in the incidence of retroesophageal right subclavian artery in the offspring ($p>0.05$, Fisher 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 (1987a) 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 "$\alpha_{2\mu}$-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 $\alpha_{2\mu}$-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 $\alpha_{2\mu}$-globulin in a manner similar to that of 2,2,4-trimethylpentane (TMP), a component of unleaded gasoline that has been shown to elicit $\alpha_{2\mu}$-globulin-related effects (Charbonneau et al., 1989). Animals treated with 1,4-dichlorobenzene develop kidney lesions characteristic of $\alpha_{2\mu}$-globulin-related toxicity, including hyaline droplet formation and cellular damage and proliferation of the P1/P2 proximal tubule regions (Bomhard et al., 1988; Lake et al., 1997). Additionally, NBR rats, a strain that does not synthesize $\alpha_{2\mu}$-globulin, showed no renal effects following a gavage exposure to 500 mg/kg of 1,4-dichlorobenzene for 4 days, whereas Fischer 344 rats showed clear evidence of $\alpha_{2\mu}$-globulin accumulation and toxicity at the same dose levels (Dietrich and Swenberg, 1991). Thus, the available evidence supports the development of $\alpha_{2\mu}$-globulin-related lesions following exposure to 1,4-dichlorobenzene.

The evidence for the involvement of $\alpha_{2\mu}$-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-dichorobenzene 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 $\alpha_{2\mu}$-globulin nephropathy, in both F_0 and F_1 male rats. Other studies of 1,2-dichorobenzene toxicity (Hollingsworth et al., 1958; NTP, 1985; Robinson et al., 1991) presented evidence of renal toxicity, but not of effects consistent with $\alpha_{2\mu}$-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 $\alpha_{2\mu}$-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 $\alpha_{2\mu}$-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 cytochrome 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 among the dichlorobenzene isomers, with the more acutely hepatotoxic isomers, 1,2- and 1,3-dichlorobenzene, showing greater involvement of cytochrome P450-based metabolism than the hepatocarcinogenic 1,4-dichlorobenzene (Nedelcheva et al., 1998). This initial metabolism likely results in a reactive intermediate, most likely an epoxide, that can bind covalently to cellular macromolecules or react with glutathione, resulting in a depletion of cellular glutathione stores. However, while these mechanisms are potentially involved in the subchronic and/or chronic toxicity of the dichlorobenzenes, their contribution has not been conclusively established.

4.4.1.2.1.1. *1,2-Dichlorobenzene*

Considerable evidence exists supporting the hypothesis that the toxicity of 1,2-dichlorobenzene results from an initial P450-related metabolism to an epoxide, followed by a reaction of that epoxide with cellular molecules. Stine et al. (1991) treated Fischer 344 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-post-exposure, and a gradual decrease throughout 72 hours post-exposure. Pretreatment with SKF-525A, a cytochrome 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 cytochrome P450 inhibitor) significantly decreased the hepatic toxicity of 1,2-dichlorobenzene.

Additional evidence for the involvement of a reactive intermediate in the hepatotoxicity of 1,2-dichlorobenzene comes from studies depleting cellular oxidant defenses or measuring indicators of oxidative stress. Pretreatment with phorone, which depletes hepatic glutathione, resulted in greatly enhanced serum ALT levels after 1,2-dichlorobenzene administration (Stine et al., 1991). In a later study (Younis et al., 2000), pretreatment of Fischer-344 or Sprague-Dawley rats with 1-aminobenzotriazole, a noncompetitive inhibitor of cytochrome P450, completely eliminated the decrease in hepatic glutathione levels and increase in oxidized glutathione (GSSG) in the bile associated with 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 intraperitoneal 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 of 1,2-dichlorobenzene, which normally produces a much greater toxicity. Pretreatment with phorone to deplete hepatic glutathione (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 cytochrome P450 enzymes to a hepatotoxic intermediate, evidenced by the increase in ALT following phenobarbital administration. The fact that glutathione depletion enhances the toxicity of 1,3-dichlorobenzene is further evidence of biotransformation to a reactive intermediate, likely an epoxide, that can react with cellular glutathione. No other data on the involvement of cytochrome P450 enzymes on the hepatotoxicity of 1,3-dichlorobenzene or data examining the possible role of glutathione conjugation or covalent binding in the toxicity of 1,3-dichlorobenzene are available.

4.4.1.2.1.3. *1,4-Dichlorobenzene*

Of the isomers of dichlorobenzene, 1,4-dichlorobenzene appears to be the least acutely hepatotoxic, as well as the isomer whose acute toxicity is least likely to be influenced by cytochrome P450-based metabolism. Exposure of male F344 rats and male $B6C3F_1$ mice to 1,4-dichlorobenzene resulted in both an increase in general cytochrome P450 activity and an induction of microsomal cytochrome P4502B1/2 protein levels, as assessed by Western blotting (Lake et al., 1997). However, while exposure to 1,4-dichlorobenzene can induce cytochrome P450 enzymes, induction of cytochrome P450 enzymes by pretreatment with phenobarbital did not result in an acute toxic response, as measured by plasma ALT levels, after a single intraperitoneal 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, intraperitoneal 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. Microsomes incubated with radio labeled 1,4-DCB and later treated with antioxidants (i.e., ascorbic acid) resulted in a decrease in *in vitro* covalent binding to macromolecules (Hissink et al., 1997c), suggesting that metabolism results in the formation of an reactive oxygen species. Additionally, studies have demonstrated that depletion of GSH levels results in an acute hepatotoxic response following administration of 100-132 mg/kg of 1,4-dichlorobenzene (Stine et al., 1991; Mizutani et al., 1994). However, unlike 1,2-dichlorobenzene, 1,4-dichlorobenzene treatment does not appear to result in increased levels

of oxidized glutathione in the liver (Gustafson et al., 2000), suggesting that if a reactive intermediate is formed, it occurs at a low concentration or does not tend to oxidize glutathione.

4.4.1.2.2. Role of cell proliferation

An issue that has received considerable discussion is the potential mechanism behind the appearance of liver tumors in mice, but not in rats, in the 2-year bioassay of 1,4-dichlorobenzene, particularly given that other isomers are much more acutely hepatotoxic and did not show evidence of hepatocarcinogenicity at similar doses.

1,4-Dichlorobenzene does not appear to function in an initiator/promoter sequence. Exposure of rats to 1,4-dichlorobenzene by gavage for 13 weeks, followed by 26-39 weeks of exposure to trisodium nitrilotriacetic acid, a known promoter, resulted in no neoplastic lesions, indicating the absence of initiating activity of 1,4-dichlorobenzene (Umemura et al., 2000). Pretreatment with diethylnitrosamine, an initiating agent, followed by 6 weeks of treatment with 1,4-dichlorobenzene did not result in the formation of preneoplastic foci (1,2,4,5-tetrachlorobenzene was used as a positive control), indicating that 1,4-dichlorobenzene does not act as a promoter (Gustafson et al., 1998).

One hypothesis suggests an effect of 1,4-dichlorobenzene on regulation of hepatic cell proliferation. The observed proliferation does 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 does not induce unscheduled DNA synthesis in the livers of rats and mice (Perocco et al., 1983; Sherman et al., 1998). Rather, the proliferation is 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. 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 was greater in the mouse 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.

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 bromodeoxyuridine (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 cumulative replicating fraction (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) at week 4 of the study. Similar increases in labeling index after 1 week of exposure were reported in the 13-week subchronic studies of $B6C3F_1$ mice (Eldridge et al., 1992; Lake et al., 1997) and F344 rats (Lake et al., 1997).

Interestingly, both of the 13-week studies reported that the increase in labeling index was no longer present at week 13 of the study in either species, although examination at 4 weeks still revealed an increased labeling index in both rats and mice (Lake et al., 1997). Additional data will be required to fully evaluate the role of this mechanism in 1,4-dichlorobenzene-induced carcinogenesis.

4.4.2. Genotoxicity

The genotoxic effects of the dichlorobenzenes are summarized in Table 4-3. In general, the results of *in vitro* examinations of dichlorobenzene genotoxicity have been negative, while *in vivo* studies, although limited, have suggested potential genotoxic effects of acute dichlorobenzene exposure.

4.4.2.1. *1,2-Dichlorobenzene*

1,2-Dichlorobenzene was negative for reverse mutation in *Salmonella typhimurium*, either with or without metabolic activation (Waters et al., 1982; Connor et al., 1985; NTP, 1985; Shimizu et al., 1983). 1,2-Dichlorobenzene treatment gave similarly negative results for reverse mutation in *Escherichia coli* without metabolic activation (Waters et al., 1982), but positive results in *S. cerevisiae* with metabolic activation (Paolini et al., 1998). In mouse lymphoma cells, 1,2-dichlorobenzene was negative for forward mutation without metabolic activation, but was positive 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 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 not added to the experiment (Loveday et al., 1990).

In vivo treatment of mice with 93.5 mg/kg of 1,2-dichlorobenzene resulted in increased micronucleus formation (Mohtashamipur et al., 1987). No other studies of the *in vivo* genotoxicity of 1,2-dichlorobenzene were located in the examined literature.

4.4.2.2. *1,3-Dichlorobenzene*

Exposure to 1,3-dichlorobenzene does not cause an increase in reverse mutation, either with or without S9 mixture, in *S. typhimurium* (Waters et al., 1982; Connor et al., 1985; Shimizu et al., 1983) 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).

Table 4-3. Results of Selected Genotoxicity Studies of Dichlorobenzenes

Test System	Results		Reference
	Without Metabolic Activation	With Metabolic Activation	
1,2-Dichlorobenzene			
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	+	Paoloni 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
Micronucleus formation in mice *in vivo*	+	NA	Mohtashamipur et al., 1987
1,3-Dichlorobenzene			
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

Table 4-3. Results of Selected Genotoxicity Studies of Dichlorobenzenes cont.

Test System	Results		Reference
	Without Metabolic Activation	With Metabolic Activation	
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
Micronucleus formation in mice *in vivo*	+	NA	Mohtashamipur et al., 1987
1,4-Dichlorobenzene			
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	+	Paoloni et al., 1988
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

Table 4-3. Results of Selected Genotoxicity Studies of Dichlorobenzenes cont.

Test System	Results		Reference
	Without Metabolic Activation	With Metabolic Activation	
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
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
Micronucleus formation in mice *in vivo*	-	NA	NTP, 1987
Micronucleus formation in mice *in vivo*	-	NA	Tegethoff et al., 2000
Micronucleus formation in mice *in vivo*	+	NA	Mohtashamipur et al., 1987
Micronucleus formation in mice *in vivo*	-	NA	Morita et al., 1997
Micronucleus formation in rat kidney *in vivo*	+	NA	Robbiano et al., 1999
Increased replicative DNA synthesis in mice *in vivo*	+	NA	Miyagawa et al., 1995
Damage to nuclear DNA in rat kidney *in vivo*	+	NA	Robbiano et al., 1999
-: negative; +: positive; =: equivocal results; ND: Not Done; NA: Not Applicable			

1,3-Dichlorobenzene did not result in an increase in replicative DNA synthesis in cultured human lymphocytes (Perocco et al., 1983).

In vivo, treatment of mice with 87.5 mg/kg of 1,3-dichlorobenzene resulted in increased micronucleus formation (Mohtashamipur et al., 1987). No other studies of the *in vivo* genotoxicity of 1,3-dichlorobenzene were located in the examined literature.

4.4.2.3. *1,4-Dichlorobenzene*

Evaluation of 1,4-dichlorobenzene for reverse mutation yielded negative results in both *S. typhimurium* (Waters et al., 1982; Connor et al., 1985; NTP, 1987; Shimizu et al., 1983) 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 following examination for forward mutations in mouse lymphoma cells (McGregor et al., 1988; NTP, 1987), but was negative in examinations of 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 in human and rat hepatocytes by 1,4-dichlorobenzene have been equivocal (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 has generally tested negative for micronucleus formation in mice (NTP, 1987; Tegethoff et al., 2000; Morita et al., 1997), 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 rat kidney (Robbiano et al., 1999). Exposure of mice to 1,4-dichlorobenzene resulted in increases in replicative DNA synthesis (Miyagawa et al., 1995).

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION—ORAL AND INHALATION

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 is on 1,4-dichlorobenzene. Several repeated dose toxicity investigations of 1,2-dichlorobenzene have been conducted and only two studies are available for 1,3-dichlorobenzene. A summary of available relevant studies on the three isomers is provided in Table 4-4. Information is available on the developmental toxicity of all three isomers, but reproductive toxicity has only been evaluated with 1,4-dichlorobenzene. Potential effects of

Table 4-4. Critical Effect Levels in Subchronic, Chronic, Developmental and Reproductive Oral Studies of Dichlorobenzene

Isomer	Species, Strain, Sex	Exposure Protocol[1]	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects[2]	Reference
1,2-DCB	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, Sprague-Dawley, M&F	0, 25, 100, or 400 mg/kg-day for 90 days	100	400	Hypertrophy, degeneration and necrosis in liver (histopathology not evaluated at 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)	89.3	179	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)	42.9, 85.7	ND	No histopathology in liver or other organs.	NTP, 1985
	Rat, Sprague-Dawley, F	50, 100, or 200 mg/kg-day, gestation days 6-15	200	ND	No maternal or developmental toxicity. Poorly reported study (abstract only). Controls not reported.	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
	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)	85.7	ND	No histopathology in liver or other organs.	NTP, 1985
1,3-DCB	Rat, Sprague-Dawley, 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

Table 4-4. Critical Effect Levels in Subchronic, Chronic, Developmental and Reproductive Oral Studies of Dichlorobenzene cont.

Isomer	Species, Strain, Sex	Exposure Protocol[1]	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects[2]	Reference
	Rat, Sprague-Dawley, F	50, 100, or 200 mg/kg-day, gestation days 6-15	200	ND	No maternal or developmental toxicity. Poorly reported study (abstract only). Controls not reported.	Ruddick et al., 1983
1,4-DCB	Dog, Beagle, M&F	0, 7, 36, or 54 mg/kg-day for 1 year[3]	7	36	Statistically significant increases in liver lesions at the mid and high doses. Statistically significant increases in absolute and relative liver, kidneys, adrenals, and thyroid weight 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. No liver histology exams.	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, hematocrit 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.	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 4-4. Critical Effect Levels in Subchronic, Chronic, Developmental and Reproductive Oral Studies of Dichlorobenzene cont.

Isomer	Species, Strain, Sex	Exposure Protocol[1]	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects[2]	Reference
1,4-DCB	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, Sprague-Dawley, 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, gestation days 6-15	250	500	Decreased maternal weight gain and increased incidences of extra ribs.	Giavini et al., 1986
	Rat, Sprague-Dawley, F	50, 100, or 200 mg/kg-day, gestation days 6-15	200	ND	No maternal or developmental toxicity. Poorly reported study (abstract only). Controls not reported.	Ruddick et al., 1983

Table 4-4. Critical Effect Levels in Subchronic, Chronic, Developmental and Reproductive Oral Studies of Dichlorobenzene cont.

Isomer	Species, Strain, Sex	Exposure Protocol[1]	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects[2]	Reference
1,4-DCB	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
	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

Table 4-4. Critical Effect Levels in Subchronic, Chronic, Developmental and Reproductive Oral Studies of Dichlorobenzene cont.

Isomer	Species, Strain, Sex	Exposure Protocol[1]	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects[2]	Reference
1,4-DCB	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

[1]Doses administered by gavage unless otherwise noted.
[2]Kidney effects not reported for male rats due to the species and sex specificity of the mechanism ($\alpha_{2\mu}$-globulin nephropathy).
[3] Doses administered via gelatin capsules.

ND - not determined
AST- aspartate aminotransferase
ALT- alanine aminotransferase
AP - alkaline phosphatase
GGTP - γ-glutamyltranspeptidase

repeated oral exposures 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 (Table 4-4) and, as such, provides the best basis for comparing differences in oral toxicity between the isomers. Based on the available subchronic and chronic hepatic effects data, and considering differences among these studies in sensitivity of endpoints at comparable dose levels, there is no clear basis for assessing the relative toxicity 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 cytochrome P450-based metabolism than with 1,4-dichlorobenzene. This initial metabolism likely results in a reactive intermediate, which can bind covalently to cellular macromolecules or react with glutathione, resulting in depletion of cellular glutathione 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. 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 (Tables 4-4). Subchronic studies in rats found indications of liver toxicity (liver lesions) in rats at doses of $\geq$179 mg/kg-day for 13 weeks, 270 mg/kg-day for 192 days, and 400 mg/kg-day for 90 days (Hollingsworth et al., 1958; NTP, 1985; Robinson et al., 1991), as well as in mice exposed to 89.3 mg/kg-day for 13 weeks (NTP, 1985). 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). Incidences of renal tubular degeneration were increased in male mice exposed to 85.7 mg/kg-day, but this is not judged to be an adverse effect due to lack of accompanying tubular degeneration or any other kidney lesions. The results of the 103-week NTP (1985) study, therefore, show that 42.9 mg/kg-day and 85.7 mg/kg-day were the chronic NOAELs in for liver and kidney effects in rats and mice. Though no compound-related incidences of nonneoplastic lesions in the liver, kidneys or any other tissues were observed at the two tested doses, these incidences were observed in the liver at the 89.3 mg/kg-day dose in a 1985 NTP subchronic study (NTP, 1985) indicating that 42.9 mg/kg-day in the chronic study is a better selection for a NOAEL.

Considering the induction of liver lesions in rats at doses $\geq$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 $\geq$270 mg/kg-day (Hollingsworth et al., 1958; Robinson et al., 1991), as well as the lack of maternal or developmental toxicity in rats gestationally exposed to 200 mg/kg-day (highest tested dose) (Ruddick et al., 1983), the LOAEL is identified as 89.3 mg/kg-day based on the subchronic

evidence for liver effects in rats (an adverse effect level has not been identified in the available chronic studies).

The subchronic LOAEL of 89.3 mg/kg-day and chronic NOAEL of 42.9 mg/kg-day for liver effects in rats 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. 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 dose levels. Hepatic effects included increased serum levels of AST at $\geq$9 mg/kg-day and increased incidences of lesions at higher doses, including hepatocellular cytoplasmic alterations of minimal to mild severity at $\geq$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 $\geq$9 mg/kg-day. Incidences of rats with moderate or marked reductions in follicular colloidal density were increased at $\geq$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 follicular gland hyperplasia occurred in mice exposed to 429 mg/kg-day of 1,4-dichlorobenzene for 103 weeks (NTP, 1987). Additionally, plasma thyroxine (T_4) concentrations were reduced in rats 24 hours after a single intraperitoneal 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 triiodothyrine (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 $\geq$9 mg/kg-day. Incidences of rats with moderate or marked pituitary cytoplasmic vacuolization were increased at $\geq$588 mg/kg/day. The pituitary lesion only occurred in males and was reportedly similar to "castration cells" found in the pituitary of gonadectomized rats (considered to be an indicator of gonadal deficiency). Serum cholesterol levels were also increased at $\geq$9 mg/kg-day and could be pituitary-related as well liver-related. The overall findings in this study suggest a possible disruption of hormonal feedback mechanisms, or target organ effects on the pituitary, hypothalamus and/or other endocrine organs. 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 red blood cell counts, hematocrit, 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.

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 summarized in Table 4-4 and 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 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.

Hepatic effects induced by subchronic and chronic oral exposures 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 induced. Increased liver weight along with mild to moderately severe liver lesions is the most sensitive effect in a chronic dog study, observed at doses as low as 36 mg/kg-day. Increased liver weight is 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 (Hollingsworth et al., 1956; Lake et al., 1997; Umemura et al., 1998), but is not considered adverse without concomitant enzymatic or histopathological 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 zone of glutamine synthetase-expressing hepatocytes) (Umemura et al., 1998), and increases in liver porphyrins in rats exposed to $\geq$50 mg/kg-day for 120 days were not considered to be toxicologically significant (Carlson, 1977). Hepatocellular hypertrophy and decreased serum triglycerides occurred in rats exposed to $\geq$214 mg/kg-day for 13 weeks (NTP, 1987; Lake et al., 1997). 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) seem 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 are 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 tested level in this species, but higher than the chronic LOAEL in mice.

Considering the information summarized above, 36 mg/kg-day is the lowest chronic LOAEL for liver effects in dogs based on liver lesions and increased absolute and relative liver weights. The chronic NOEL in the dog study is 7 mg/kg-day (Monsanto Company, 1996). The chronic LOAEL for liver effects in mice (the most sensitive species in rodent studies) is 214 mg/kg-day based on hepatocellular degeneration (NTP, 1987). There is no chronic NOAEL in mice because 214 mg/kg-day is the lowest tested chronic dose in this species. The only data on liver effects in mice at doses below this chronic LOAEL are 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), but 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 histological alterations (e.g., hepatocellular hypertrophy) at $\geq$214 mg/kg-day, and necrotic and degenerative effects at $\geq$270 mg/kg-day (Eldridge et al., 1992; Hollingsworth et al., 1956; Lake et al., 1997; NTP, 1987; Umemura et al., 1998), but no hepatic histopathology occurred at doses ranging from 107 to 429 mg/kg-day in chronic rat studies (NTP, 1987). Considering the clearly adverse liver effects 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 is reported in a high dose male and at all levels in the females in the chronic dog study (Monsanto Company, 1996). It was concluded that the lesion could be associated to the test chemical at the mid and high dose in the females where it was accompanied by increased kidney weights and grossly observed renal discoloration. Renal changes, including hyaline droplet accumulation, increased kidney weights, and tubular lesions, are characteristically observed effects of subchronic and chronic oral exposure to 1,4-dichlorobenzene in male rats at doses $\geq$75 mg/kg-day (Bomhard et al., 1988; Lake et al., 1997; NTP, 1987). These findings are detailed in Section 4.2.1.3, but are not further discussed here or included in Table 4-4 because there is a scientific consensus that they are related to the $\alpha_{2\mu}$-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 $\geq$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 $\geq$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 $\geq$214 mg/kg-day for 103 weeks (NTP, 1987). The results of the NTP (1987) study, therefore, indicate 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 is 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, hematocrit, 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 provide no indications that 1,4-dichlorobenzene is 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 the 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 with occasional partial tail loss (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 is 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 is 36 mg/kg-day, which is the same as the LOAEL for kidney effects in both male and 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 pre-weaning period, including decreased birth weight and neonatal survival in F_1 and F_2 pups, at doses ≥90 mg/kg-day. This finding indicates that perinatal developmental toxicity is another sensitive endpoint. The 7 mg/kg-day NOEL and 36 mg/kg-day LOAEL for hepatotoxicity (Monsanto Company, 1996) are the critical effect levels for oral exposure to 1,4-dichlorobenzene.

4.5.2. Inhalation

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 had no effects on standard blood and urine indices, as shown by 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 (Girard et al., 1969; IARC, 1982). Although none of these cases had exposure to unchlorinated benzene (a known human leukemogen), the reports are insufficient for establishing that 1,2-dichlorobenzene was the causal agent. A cohort mortality study was conducted of 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 non-Hodgkin lymphoma, although the risk estimates were based on a small number of observations. The only information on possible hematological effects of inhaled 1,2-dichlorobenzene in animals is 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 exams was not indicated.

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 of 100 ppm 1,2-dichlorobenzene is reported to be irritating to the eyes and respiratory passages (Elkins, 1950). This limited information on irritative effects of 1,2-dichlorobenzene in humans is consistent with histological 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 histological alterations were observed in the respiratory epithelium of the trachea or lungs. The mouse data show that the upper respiratory tract is 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 is considered to be the LOAEL for nasal olfactory lesions in the Zissu (1995) study. A NOAEL cannot be determined.

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 (Dow Chemical, 1981; Hayes et al., 1985). 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 similarly exposed to 49 ppm only and the rabbits and monkeys were similarly exposed to 93 ppm only, but findings in the latter species are compromised by small numbers of animals (2 rabbits/sex and 2 female monkeys). No compound-related histopathological 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 is the LOAEL in this study. The report does not indicate if respiratory tract examinations were conducted in any species.

In the reproductive toxicity study, male and female rats were exposed to 50, 150, or 394 ppm levels 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). $\alpha_{2\mu}$-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 are not considered to be adaptive and not adverse, indicating that the NOAEL and LOAEL for systemic toxicity are 50 ppm and 150 ppm, respectively, based on decreased weight gain. Evaluations of the respiratory tract were not performed in this study. There were no effects on reproduction in either generation, indicating that the NOAEL for reproductive toxicity is 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 400 ppm on days 6-15 (rats) or 6-18 (rabbits) of gestation (Hayes et al., 1985; Dow Chemical, 1981). A maternal LOAEL of 100 ppm is identified for decreased body weight gain in both species. A maternal NOAEL is 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 are identified for developmental toxicity.

The subchronic, reproductive, and developmental toxicity studies all suggest that body weight is a sensitive endpoint of inhaled 1,2-dichlorobenzene in rats and rabbits. The LOAELs for this effect is 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, and 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 is a short term effect level, the most sensitive effect of subchronic or chronic inhalation exposure to 1,2-dichlorobenzene cannot be reliably determined.

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 and 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 provide indications that the liver and nervous system are systemic targets of toxicity in humans, but are limited by lack of adequate quantitative exposure information and/or verification that 1,4-dichlorobenzene was the only factor associated with the effects (Cotter, 1953; Miyai et al., 1988; Reygagne et al., 1992). The hepatic, neurologic, and eye/nose irritation findings in humans are consistent with effects observed in exposed animals, as summarized below.

Information on the inhalation effects of 1,4-dichlorobenzene in animals includes results of a multispecies subchronic toxicity study (Hollingsworth et al., 1956), a subchronic immunotoxicity study in guinea pigs (Suzuki et al., 1991), and chronic toxicity studies in rats and mice (Imperial Chemical Industries Limited, 1980; Riley et al., 1980). In the 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 and monkeys exposed to levels of 96 or 158 ppm are limited by small numbers of animals (1-2/group). Hepatic changes were observed, including increased relative liver weight and slight histological 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 the 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 $\leq$50 ppm for 12 weeks (highest tested concentration, exposure schedule not specified) (Suzuki et al., 1991). In 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 (Imperial Chemical Industries Limited, 1980; Riley et al., 1980). There were no exposure-related histopathological 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 wks of exposure), but the toxicological significance is questionable due to the negative histopathology findings and lack of related clinical chemistry effects, indicating that a chronic NOAEL of 500 ppm was identified in rats. Evaluation of the mouse data is limited by insufficiencies in the available summary of the study, precluding identification of a chronic NOAEL or LOAEL in this species.

Additional data on effects of inhaled 1,4-dichlorobenzene are provided by reproduction studies in rats and mice (Anderson and Hodge, 1976; Tyl and Neeper-Bradley, 1989) and developmental toxicity studies in rats and rabbits (Hayes et al., 1985; Hodge et al., 1977). A 2-generation reproduction 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, although systemic toxicity occurred at all dose levels in F_0 and F_1 adult rats (Tyl and Neeper-Bradley, 1989). Changes indicative of $\alpha_{2\mu}$-globulin nephropathy were found in adult males of both generations at ≥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 ≥66 ppm, F_1 males and F_0 females at ≥211 ppm, and F_1 females at 538 ppm, and absolute liver weights were increased in adult F_0 adult males at ≥211 ppm, and in F_1 males and F_0 and F_1 females at 538 ppm. The increases in liver weight were more pronounced in males than females and statistically significant in these groups, but toxicological significance is questionable due to a lack of accompanying degenerative histopathological effects. The only histopathological finding in the liver was hepatocellular hypertrophy in both sexes and generations at 538 ppm. The liver effects are considered adaptive rather than adverse. 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 are 211 and 538 ppm based on the evidence for parental clinical signs and postnatal toxicity in the offspring. This study also identified a NOAEL of 538 ppm for reproductive toxicity. The 538 ppm reproductive NOAEL in rats is supported by a NOAEL of 450 ppm for reproductive performance in male mice that were exposed for 6 hours/day for 5 days prior to weekly mating with unexposed females for 8 weeks (Anderson and Hodge, 1976). 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 is 500 ppm. A developmental study in which rabbits were exposed to 100-800 ppm for 6 hours/day on gestation days 6-18 found evidence of fetotoxicity (a minor variation of the circulatory system) only at 800 ppm, which was also maternally toxic as shown by body weight loss early in gestation (Hayes et al., 1985), indicating that 800 ppm is a LOAEL for maternal and developmental effects in rabbits.

The available animal data identify adult systemic toxicity (CNS and other clinical signs) and developmental toxicity (increased stillbirths and perinatal mortality) as critical effects of inhaled 1,4-dichlorobenzene. The NOAEL and LOAEL for these effects are 211 and 538 ppm,

based on the findings in rats in the multigeneration reproduction study (Tyl and Neeper-Bradley, 1989). There is 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 at concentrations ≤538 ppm (Tyl and Neeper-Bradley, 1989). Developmental toxicity was only found in rats exposed to 800 ppm, a level that was also maternally toxic and higher than the LOAEL for hepatic effects. The animal database lacks fully adequate information on respiratory tract effects of 1,4-dichlorobenzene, an important limitation because both 1,4- and 1,2-dichlorobenzene are known nose and eye irritants in humans, and the olfactory epithelium is a sensitive target of inhaled 1,2-dichlorobenzene in mice.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION—SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION

4.6.1. 1,2-Dichlorobenzene

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 doses of 1,2-dichlorobenzene for 103 weeks (NTP, 1985). This is a well-designed chronic study with respect to exposure duration and scope of histological examinations, but it is unclear whether an MTD was achieved in either species.

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, although positive in *B. subtilis* (Connor et al., 1985; Shimizu et al., 1983; NTP, 1987; Paolini et al., 1998; 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 (Perocco et al., 1983; Williams et al., 1989). Sister-chromatid exchanges were induced in Chinese hamster ovary (CHO) cells with activation, although chromosomal aberrations were not (Loveday et al., 1990). *In vivo* exposure induced micronucleus formation in mice (Mohtashamipur et al., 1987).

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 and the uncertainty as to whether the MTD was reached. Using the draft cancer guidelines (U.S. EPA, 1999), the available carcinogenicity data for 1,2-dichlorobenzene are considered *inadequate for an evaluation of human carcinogenic potential*.

4.6.2. 1,3-Dichlorobenzene

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 intraperitoneally exposed to 1,3-dichlorobenzene (Mohtashamipur et al., 1987).

EPA concludes that *the data are inadequate for an evaluation of human carcinogenic potential for 1,3-dichlorobenzene*, under the draft revised guidelines for carcinogen risk assessment (U.S. EPA, 1999). These assessments are based on a lack of human and animal carcinogenicity data.

4.6.3. 1,4-Dichlorobenzene

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 (NTP, 1987; Chlorobenzene Producers Association, 1997; Imperial Chemical Industries Limited, 1980; Riley et al., 1980), as well as from subchronic initiation-promotion studies in rats (Gustafson et al., 1998; Umemura et al., 2000).

Chronic oral bioassays were conducted in rats and mice that were exposed to 107 or 214 mg/kg-day (male rats) or 214 or 429 mg/kg-day (female rats and mice of both sexes) doses of 1,4-dichlorobenzene for 103 weeks (NTP, 1987). Kidney tumors were induced in the male rats, as shown by a dose-related increase in the incidence of renal tubular cell adenocarcinomas that was statistically significantly greater than controls in the high-dose group. The 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 incidences. No indications of carcinogenicity were found in the female rats. Findings in the mice included liver cancer in both sexes, as shown by positive dose-related trends for hepatocellular adenomas and carcinomas, with incidences in the low-dose males and high-dose males and females significantly greater than in the controls. Hepatoblastoma, an extremely rare form of hepatocellular carcinoma, also occurred in a few of the high-dose male mice. The incidence of hepatoblastoma was increased, but not quite statistically significant, although comparison to historical control incidences suggested that the finding was likely related to exposure. Other neoplastic effects included marginal increases in adrenal pheochromocytomas in the male mice. The only other information regarding carcinogenicity of

oral exposure are from two-stage studies that found no indications of kidney tumor initiation or liver tumor promotion in rats (Gustafson et al., 1998; Umemura et al., 2000). 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 trisodium nitrilotriacetic acid 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 intraperitoneal injection of *N*-nitrosodiethylamine, followed 2 weeks later by oral promotion with ≤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 that were exposed to 75 or 500 ppm of 1,4-dichlorobenzene for 5 hours/day, 5 days/week for up to 76 weeks (rats) or 57 weeks (mice), followed by 36 weeks (rats) or 19 weeks (female mice) without exposure (Imperial Chemical Industries Limited, 1980; Riley et al., 1980). There were no neoplastic or any other histopathological changes in the liver, kidneys, or other tissues in the rats or female mice. The adequacy of these studies for carcinogenicity evaluation is limited by failure to reach the maximum tolerated dose, 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 from fighting and probable respiratory infection), as well as unavailability of a complete study report. Inhalation carcinogenicity data are also available from an inadequately reported summary of a Japanese study in which rats and mice of both sexes were exposed to 20, 75, or 300 ppm of 1,4-dichlorobenzene on 5 days/week for 104 weeks (Chlorobenzene Producers Association, 1997). Liver tumors were increased in male and female mice at the highest concentration, but the adequacy of this study cannot be evaluated due to the lack of sufficient information on experimental design and results.

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 Chinese hamster ovary 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 mammalian cell evaluations for chromosomal aberrations, sister-chromatid exchanges, and micronucleus formation, were equivocal and inconsistent, with findings that included both positive and negative effects (Anderson et al., 1990; Carbonell et al., 1991; Canonero et al., 1997; NTP, 1987; Mohtashamipur et al., 1987; Miyagawa et al., 1995; Morita et al., 1997; Robbiano et al., 1999; Tegethoff et al., 2000). In animals, the preponderance of studies and overall weight of evidence indicate that 1,4-dichlorobenzene is non-genotoxic. 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.

The human relevance of the 1,4-dichlorobenzene-induced kidney tumors in rats and liver tumors in mice in the NTP (1987) bioassay has been extensively studied and debated. Regarding effects in the kidney, there is a widespread scientific consensus that 1,4-dichlorobenzene causes both renal toxicity and tumors through a non-DNA-reactive mechanism that is specific to male rats and is not present in female rats or other species, including humans (Barter and Sherman, 1999; IARC, 1999; U.S. EPA, 1991b). Substantial evidence indicates that the renal effects are produced by a sequence of events initiated by binding of 1,4-dichlorobenzene with the male rat-specific protein $\alpha_{2\mu}$-globulin. $\alpha_{2\mu}$-Globulin nephropathy is characterized by a series of histopathological 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 (Bomhard et al., 1988; Charbonneau et al., 1989; Lake et al., 1997; NTP, 1987). Based on widely recognized criteria for establishing the role of $\alpha_{2\mu}$-globulin nephropathy in male rat renal carcinogenesis, it is generally accepted that $\alpha_{2\mu}$-globulin-associated kidney tumors are not relevant to humans (Barter and Sherman, 1999; IARC, 1999; U.S. EPA, 1991b).

In contrast to the kidney tumors in male rats, the mechanism by which 1,4-dichloro-benzene 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 the mouse liver tumors is non-genotoxic and is based on sustained mitogenic stimulation and proliferation of the 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 found that liver tumor incidences were only increased in mice that also showed hepatotoxic effects, but not in low-dose female mice, which had little or no hepatotoxicity. The proliferation is 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, based on evidence for decreases in apoptosis and increases in BrdU labeling index, DNA synthesis, or cumulative replicating fraction in livers of exposed mice (Eldridge et al., 1992; James et al., 1998; Lake et al., 1997; Sherman et al., 1998; Umemura et al., 1992, 1996, 1998). However, similar effects were found in the livers of exposed rats, even though 1,4-dichlorobenzene did not induce liver tumors in rats (Eldridge et al., 1992; James et al., 1998; Hasmall et al., 1997; Lake et al., 1997; Sherman et al., 1998; Umemura et al. 1992, 1996, 1998). Additionally, the mitogenic effects of 1,4-dichlorobenzene may not be sustained throughout long-term exposure (Eldridge et al., 1992; Lake et al. 1997), and NTP (1987) did not report hepatic hyperplasia among responses significantly elevated following chronic exposure to 1,4-dichlorobenzene, although other hepatotoxic effects were noted. Thus, the evidence supporting a sustained proliferative response following 1,4-dichlorobenzene exposure as the mode of action for 1,4-dichlorobenzene-induced tumor formation is incomplete.

Evidence of animal carcinogenicity is based on findings of increased tumor incidences in male rat kidneys and in the livers of male and female mice following oral exposure. The kidney tumors in rats are not relevant to humans because the mechanism is specific to male rats. The mechanistic basis of the mouse liver tumors has not been adequately defined. The adequacy of

carcinogenic evaluation via inhalation route is limited due to the failure to reach the maximum tolerated dose, less-than-lifetime exposure durations, and short observation periods in both species (Riley et al. ,1980; Imperial Chemical Industries Limited, 1980). In addition, there are insufficient data available to consider a route to route extrapolation. In view of this, a positive or a negative carcinogenicity Weight of Evidence conclusion based on the inhalation route is not feasible at this time. Therefore, under the draft revised cancer guidelines (U.S. EPA, 1999), 1,4-dichlorobenzene is considered *likely to be carcinogenic* in humans.

4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.7.1. Possible Childhood Susceptibility

Limited information regarding possible adverse effects of dichlorobenzenes in children are 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. These case reports are consistent with an expectation that health effects in children and adults are similar. Although there are no known differences in the disposition of dichlorobenzenes in adults and children, the available data are insufficient to substantiate this claim.

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 (Bio/dynamics, 1989; Bornatowicz et al., 1994; Giavini et al., 1986; Hayes et al., 1985; Hodge et al., 1977; Ruddick et al., 1983; Tyl and Neeper-Bradley, 1989). These studies provide no indications 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.

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, histopathological changes in the thyroid were generally more severe in males than females (McCauley et al., 1995). This study also found histopathology in the pituitary of male rats, but not in females. The pituitary lesion was reported to be similar to those 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 one chronic (NTP, 1985) and three subchronic studies in rats and mice (Hollingsworth et al., 1958; NTP, 1985; Robinson et al., 1991). 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, 5 days/week for 103 weeks (NTP, 1985). The only exposure-related effect in either species was a significantly increased incidence of renal tubular regeneration in the high-dose male mice. This renal alteration is not judged to be an adverse effect due to a lack of accompanying tubular degeneration or any other kidney lesions, indicating that both of the dose levels in this study are NOAELs and that insufficient data are available to identify a critical effect for chronic exposure.

The subchronic studies identify the liver as the most sensitive target for repeated oral exposures to 1,2-dichlorobenzene. As discussed in Section 4.5.1.1, incidences of degenerative liver lesions were significantly increased in rats exposed to 179-400 mg/kg-day for $\geq$13 weeks (Hollingsworth et al., 1958; NTP, 1985; Robinson et al., 1991) and mice exposed to 179 mg/kg-day for 13 weeks (NTP, 1985). The liver was also affected in rats exposed to lower doses of 89.3-135 mg/kg-day for $\geq$13 weeks (Hollingsworth et al., 1958; NTP, 1985; Robinson et al., 1991), but the effects at these levels were essentially limited to increases in relative liver weight and in serum ALT and slight dose-related increases in serum cholesterol, serum protein, and decreases in serum triglycerides. In addition, individual hepatocellular necrosis and focal hepatic necrosis was observed in one female rat (89.3 mg/kg-day) and one male rat (89.3 mg/kg-day) and two female rats (89.3 mg/kg-day) respectively (NTP, 1985). Increased serum ALT is an inconsistent finding because it was induced in rats exposed to $\geq$100 mg/kg-day for 90 days (Robinson et al., 1991), but not in rats exposed to $\geq$89.3 mg/kg-day for 13 weeks (NTP, 1985). Additionally, the increase in serum ALT was not dose-related, and serum levels of other liver-associated enzymes were not increased in either the Robinson et al. (1991) study (AST, LDH and AP) or the NTP (1985) study (AP and GGTP). The lowest subchronic effect level is 89.3 mg/kg-day, based on increased liver weight in the NTP (1985) study. In this study, F344 rats (10/sex/group) and B6C3F$_1$ mice (10/sex/group) were administered 1,2-dichlorobenzene in corn oil by gavage in duration-adjusted doses of 0, 21.4, 42.9, 89.3, 179 or 357 mg/kg-day, 5

days/week 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 incidences of liver lesions were significantly increased in both species at 179 mg/kg-day, as shown in Table 5-1.

Table 5-1. Liver Lesions in Rats and Mice Exposed to 1,2-Dichlorobenzene for 13 Weeks (NTP, 1985)

(Individual cell or focal necrosis; centrilobular degeneration also occurred in the high-dose group)	Duration-adjusted Oral Dose (mg/kg-day)					
	0	21.4	42.9	89.3	179	357
male rats	0/10	ND	ND	1/10	4/9*	8/10*
female rats	0/10	ND	ND	3/10	5/10*	9/10*
male mice	0/10	ND	ND	0/10	4/10*	9/10*
female mice	0/10	ND	ND	0/10	0/10	9/10*

*Significantly different ($p<0.05$) from control incidence; Fisher Exact Test performed by Syracuse Research Corporation.

ND - no histological examinations conducted in this group.

The occurrence of hepatocellular necrosis coupled with an increase in relative liver weight and changes in serum chemistry support the choice of the 89.3 mg/kg-day dose as a LOAEL from the NTP (1985) subchronic study. This selection is further augmented by significant increases in relative liver weight along with increases in relative weights of other organs at the high dose group (400 mg/kg-day) in both sexes in the Robinson et al. (1991) study. A significant increase in relative liver weight at the 100 mg/kg-day dose group in both sexes was also observed in the 1991 study. In addition, ALT values were significantly elevated in males dosed with 100 and 400 mg/kg-day; BUN was also significantly increased in the males at the 400 mg/kg-day level and both males and females showed increased total bilirubin in the high dose group compared to controls. Histopathology at the high dose level in the Robinson et al. study revealed statistically significant increases in liver lesions. A NOAEL of 25 mg/kg-day was identified in the 1991 study. A NOAEL was not identified in the NTP (1985) subchronic study since histopathology examinations were not conducted at the two lower doses (21.4 and 42.9 mg/kg-day). Between the two identified NOAELs (42.9 and 85.7 mg/kg-day) in the chronic NTP study (1985) and considering the effects observed at the 89.3 mg/kg-day dose in the NTP subchronic study, the NOAEL of 42.9 mg/kg-day is the most appropriate basis for the derivation of an RfD for 1,2-dichlorobenzene.

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

The NOAEL/LOAEL approach is an appropriate method for deriving an RfD for 1,2-dichlorobenzene. As discussed in the previous section, no effects occurred in the only chronic oral study of 1,2-dichlorobenzene, in which NOAELs of 42.9 and 85.7 mg/kg-day were identified in rats and mice exposed for 103 weeks (NTP, 1985). Subchronic data show that liver is the

critical target, and the LOAEL of 89.3 mg/kg-day was identified based on hepatic histopathology in rats and mice exposed for 13 weeks (NTP, 1985). Using this approach, the highest chronic NOAEL of 42.9 mg/kg-day is the basis for the RfD.

The lack of a LOAEL in the 103-week study precludes analyzing the chronic data using benchmark dose (BMD) analysis. The BMD analysis was performed on the 13-week liver histopathology data (Table 5-1) to compare points of departure (the lower 95% confidence limit on the BMD [BMDL]) for subchronic effects with the chronic NOAEL. All dichotomous models in the EPA Benchmark Dose Software (version 1.3.1) were fit to the incidence data for liver lesions in the most sensitive animals (male and female rats and male mice). Akaike's Information Criteria (AIC) was used to assess the model with the best fit in each data set, and the best-fitting model was used to calculate a BMD associated with 10% extra risk for liver toxicity and its BMDL (Appendix B1). The Quantal-quadratic, Quantal-linear and Probit models provided the best fits of the male rat, female rat, and male mouse incidence data, respectively (Table B1-2). The BMDs and BMDLs (rounded values) are, respectively, 86.1 and 68.1 mg/kg-day for the male rats, 22.0 and 14.7 mg/kg-day for the female rats, and 126.1 and 82.1 mg/kg-day for the male mice.

The lower of the two chronic NOAELs among 42.9 and 82.7 mg/kg-day was selected as the basis for the RfD derivation for three reasons. First, BMDL ranges between 14.7 mg/kg-day and 82.1 mg/kg-day were calculated using the NTP subchronic study with 14.7 mg/kg-day in female rats being the lowest BMDL. However, the subchronic study size was too small to adequately differentiate the liver effects between the treated and control groups. Second, the subchronic LOAEL would appear to have minimal severe effect. Finally, there was a lack of liver effects at a slightly lower dose (120 mg/kg-day) in the chronic study compared to liver effects at a dose of 125 mg/kg-day in the subchronic study. Since there is a higher confidence in a chronic study when compared to a subchronic study, the chronic NOAEL of 42.9 mg/kg-day (NTP, 1985) was judged to be the most appropriate value on which to base the oral RfD.

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

To derive the RfD for 1,2-dichlorobenzene, the chronic NOAEL of 42.9 mg/kg-day is divided by a total uncertainty factor of 300: 10 for interspecies extrapolation, 10 for interindividual variability, and 3 for database deficiencies.

A 10-fold uncertainty factor is used to account for the interspecies variability in extrapolating from laboratory animals (rats) to humans. 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 insufficient as a basis for reducing the uncertainty factor for interspecies extrapolation.

A 10-fold uncertainty factor is used to account for variation in sensitivity within human populations. No effects on developing fetuses were reported in a study, reported only as an

abstract, in which rats were gestationally exposed to oral doses of 200 mg/kg-day, indicating that developmental toxicity of 1,2-dichlorobenzene, if it does occur, would only occur at levels higher than the critical LOAEL for systemic toxicity (liver effects). However, 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 3-fold uncertainty factor is used to account for deficiencies in the database. There is no information on the toxicity of 1,2-dichlorobenzene in orally-exposed humans. A limited amount of information is available on health effects in people who were occupationally exposed to 1,2-dichlorobenzene, but the data are insufficient for identifying sensitive systemic endpoints in humans or for other risk assessment purposes (see Section 4.5.2.1). Regarding chronic oral toxicity of 1,2-dichlorobenzene in animals, the only available studies (NTP, 1985) were conducted in two species and are generally well-designed. The NTP (1985) studies in rats and mice are limited by the use of only two dose levels and an apparent failure to achieve an MTD in either species, but subchronic studies are sufficient to identify the liver as a critical target, as well as a critical LOAEL for hepatotoxicity. The oral database for 1,2-dichlorobenzene lacks adequate assessments of neurotoxicity and immunotoxicity, as well as endpoints known to be sensitive to other isomers of dichlorobenzene (e.g., thyroid and pituitary, as shown by oral testing with 1,3-dichlorobenzene). The only information on developmental toxicity is from a poorly reported study (Ruddick et al., 1983) that found no evidence of maternal or fetal effects in rats at dose levels higher than the critical LOAEL for systemic effects; data on developmental toxicity in a second species are lacking. The primary limitations of the oral data base are the lack of an adequate developmental toxicity study and reproductive toxicity study in either sex, although an inhalation 2-generation study of 1,2-dichlorobenzene in rats has been conducted (Bio/dynamics, 1989). Because the inhalation study found no effects on reproduction in either generation at exposure levels higher than those causing liver effects in the parental animals, it can be used to partially address the datagap for oral exposure. Therefore, an uncertainty factor of 3 is used for database deficiencies.

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

$$\begin{aligned} \text{RfD} &= \text{NOAEL} \div \text{UF} \\ &= 42.9 \text{ mg/kg-day} \div 300 \\ &= 0.143 \text{ mg/kg-day} \end{aligned}$$

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, pituitary, and liver occurred at all tested dose levels (Table 5-2). This study was selected as the principal study for derivation of the RfD for 1,3-dichlorobenzene. Collectively, the data for male rats (which were more responsive than female rats) in Table 5-2 identify thyroid effects (reduced follicular colloidal density) and pituitary effects (cytoplasmic vacuolation in par distalis) as the critical effects from subchronic exposure. Liver lesions (increased incidence of hepatocellular cytoplasmic alterations) occurred at higher dose levels than the lowest doses that induced thyroid and pituitary effects (Table 5-2). Mean serum levels of AST and cholesterol were statistically significantly increased in all male exposed groups compared with control means, but other serum markers of liver damage such as activities of ALT and LDH were not significantly increased in exposed groups (Table 5-2). 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 and pituitary are considered to be adverse. The vacuolation in the par distalis indicates cytotoxic effects in the pituitary, and the reduced follicular colloidal density in the thyroid is indicative of thyroid stimulation (Gershon and Nunez, 1988). In addition, McCauley et al. (1995) speculated that the elevated serum cholesterol concentrations may be related to pituitary damage, rather than liver damage. In the absence of data to indicate otherwise, the thyroid and pituitary effects are assumed to be critical effects relevant to humans who may chronically ingest 1,3-dichlorobenzene and are selected to serve as the basis of the chronic RfD.

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

Potential points of departure for the RfD were derived by benchmark dose analysis of the thyroid and pituitary data in Table 5-2. All dichotomous models in the EPA Benchmark Dose Software (version 1.3.1) were fit to the male rat incidence data for: 1) reduced follicular colloidal density in the thyroid, and 2) cytoplasmic vacuolation in the pars distalis of the pituitary. For each variable, Akaike's Information Criteria (AIC) was used to select the best fitting model from which benchmark doses (BMDs) and their lower 95% confidence limits (BMDLs) were calculated, using a benchmark response (BMR) of 10% extra risk.

For the thyroid incidence data, the Gamma, Multi-stage, Quantal-linear, and Weibull model runs obtained the same model (power parameters were restricted to be ≥ 1), which provided a better fit than the logistic, quantal-quadratic, or probit models (Appendix B2). The chi-square goodness-of-fit statistics for all of these models indicated poor fits ($p<0.1$), but a graph of the observed incidences of thyroid lesions and Gamma-model-predicted incidences showed a reasonable visual fit (Appendix B2). Thus, the BMD and BMDL predicted from the Gamma model, 4.09 and 1.9 mg/kg-day, respectively, were selected as the best benchmarks for thyroid lesions in male rats (Appendix B2).

Table 5-2. Liver, Thyroid, and Pituitary Effects Observed in Male Rats Orally Exposed to 1,3-Dichlorobenzene for 90 Days (McCauley et al., 1995)

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]
mean serum AST (U/L) ±SD	43.7±37.7	87.6±24.7[b]	109.8±9.5[c]	88.0±23.3[b]	82.8±13.8[b]
mean serum cholesterol (mg/dL) ±SD	73.5±1.4	96.6±1.7[b]	111.1±1.6[b]	157.9±12.5[b]	89.5±1.5[b]
mean serum ALT (U/L) ±SD	46.8±7.7	40.8±9.7	43.3±4.5	38.5±8.2	59.3±11.0
mean serum LDH (U/L) ±SD	1762±765	623±466	798±238	778±530	735±288
thyroid, reduced follicular colloidal density	2/10	8/10[a]	10/10[a]	8/9[a]	8/8[a]
pituitary, cytoplasmic vacuolation in pars distalis	2/10	6/10	6/10	10/10[a]	7/7[a]

[a] Significantly ($p<0.05$) different from control; Fisher Exact Test performed by Syracuse Research Corporation.
[b] Reported to be significantly higher ($p\leq0.05$) than control mean by study authors.
[c] This value was not reported to be significantly higher than control mean.

For the pituitary cytoplasmic vacuolation incidence data, the Gamma, Quantal-linear, and Weibull model runs obtained the same model (power parameters restricted ≥1), which provided a nearly equivalent fit as the Probit model. The other models fit the data less well, using the AIC as the fit indicator (Appendix B2). The BMD and BMDL from the Gamma model were 4.08 and 2.10 mg/kg-day, whereas the BMD and BMDL from the Probit model were 7.79 and 4.46 mg/kg-day. Given the similarities of these BMDLs, their average, 3.3 mg/kg-day, is selected as the BMDL for pituitary cytoplasmic vacuolation in male rats.

Since the BMDLs for thyroid lesions (1.9 mg/kg-day) and pituitary lesions (3.3 mg/kg-day) are similar, and the effects may be related to each other, the point of departure for the RfD is selected as the average of these values, 2.6 mg/kg-day.

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

To derive the RfD, the average BMDL of 2.6 mg/kg-day for reduced thyroidal colloidal density and cytoplasmic vacuolation in the pituitary of male rats exposed to 1,3-dichlorobenzene was divided by a total uncertainty factor of 3000: 10 for interspecies variability, 10 for interindividual variability, 10 for extrapolation from subchronic to chronic exposure, and 3 for database deficiencies.

A 10-fold uncertainty factor was used to account for uncertainty in extrapolating from rats to humans (i.e., interspecies variability). No information is available on the toxicity of ingested 1,3-dichlorobenzene in humans, or on differences that may exist between animals and humans in

the disposition of, or response to, ingested 1,3-dichlorobenzene. In the absence of data to the contrary, the pituitary and thyroid effects observed in subchronically exposed rats are assumed to be relevant to humans chronically exposed to ingested 1,3-dichlorobenzene.

A 10-fold uncertainty factor was used to account for variation in sensitivity to 1,3-dichlorobenzene within human populations. There were no effects on developing fetuses of rat dams exposed to a dose of 200 mg/kg-day, suggesting that developmental effects from 1,3-dichlorobenzene, if they occur, would only occur at dose levels higher than those inducing thyroid or pituitary effects in subchronically exposed rats (9-147 mg/kg-day). However, this study was inadequately reported. 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. The rat subchronic toxicity study identified male rats as more susceptible to the thyroid, pituitary, and liver effects of 1,3-dichlorobenzene, but additional information on possible gender differences in toxicokinetics or toxicodynamics is not available.

A 10-fold uncertainty factor was used to account for extrapolating from subchronic oral exposure to chronic oral exposure. Although the modes of action whereby 1,3-dichlorobenzene may produce cytotoxic effects on the pituitary and stimulate 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 3-fold uncertainty factor was used to account for deficiencies in the database. Some of the uncertainty in the database is addressed by the factors used for uncertainty in other areas (e.g., interspecies variability). The only information on the systemic toxicity of repeated oral exposure to 1,3-dichlorobenzene comes from the subchronic rat study reporting thyroid and pituitary effects at doses $\geq$9 mg/kg-day (McCauley et al., 1995). This is a well-designed study that 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 found no evidence for maternal toxicity or developmental toxicity in rats at a dose level of 200 mg/kg-day (Ruddick et al., 1983), but the data are not well reported. The oral-exposure database for 1,3-dichlorobenzene contains no chronic toxicity data and lacks assessments of developmental toxicity in a second animal species, reproductive toxicity in males or females, neurotoxicity and immunotoxicity.

The RfD for 1,3-dichlorobenzene is calculated as follows:

$$\begin{aligned} \text{RfD} &= \text{BMDL} \div \text{UF} \\ &= 2.6 \text{ mg/kg-day} \div 3000 \\ &= 9\text{x}10^{-4} \text{ mg/kg-day} \\ &= 0.9 \text{ µg/kg-day} \end{aligned}$$

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 toxicity of 1,4-dichlorobenzene has been assessed in a number of studies of 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 thyroids 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 indicate that developmental effects from gestational exposure, along with maternal weight gain effects, 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) indicate 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-DCB in male and female beagle dogs 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 (adjusted doses; 0, 7, 36, 107 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 (71 mg/kg-day) during the third week of exposure for males and further reduced to 75 mg/kg-day (54 mg/kg-day) for both sexes at the beginning of week six. 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 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 undetected. 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 include statistically significant liver lesions (Table 5-3) and increase in absolute and relative organ weights (liver, kidneys, adrenals, and thyroid) at the mid and high dose levels (Table 5-4). 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 levels in the females. The authors concluded that the lesion could be associated to the test chemical at the mid and high dose in the females where it was accompanied by increased kidney weights and grossly observed renal discoloration (Monsanto Company, 1996).

In summary, hepatotoxicity is 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 NOAEL of 7 mg/kg-day and a LOAEL of 36 mg/kg-day is selected as the principal study for RfD derivation.

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

Compound related liver lesions (diffuse hepatocellular hypertrophy, multifocal chronic inflammation, and multifocal hepatocyte pigment deposition in males and diffuse hepatocellular hypertrophy in females) in both male and female beagle dogs were analyzed by benchmark dose modeling because there was a statistically significant increase in liver lesions in the mid and high dose groups. All dichotomous models in the EPA Benchmark Dose Software (version 1.3.2) were fit to the incidence data for liver lesions in male and female beagle dogs (Table 5-5). All models, except the Probit, Quantal-linear and Quantal-quadratic models (male beagle dogs) (Table 5-6) adequately ($p>0.1$) fit the liver lesions as indicated by the chi-square goodness-of-fit statistic (U.S. EPA, 2003). Based on the Log-logistic BMDL of 0.237 mg/kg-day, liver lesions (multifocal chronic inflammation) in male beagle dogs were more sensitive compared to the lesions in the female dogs (Table 5-6).

Table 5-3. Summary of Liver Histopathology Incidence in Female and Male Beagle Dogs Exposed to 1,4-Dichlorobenzene in Gelatin Capsules (Monsanto Company, 1996)

Liver Histopathology	Dose Group (mg/kg-day)							
	M[a]0	F[b]0	M[a]7	F[b]7	M[a]36	F[b]36	M[a]54	F[b]54
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, Miltifocal Hyperplasia	0	0	0	0	0	0	1	1
Diffuse Hepatocellular Hypertrophy	0	0	0	0	3[c]	2[c]	5[c]	4[c]
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 Inflamation	0	0	0	0	0	0	0	1
Focal Chronic Inflamation	0	0	1	0	0	1	0	0
Multifocal Chronic Inflamation	2	5	3	4	5	3	4	3
Focal Portal Inflamation	0	0	0	1	0	1	0	0
Multifocal Portal Inflamation	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] Male dogs

[b] Female dogs

[c] Statistically significant at $p \leq 0.01$, Fisher's exact test, one-tailed

Table 5-4. Absolute and Relative Liver Weights of Female and Male Beagle Dogs Exposed to 1,4-Dichlorobenzene in Gelatin Capsules (Monsanto Company, 1996)

Effect	Dose (mg/kg-day)						
	0	7	% Control	36	% Control	54	% Control
Absolute Liver Weight (gm) Male	379.8	318.64	84	473.22	125	531.9[a]	140
Absolute Liver Weight (gm) Female	261.8	291.42	111	388.68	148	407.4[b]	156
Relative Liver Weight (%) Male	2.7738	2.8821	104	3.9663[b]	143	4.726[b]	170
Relative Liver Weight (%) Female	2.7078	3.0504	113	4.2028[b]	155	4.6040[b]	170

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

Table 5-5. BMD Modeling of Incidence Data for Liver Lesions in Male Beagle Dogs Exposed to 1,4-Dichlorobenzene (Monsanto Company, 1996). BMDs and BMDLs were calculated based on a BMR of 10% extra risk for the lesion.

Model	AIC	Chi-square p-value	BMD (mg/kg-day)	BMDL (mg/kg-day)
Diffuse Hepatocellular Hypertrophy				
Gamma	8.88452	0.08	24.234	6.09995
Log-Logistic	8.73462	0.00	31.1502	7.7263
Multistage-3 degrees	9.15306	0.25	16.6264	3.78861
Probit	10.7301	0.00	32.0714	7.47999
Quantal Quadratic	10.0978	0.81	10.766	7.68502
Weibull	10.7301	0.00	28.2718	6.05214
Multifocal Chronic Inflammation				
Gamma	24.8958	1.91	2.9798	1.29394
Log-Logistic	26.4232	1.43	1.16546	0.237025
Multistage-3 degrees	24.8958	1.91	2.97979	1.29394
Quantal Linear	24.8958	1.91	2.97971	1.29394
Weibull	24.8958	1.91	2.97971	1.29394
Multifocal Hepatocyte Pigment Deposition				
Gamma	18.045	0.51	18.0286	5.1137
Log-Logistic	18.0067	0.46	17.4673	3.64104
Multistage-3 degrees	16.2062	0.72	20.9665	5.00917
Probit	17.879	0.37	17.542	8.77067
Quantal Linear	16.3776	0.58	10.2144	4.90518
Quantal Quadratic	16.2062	0.72	20.9665	14.5079
Weibull	18.1169	0.55	17.0605	5.06598

Table 5-6. BMD Modeling of Incidence Date for Liver Lesions in Female Beagle Dogs Exposed to 1,4-Dichlorobenzene (Monsanto Company, 1996). BMDs and BMDLs were calculated based on a BMR of 10% extra risk for the lesion.

Model	AIC	Chi-square p-value	BMD (mg/kg-day)	BMDL (mg/kg-day)
Diffuse Hepatocellular Hypertrophy				
Gamma	15.7361	0.00	23.4038	4.10099
Log-Logistic	15.7387	0.00	24.1591	4.26188
Multistage-3 degrees	13.7758	0.02	21.6045	4.06118
Probit	15.7342	0.00	24.6023	6.4818
Quantal Linear	15.8457	1.44	5.60153	2.97246
Quantal Quadratic	14.1096	0.26	14.9645	10.8004
Weibull	15.7758	0.02	21.6108	4.06118

For the male liver lesion (multifocal chronic inflammation) analysis, the Gamma, Multistage, Linear, and Weibull models were a better fit to the data than the Log-logistic on the basis of the Akaike's Information Criterion (AIC), but the Log-logistic model was characterized by the closest match between predicted and observed response, as evidenced by the lowest chi-square value. In addition, in this instance, the Gamma, Multistage, and Weibull models were equivalent to the Linear models, as all but one of the model parameters for each of the Gamma, Multistage, and Weibull were constrained by their predefined lower bounds. The end result was that only one parameter needed to be estimated for the Gamma, Multistage, Linear models, and Weibull while two parameters were estimated for the Log-logistic model. Presumably, the Gamma, Multistage, and Weibull models would have yielded lower BMDLs had the parameter lower-bound constraints been removed. In general, a 2-parameter model would be superior to a 1-parameter model for fitting dose-response data. In this case, although the 1-parameter linear model appeared to fit the data slightly better than the 2-parameter Log-logistic model, the difference in goodness-of-fit was inconsequential. Therefore, the Log-logistic BMDL of 0.237 mg/kg-day for 10% extra risk of liver lesions in the male beagle dogs was chosen as the point-of-departure for the RfD because it was the most sensitive measure of toxicity and it arose from an unconstrained 2-parameter model.

For comparison purposes, the mean relative organ weights for liver, kidneys, adrenals and thyroid were also analyzed using the benchmark dose approach. Linear models with a constant variance or a non-homogenous variance in the EPA Benchmark Dose Software (version 1.3.2) were fit to the mean relative liver, kidneys, adrenals and thyroid weight data in Table 5-4. Log-likelihood ratio tests for mean relative liver weights in male and female beagle dogs showed that the data were appropriate for modeling. Using the relative deviation at a Bench Mark Response (BMR) of 10%, the BMDs and BMDLs for liver weights from the various continuous models were somewhat similar for both male and female dogs (Table 5-7; kidney, adrenal and thryoid BMDs and BMDLs shown in Appendix B3). The BMDs and BMDLs for relative liver weights in male and female dogs ranged from 10.21584 to 15.6199 mg/kg-day and 7.65337 to 7.89713 mg/kg-day respectively (Table 5-7).

Table 5-7. BMD Modeling of Relative Liver Weights in Male and Female Beagle Dogs Exposed to 1,4-dichlorobenzene in Gelatin Capsules (Monsanto Company, 1996). BMDs and BMDLs were calculated based on a BMR of 10% relative deviation for the relative liver weights.

Model	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Male Dogs			
Polynomial Linear	-9.585979	10.1584	7.65337
Polynomial-2 Degrees	-7.886115	13.4342	7.78095
Power	-3.991585	15.6199	7.80525
Female Dogs			
Polynomial-Linear	-5.550795	10.6472	7.89713
Polynomial-2 Degrees	-5.550795	10.6472	7.89713
Power	-1.550795	10.6472	7.89713

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

To derive the RfD, the $BMDL_{10}$ of 0.237 mg/kg-day for liver lesions from a 1-year chronic toxicity study in beagle dogs exposed to 1,4-dichlorobenzene was divided by a total uncertainty factor of 100: 10 for interspecies variability, and10 for interindividual variability.

A 10-fold uncertainty factor was used to account for uncertainty in extrapolating from dogs to humans (i.e., interspecies variability). Limited information is available on the toxicity of ingested 1,4-dichlorobenzene in humans, or on differences that may exist between animals and humans in the disposition of, or response to, ingested 1,4-dichlorobenzene. In the absence of data to the contrary, the liver lesions in the mid and high dose male and female beagle dogs and significant increases in relative organ weights in male and female dogs is assumed to be relevant to humans chronically exposed to ingested 1,4-dichlorobenzene.

A 10-fold uncertainty factor was used to account for variation in sensitivity to 1,4-dichlorobenzene within human populations. However, the degree to which humans of varying gender, health status, or genetic makeup may vary in disposing of, or responding to, ingested 1,4-dichlorobenzene has not been studied.

The animal oral toxicity database is substantial and generally adequate, including chronic toxicity studies 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). Effects of oral exposure to 1,4-dichlorobenzene on various organs was evaluated along with effects in the hematopoietic system. Based on these results, an uncertainty factor of 1 was applied for data base adequacy.

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

$$\begin{aligned} RfD &= BMDL \div UF \\ &= 0.237 \text{ mg/kg-day} \div 100 \\ &= 0.0024 \text{ mg/kg-day} \end{aligned}$$

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 (Girard et al., 1969; IARC, 1982). 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 (Elkins, 1950; Hollingsworth et al., 1958). None of the human data are sufficient for risk assessment, as discussed in Section 4.5.2.1.

The observation of irritative effects of 1,2-dichlorobenzene in occupationally-exposed humans is consistent with histological 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 histological alterations were observed in the trachea or lungs. 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 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 a sensitive maternal systemic endpoint, occurring at 93-150 ppm in rats and rabbits (Bio/dynamics, 1989; Hayes et al., 1985; Hollingsworth et al., 1958), and there were no effects on reproduction or developmental toxicity in these species at concentrations below 394-400 ppm (Bio/dynamics, 1989; Dow Chemical, 1981; Hayes et al., 1985).

The 14-day mouse study showed that the upper respiratory tract is 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 (PBPK, BMD, etc.)*

Not applicable.

5.2.1.3. *RfC Derivation—Including Application of Uncertainty Factors (UFs) and Modifying Factors (MFs)*

Not applicable.

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 vary dramatically following 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 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. 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 (PBPK, BMD, etc.)*

Not applicable.

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

Not applicable.

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 suggest that the liver and nervous system are systemic targets of toxicity in humans, but are limited by lack of adequate quantitative exposure information and/or verification that 1,4-dichlorobenzene was the sole causal factor (Cotter, 1953; Miyai et al., 1988; Reygagne et al., 1992). The hepatic, neurologic and eye/nose irritation observations in humans are consistent with effects observed in animals exposed to high concentrations of the chemical.

The inhalation toxicity of 1,4-dichlorobenzene in animals was evaluated in several studies that involved subchronic, chronic and multigeneration exposures, mainly in rats as discussed in Section 4.5.2.3. Daily and weekly exposures were similar (i.e., 5-7 hours/day and 5 days/week) and are not detailed below to facilitate comparisons between the studies. The findings show a general pattern in which increased liver weight was the predominant effect at tested exposure levels below those inducing overt toxicity. Liver weight was increased in guinea pigs exposed to $\geq$96 ppm and rats exposed to $\geq$158 ppm for 5-7 months (Hollingsworth et al., 1956), rats exposed to 500 ppm for 76 weeks (Imperial Chemical Industries Limited, 1980), and rats exposed to $\geq$66 ppm for 15-17 weeks in a 2-generation reproduction study (Tyl and Neeper-Bradley, 1989), but increases in liver weight in the absence of concomitant enzymatic and histopathological changes is not considered to be adverse. Hepatic histological changes were observed in rats at 158 ppm (cloudy swelling, congestion or granular degeneration), but considered of questionable significance by the investigators, and were not reported at 358 ppm in the same study (Hollingsworth et al., 1956), indicating that neither 158 or 358 ppm is a reliable LOAEL for liver pathology in rats. Hepatic histological effects were also observed in guinea pigs at 341 ppm and seem to have been more severe (cloudy swelling with fatty degeneration, focal necrosis and slight cirrhosis) than in rats, but only occurred in some of the animals (number not reported) (Hollingsworth et al., 1956). These findings suggest that 341 ppm is a LOAEL for liver histopathology in guinea pigs, but confidence is low due to imprecise and brief qualitative reporting of the results, a general limitation of the Hollingsworth et al. (1956) study.

Liver histopathology was described as slight to moderate (cloudy swelling and central necrosis) in guinea pigs, rats and rabbits exposed to 798 ppm, and overt signs of toxicity (e.g., marked tremors, weight loss, eye irritation and unconsciousness) were found in all of these species at the same level (Hollingworth et al., 1956), showing that this concentration is a LOAEL for 1,4-dichlorobenzene. Similar clinical signs, including tremors, salivation, and ocular and nasal discharges, as well as non-adverse hepatic histological alterations (hepatocellular hypertrophy without degenerative changes) consistent with the increased liver weight, occurred in adult F_0 and F_1 rats exposed to 538 ppm for 15-17 weeks in the 2-generation reproduction study (Tyl and Neeper-Bradley, 1989). Other effects at 538 ppm included reduced gestational and lactational 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, establishing that this concentration is also a LOAEL in rats. Survival at lactation day 4 was the only pup viability index that was significantly reduced on a per litter basis (Table 5-8). Considering the available data, the lowest subchronic LOAEL in rats is 538 ppm based on toxicity in adult animals in the 2-generation study, including signs of neurotoxicity and eye and nasal irritation, as well as postnatal developmental toxicity in their pups (Tyl and Neeper-Bradley, 1989). The only effect that was clearly and consistently exposure-related at doses lower than 538 ppm was increased liver weight at 211 ppm in the same study, but this is not considered to be adverse due to lack of any accompanying histological changes.

Table 5-8. Selected Effects in Rats Exposed to 1,4-Dichlorobenzene for Two Generations (Tyl and Neeper-Bradley, 1989)

Developmental Effect	Exposure Concentration (ppm)			
	0	**66**	**211**	**538**
4-day survival index[1] in F_1 pups [mean ± SD (no. litters)]	93.8 ± 20.33 (n=24)	97.5 ± 3.57 (n=20)	92.7 ± 21.07 (n=27)	82.0* ± 29.25 (n=22)
4-day survival index[1] in F_2 pups [mean ± SD (no. litters)]	99.1 ± 2.25 (n=22)	99.4 ± 2.80 (n=20)	99.3 ± 1.99 (n=24)	71.3* ± 41.96 (n=21)

*Significantly different ($p<0.05$) from control group as reported by study investigators
[1]4-Day survival index = no. pups surviving 4 days ÷ total no. live pups at birth

In summary, the critical LOAEL in rats is 538 ppm based on clinical signs of toxicity in adults and postnatal developmental toxicity in their offspring (Tyl and Neeper-Bradley, 1989). The highest reliable NOAEL below the rat and guinea pig LOAELs is 211 ppm in rats in the 2-generation study (Tyl and Neeper-Bradley, 1989). The F_0 and F_1 rats in this study were exposed for 6 hours/day, 5 days/week for 10-11 weeks before mating and subsequently through the F_1 and F_2 generations. There is no evidence that reproductive toxicity or prenatal developmental toxicity are critical effects of inhaled 1,4-dichlorobenzene in rats (Hayes et al., 1985; Hodge et al., 1977; Tyl and Neeper-Bradley, 1989), as discussed in Section 4.5.2.3.

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

Potential points of departure for the RfC were derived by benchmark dose analysis of the F_1 and F_2 pup postnatal survival data in Table 5-4. None of the continuous variable models in the EPA Benchmark Dose Software (version 1.3.1) adequately ($p>0.1$) fit the F_1 or F_2 survival data as assessed by the chi-square goodness-of-fit statistic. Linear models with either an assumed constant variance or with variance modeled as a power function of the mean were fit to the F_1 pup survival data using EPA Benchmark Dose Software (version 1.3.1). Log-likelihood ratio tests indicated that both models adequately described the data, and that a non-homogeneous variance model was more consistent with the data than a constant variance model (Appendix B4). Akaike's Information Criteria (AIC) for the non-homogeneous variance model was slightly lower than the AIC for the constant variance model, indicating a better fit of the data. The non-homogeneous variance model therefore was selected to calculate the BMC and BMCL for reduced 4-day survival in F_1 rat pups, using a 5% decrease in pup survival index (compared with the control) as the BMR. A 5% decrease was selected (instead of 10% or 1 standard deviation change from the control), because the effect (decreased postnatal survival) is severe and one that would be of high concern if it occurred in human populations. The BMC and BMCL are 146 and 93 ppm, respectively. The BMCL of 93 ppm is selected as the point of departure for the RfC for 1,4-dichlorobenzene.

5.2.3.3. *RfC Derivation—Including Application of Uncertainty Factors (UFs) and Modifying Factors (MFs)*

To calculate the RfC for 1,4-dichlorobenzene, the BMCL of 93 ppm (559 mg/m^3) in rats (Tyl and Neeper-Bradley, 1989) is first duration-adjusted for intermittent exposure, as follows (U.S. EPA, 1994b):

$$BMCL_{ADJ} = (BMCL)\ (hours/24\ hours)\ (days/7\ days)$$
$$= (559\ mg/m^3)\ (6/24)\ (5/7)$$
$$= 99.8\ mg/m^3$$

1,4-Dichlorobenzene exhibits its toxic effects outside of the respiratory tract and consequently is treated as a category 3 gas for purposes of calculating the RfC. The human equivalent concentration (HEC) for extrarespiratory effects produced by a category 3 gas is calculated by multiplying the duration-adjusted BMCL 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 rats and humans. Using a default value of 1 for the ratio of partition coefficients, the $BMCL_{HEC}$ becomes 99.8 mg/m^3:

$$BMCL_{HEC} = (BMCL_{ADJ}) \times [(H_{b/g})_{RAT} / (H_{b/g})_{HUMAN}],$$
$$= 99.8\ mg/m^3 \times [1] = 99.8\ mg/m^3$$

The $BMCL_{HEC}$ of 99.8 mg/m^3 for reduced postnatal pup survival in a 2-generation reproduction study in rats is used as the point of departure for calculating the RfC. The RfC was derived by dividing the $BMCL_{HEC}$ by a total uncertainty factor of 100: 3 for interspecies extrapolation, 10 for interindividual variability, and 3 for database deficiencies.

A 3-fold uncertainty factor is 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 human equivalent exposure for time and concentration ($BMCL_{HEC}$)]. Accordingly, only the pharmacodynamic area of uncertainty remains as a partial factor for interspecies uncertainty ($10^{0.5}$ or approximately 3).

A 10-fold uncertainty factor is used to account for variation in sensitivity within human populations. Results of studies in rats and rabbits (Hayes et al., 1985; Hodge et al., 1977) indicate that teratogenic and fetotoxic effects from gestational exposure to 1,4-dichlorobenzene, if they occur, would only occur at exposure levels that are maternally toxic and similar to or higher than cross-generational doses inducing developmentally toxic effects during early postnatal periods. The 2-generation study in rats (Tyl and Neeper-Bradley, 1989) indicates that the early postnatal period is a susceptible age/developmental period for toxicity to 1,4-dichlorobenzene, but the degree to which humans of varying gender, health status, or genetic makeup may vary in disposition of or response to the chemical has not been studied.

A 3-fold uncertainty factor is used to account for deficiencies in the database. Available information on health effects in people is insufficient for identifying sensitive systemic endpoints in humans. The chronic inhalation toxicity of 1,4-dichlorobenzene was investigated in two species (rats and mice), but both studies have limitations. The chronic study in rats (Imperial Chemical Industries Limited, 1980) is limited by failure to achieve a clear effect level and a less-than-lifetime exposure duration (76 weeks). The chronic mouse study also lacks an effect level and lifetime exposure duration (57 weeks), and is further limited by unavailability of an adequate report. Information on the systemic toxicity of subchronic inhalation exposure is available from a multiple species study, but some of the data are compromised by reporting insufficiencies (Hollingsworth et al., 1956). The prenatal developmental toxicity of inhaled 1,4-dichlorobenzene has been sufficiently studied (Hayes et al., 1985; Hodge et al., 1977). The two-generation reproductive study (Tyl and Neeper-Bradley, 1989) was generally well conducted but the spacing of the exposure levels limits characterization of exposure-response relationships (essentially all effects occurred at the highest of three tested concentrations). The chronic inhalation study in rats showed no exposure-related changes in the nasal passages or other parts of the respiratory tract in rats exposed to 500 ppm of 1,4-dichlorobenzene (Imperial Chemical Industries Limited, 1980), but additional studies are needed to fully characterize respiratory system effects of the chemical.

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

$$\begin{aligned} \text{RfC} &= \text{BMCL}_{\text{HEC}} \div \text{UF} \\ &= 99.8\ \text{mg/m}^3 \div 100 \\ &= 1.0\ \text{mg/m}^3 \end{aligned}$$

5.3. CANCER ASSESSMENT

5.3.1. 1,2-Dichlorobenzene

Available carcinogenicity data for 1,2-dichlorobenzene are inadequate, precluding quantitative assessment of oral and inhalation cancer risk for this isomer.

5.3.2. 1,3-Dichlorobenzene

No data are available on the carcinogenicity of 1,3-dichlorobenzene, precluding quantitative assessment of oral and inhalation cancer risk for this isomer.

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 ($\alpha_{2\mu}$-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. Additionally observed in the high-dose male mice were four cases of hepatoblastoma, an extremely rare type of hepatocellular carcinoma. 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 for 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 5-9. The doses shown are average daily doses in the gavage study. Animals dying before the first appearance of liver tumors 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 5-9. Tumor Incidence Data Used for Dose-Response Assessment for 1,4-Dichlorobenzene

Species/ Strain/Sex	Tumor Type and Location	0 (mg/kg-day)	214 (mg/kg-day)	429 (mg/kg-day)
Male $B6C3F_1$ Mouse	Hepatocellular adenoma or carcinoma	17/44	22/40	40/42
Female $B6C3F_1$ Mouse	Hepatocellular adenoma or carcinoma	15/44	10/44	36/44

Data taken from NTP (1987). Denominators were adjusted for early mortality, as per U.S. EPA (1987).

5.3.3.1.3. Dose Conversion

In accordance with the proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), 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 is accomplished as follows:

$$HED = Dose \times \sqrt[4]{W / 70kg} * (Le / L)^3$$

where:

HED = human equivalent dose
Dose = average daily dose in animal study
W = animal body weight (kg)
70 kg = reference human body weight
Le = duration of experiment
L = lifespan of the animal

For the NTP (1987) study, the duration of the study was equal to the lifespan of the mice (103 weeks). 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 5-10.

Table 5-10. HEDs Corresponding to Average Daily Animal Doses in NTP (1987) Using a $BW^{3/4}$ Scaling Factor and Time-weighted Average Body Weights for Male and Female Mice from the Study

Animal 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

5.3.3.1.4. Extrapolation Method(s)

According to U.S. EPA (1999) *Draft Revised Guidelines for Carcinogen Risk Assessment*, both a linear and a non-linear approach to dose-response assessment can be taken for agents that are not DNA reactive and for which the plausible mode of action is consistent with non-linearity, but not fully established. As discussed in Section 4.4.1.2, available evidence indicates that the mechanism leading to the formation of the mouse liver tumors following 1,4-dichlorobenzene ingestion is non-genotoxic and 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. Thus, the evidence supporting a sustained proliferative response as the mode of action for 1,4-dichlorobenzene-induced tumor formation is incomplete, which precludes the application of a non-linear approach to quantify the carcinogenic risk from exposure to1,4-dichlorobenzene. A linear approach for the derivation of a quantitative estimate of cancer risk for ingested 1,4-dichlorobenzene was taken.

A linear approach results in calculation of an oral slope factor that describes the cancer risk per unit dose of the chemical at low doses. In accordance with the 1999 *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999), a linearized multistage model (Global86) was fit to the data, and cancer slope factors (95% upper confidence limits on the low dose slope q_1*) were calculated by the model.

5.3.3.1.5. Oral Slope Factor

The results of the linear analyses are shown in Tables 5-11 (male data) and 5-12 (female data). The q_1* values were calculated by Global86. Background tumor incidence was estimated in the model, and calculations were based on extra risk. The q_1* based on the male data ($1.3x10^{-2}$ per mg/kg-day) is an order of magnitude greater than that based on the female data ($3.3x10^{-3}$ per mg/kg-day). The largest of the calculated slope factors, which is most protective of human health, is chosen as the slope factor for the chemical ($1.3x10^{-2}$ per mg/kg-day), based upon the combined incidence of hepatocellular adenomas or carcinomas in male $B6C3F_1$ mice.

Table 5-11. q_1* Values Based on Combined Hepatocellular Adenoma or Carcinoma Incidence Data in Male $B6C3F_1$ Mice

0 (mg/kg-day)	33[a] (mg/kg-day)	66[a] (mg/kg-day)	q_1^{*}[b] $(mg/kg-day)^{-1}$
17/44	22/40	40/42	$1.3x10^{-2}$

[a] HED calculated as described in Section 5.3.3.3, above.
[b] q1* calculated by GLOBAL86 (background estimated in model, based on extra risk, 2° polynomial chosen by GLOBAL86)

Table 5-12. q_1* Values Based on Combined Hepatocellular Adenoma or Carcinoma Incidence Data in Female $B6C3F_1$ Mice

0 (mg/kg-day)	31[a] (mg/kg-day)	63[a] (mg/kg-day)	q_1^{*}[b] $(mg/kg-day)^{-1}$
15/44	10/44	36/44	$3.3x10^{-3}$

[a] HED calculated as described in Section 5.3.3.3, above.
[b] q1* calculated by GLOBAL86 (background estimated in model, based on extra risk, 3° polynomial chosen by GLOBAL86)

5.3.3.2. *Inhalation Exposure*

Available inhalation carcinogenicity data for 1,4-dichlorobenzene are inadequate, precluding quantitative assessment of inhalation cancer risk for this isomer. An increase in liver tumors in male and female mice was reported in an unpublished study from the Japanese literature, but the adequacy of cannot be evaluated due to a lack of sufficient information on experimental methods and results in the available summary (Chlorobenzene Producers Association, 1997). Earlier inhalation bioassays (Imperial Chemical Industries Limited, 1980; Riley et al., 1980) did not find tumor increases in exposed rats or mice, but were not adequate studies due to failure to reach the maximum tolerated dose, less-than-lifetime exposure durations, and short observation periods.

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 subchronic animal studies identify the liver as the most sensitive target for repeat oral exposures to 1,2-dichlorobenzene (NTP, 1985).

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 of inhalation exposures 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. The subchronic inhalation studies did not examine the respiratory tract, indicating that a critical effect for long-term exposures cannot 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, but these may not have been adequate tests of carcinogenicity due to uncertainty as to whether the MTD was reached. Using the draft revised cancer guidelines (U.S. EPA, 1999), the available carcinogenicity data for 1,2-dichlorobenzene are considered inadequate for an evaluation of human 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 exposures 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 and a poorly reported developmental toxicity study in rats. Based on the subchronic data (McCauley et al., 1995), the thyroid and pituitary are identified as particularly sensitive targets of repeated oral exposures to 1,3-dichlorobenzene.

No information is available regarding the carcinogenicity of 1,3-dichlorobenzene in humans or animals. In accordance with the draft revised cancer guidelines (U.S. EPA, 1999), the data are inadequate for an evaluation of human 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 and pharmaceuticals, 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 available 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 are the best studied and most consistently observed systemic findings. A limited amount of data indicate 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, at doses lower than those causing systemic effects in the subchronic and chronic toxicity studies. Among all the observed effects, the liver was identified as the most sensitive endpoint (beagle dog study, Monsanto Company, 1996) for oral exposure to 1,4-dichlorobenzene.

The inhalation toxicity of 1,4-dichlorobenzene in animals was evaluated in several studies involving subchronic, chronic, gestational and multigenerational exposures, mainly in rats. The findings show a general pattern in which increased liver weight was the predominant effect at tested exposure levels below those inducing overt toxicity. The increases in liver weight were generally considered to be adaptive and not adverse due to lack of accompanying hepatic histopathology. There is no indication that inhaled 1,4-dichlorobenzene is a reproductive or

prenatal developmental toxicant in animals. A 2-generation study showed that the critical effects of inhalation exposure are clinical signs of toxicity in adult rats, including neurotoxicity and eye and nasal irritation, and postnatal developmental toxicity in their offspring, including reduced neonatal survival in F_1 and F_2 pups (Tyl and Neeper-Bradley, 1989).

Oral cancer bioassays were conducted in male and female rats and mice that were chronically exposed to 1,4-dichlorobenzene. The rat study found no tumor increases in females and, in males, an increase in the incidence of renal tubular adenomas or adenocarcinomas, which are associated with male rat-specific hyaline droplet ($\alpha_{2\mu}$-globulin) nephropathy and not relevant to carcinogenicity in humans. The mouse study showed increased incidences of hepatocellular neoplasms in both sexes, indicating that there was clear evidence of carcinogenicity in this species (NTP, 1987). An increase in liver tumors in male and female mice was also reported in an unpublished inhalation study from the Japanese literature, but evaluation of the adequacy of this study is precluded by inadequate reporting. Other inhalation bioassays of 1,4-dichlorobenzene did not find tumor increases in exposed rats or mice, but were not adequate studies due to failure to reach the maximum tolerated dose, less-than-lifetime exposure durations, and short observation periods. The kidney tumors in rats are not relevant to humans because the mechanism is specific to male rats, and the mechanistic basis of the mouse liver tumors has not been adequately defined. Therefore, under the draft revised cancer guidelines (U.S. EPA, 1999), 1,4-dichlorobenzene is considered likely to be carcinogenic in humans.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

6.2.1.1. *1,2-Dichlorobenzene*

The NOAEL/LOAEL approach was used to derive an RfD of 0.143 mg/kg-day for 1,2-dichlorobenzene based on liver toxicity in rats. No effects occurred in the only chronic oral study of 1,2-dichlorobenzene, which identified a two NOAELs of 42.7 and 85.7 mg/kg-day (NTP, 1985). Subchronic data were used to show that liver is the critical target, and a LOAEL of 89.3 mg/kg-day was identified for hepatic histopathology (NTP, 1985). The lower chronic NOAEL was used as the basis of the RfD. The lack of a LOAEL in the chronic study precluded analyzing the chronic data using benchmark dose analysis. BMD analysis was performed on the subchronic liver histopathology data to compare BMDLs for subchronic effects with the chronic NOAEL. The lower of the two chronic NOAELs among 42.9 and 82.7 mg/kg-day was selected as the basis for the RfD derivation for three reasons. First, BMDL ranges between 14.7 mg/kg-day and 82.1 mg/kg-day were calculated using the NTP subchronic study with 14.7 mg/kg-day in female rats being the lowest BMDL. However, the subchronic study size was too small to adequately differentiate the liver effects between the treated and control groups. Second, the subchronic LOAEL would appear to have minimal severe effect. Finally, there was a lack of liver effects at a slightly lower dose (120 mg/kg-day) in the chronic study compared to liver effects at a dose of 125 mg/kg-day in the subchronic study. Since there is a higher confidence in a chronic study when compared to a subchronic study, the chronic NOAEL of 42.9 mg/kg-day (NTP, 1985) was

judged to be the most appropriate value on which to base the oral RfD. The RfD was derived by dividing the chronic NOAEL by a total uncertainty factor of 300: 10 for interspecies extrapolation, 10 for interindividual variability, and 3 for database deficiencies.

6.2.1.2. *1,3-Dichlorobenzene*

An RfD of 0.9 μg/kg-day was based on an average $BMDL_{10}$ of 2.6 mg/kg-day for histopathologic lesions in the thyroid (reduced colloidal density) and pituitary (cytoplasmic vacuolation), which were observed in rats in the only available systemic toxicity study (subchronic) of 1,3-dichlorobenzene (McCauley et al., 1995). The BMDLs for thyroid lesions (1.9 mg/kg-day) and pituitary lesions (3.3 mg/kg-day) are similar, and the effects may be related to each other, indicating that was appropriate to use the average of these values, 2.6 mg/kg-day, as the point of departure for the RfD. The RfD was derived by dividing the average $BMDL_{10}$ by a total uncertainty factor of 3000: 10 for interspecies variability, 10 for interindividual variability, 10 for extrapolation from subchronic to chronic exposure, and 3 for database deficiencies.

6.2.1.3. *1,4-Dichlorobenzene*

An RfD of 2.4E -3 was based on a $BMDL_{10}$ of 0.237 mg/kg-day for liver lesions in a 1-year chronic toxicity study in dogs exposed to 1,4-dichlorobenzene. The BMDL was calculated using a benchmark response (BMR) of 10% extra risk. The RfD was derived by dividing the $BMDL_{10}$ by a total uncertainty factor of 100: 10 for interspecies variability, and10 for interindividual variability.

6.2.2. Noncancer/Inhalation

6.2.2.1. *1,2-Dichlorobenzene*

An RfC was not calculated for 1,2-dichlorobenzene due to inadequate data on effects of long-term exposures. A 14-day study (Zissu, 1995) showed that the upper respiratory tract is a sensitive target for inhalation exposures to 1,2-dichlorobenzene, as serious nasal olfactory lesions occurred in mice at concentrations below 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 available mechanistic evidence suggests that hepatic metabolism to a reactive intermediate may be of considerable importance in toxicity, and

the extent of hepatic metabolism is likely to vary dramatically following 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 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 1.0 mg/m^3 was based on a $BMCL_{5\ (HEC)}$ of 99.8 mg/m^3 for reduced postnatal survival in F_1 rat pups in the 2-generation reproduction study of inhaled 1,4-dichlorobenzene (Tyl and Neeper-Bradley, 1989). The BMCL was calculated using a using a 5% decrease in pup survival index (compared with the control) as the BMR. A 5% decrease was selected (instead of 10% or 1 standard deviation change from the control), because the effect (increased postnatal deaths) is severe and one that would be of high concern if it occurred in human populations. The RfC was derived by dividing the $BMDC_{5\ (HEC)}$ by a total uncertainty factor of 100: 3 for interspecies variability, 10 for interindividual variability, and 3 for database deficiencies. An uncertainty factor of 3 is used to account for the interspecies variability in extrapolating from rats to humans because uncertainty in the extrapolation is partially addressed by the dosimetry adjustment [i.e., the calculation of the human equivalent exposure for time and concentration ($BMCL_{HEC}$)].

6.2.3. Cancer/Oral and Inhalation

6.2.3.1. *1,2-Dichlorobenzene*

Available carcinogenicity data for 1,2-dichlorobenzene are inadequate, precluding quantitative assessment of oral and inhalation cancer risk for this isomer.

6.2.3.2. *1,3-Dichlorobenzene*

No data are available on the carcinogenicity of 1,3-dichlorobenzene, precluding quantitative assessment of oral and inhalation cancer risk for this isomer.

6.2.3.3. *1,4-Dichlorobenzene*

There is clear evidence that ingested 1,4-dichlorobenzene was carcinogenic in animals. The NTP (1987) bioassay found increased incidences of liver tumors in mice, and incidence data on hepatocellular adenomas and carcinomas in this study were used for cancer dose-response assessment for oral exposure. Available mechanistic data on 1,4-dichlorobenzene indicate that it is appropriate to use the linear approach for dose-response assessment. Linear analysis showed that the largest slope factor, which is most protective of human health, is 1.3×10^{-2} per mg/kg-day, based upon the combined incidence of hepatocellular adenomas or carcinomas in male mice. The margin of exposure analysis derived a point of departure (LED_{10}) of 9.6 mg/kg-day based on the

liver tumor incidences in male mice. Areas of additional uncertainty in the margin of exposure analysis include the basis for the point of departure (tumor incidence, as compared with a hypothetical derivation based on a key precursor that would provide a more sensitive measurement endpoint that could be detected earlier and at lower doses), and the steepness of the dose response curve.

Available inhalation carcinogenicity data for 1,4-dichlorobenzene are inadequate, precluding quantitative assessment of inhalation cancer risk.

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APPENDIX B1

Benchmark dose modeling of incidence data for degenerative liver lesions in rats and mice orally exposed to 1,2-dichlorobenzene for 13 weeks.

All dichotomous models in the EPA Benchmark Dose Software (version 1.3.1) were fit to the incidence data for degenerative liver lesions in male and female rats and male mice as shown in Table B1-1.

Table B1-1. Incidence of liver lesions observed in rats and mice orally exposed to 1,2-dichlorobenzene for 13 weeks (NTP, 1985).

Lesions: individual cell or focal necrosis; centrilobular degeneration in high-dose group	Duration-adjusted dose (mg/kg-day)					
	0	21.4	42.9	89.3	179	357
male rat	0/10	ND	ND	1/10	4/9†	8/10†
female rat	0/10	ND	ND	3/10	5/10†	9/10†
male mouse	0/10	ND	ND	0/10	4/10†	9/10†

† Significantly ($p<0.05$) different from control; Fisher Exact Test performed by Syracuse Research Corporation.
ND - no histological examinations conducted in this group.

As shown in Table B1-2, the chi-square goodness-of-fit statistic indicated that all models provided statistically adequate ($p>0.1$) fits of each data set. For each data set, Akaike's Information Criteria (AIC) was used to select the best fitting model from which benchmark doses (BMDs) and their lower 95% confidence limits (BMDLs) were calculated, using a benchmark response (BMR) of 10% extra risk.

The Quantal-quadratic, Quantal-linear, and Probit models provided the best fits of the male rat, female rat, and male mouse incidence data, respectively (Table B1-2). The BMDs and BMDLs (rounded values) were 86.1 and 68.1 mg/kg-day for the male rats, 22.0 and 14.7 mg/kg-day for the female rats, and 126.1 and 82.1 mg/kg-day for the male mice. Graphs of observed versus model predicted incidences for liver lesions are shown in Figures B1-1, B1-2, and B1-3.

Table B1-2. BMD modeling of incidence data for liver lesions in male and female rats and male mice exposed to 1,2-dichlorobenzene (NTP, 1985). BMDs and BMDLs were calculated based on a BMR of 10% extra risk for the lesion.

Model	AIC	Chi-square p-value	BMD (mg/kg-day)	BMDL (mg/kg-day)
male rats				
Gamma	32.996	0.941	82.23	25.22
Logistic	32.910	0.983	85.66	31.71
Multi-stage (3-degree)	33.155	0.869	76.72	24.62
Probit	32.895	0.990	87.18	42.53
Quantal-linear	33.001	0.612	31.86	20.41
Quantal-quadratic	31.207	0.952	86.05	68.07
Weibull	33.105	0.893	76.27	24.80
female rats				
Gamma	36.875	0.864	44.25	15.30
Logistic	37.181	0.744	51.54	10.45
Multi-stage (3-degree)	36.638	0.972	30.27	15.60
Probit	37.120	0.765	53.90	27.56
Quantal-linear	35.428	0.855	22.04	14.66
Quantal-quadratic	36.009	0.638	68.49	54.77
Weibull	36.806	0.893	41.67	15.38
male mice				
Gamma	24.770	0.755	123.44	73.16
Logistic	24.605	0.812	125.59	78.97
Multi-stage (4-degree)	25.525	0.280	119.51	48.20
Probit	24.408	0.860	126.07	82.05
Quantal-linear	30.420	0.136	31.98	20.44
Quantal-quadratic	26.569	0.692	83.38	65.53
Weibull	25.450	0.611	113.78	61.86

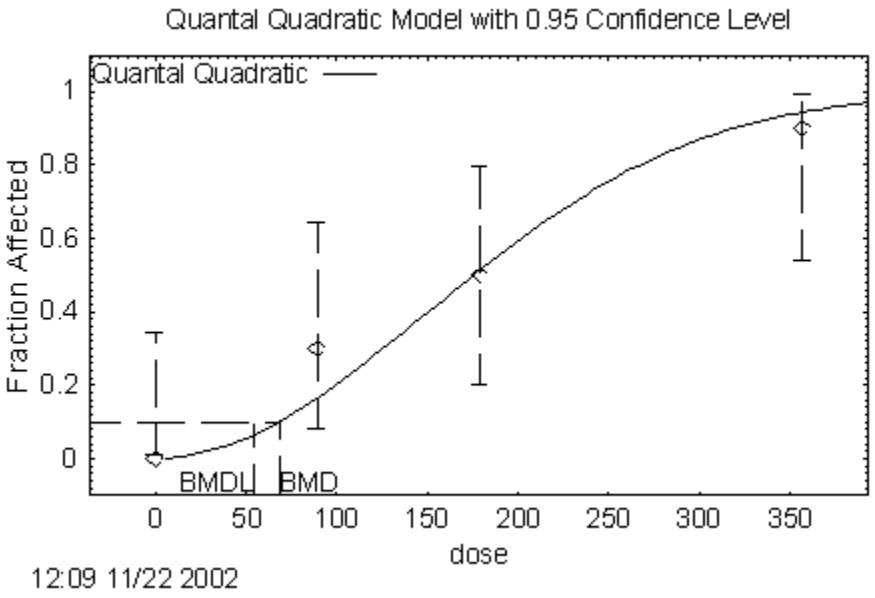

Figure B1-1. Observed incidences of liver lesions in female rats exposed to 1,2-dichlorobenzene for 13 weeks and incidences predicted by the Quantal-quadratic model.

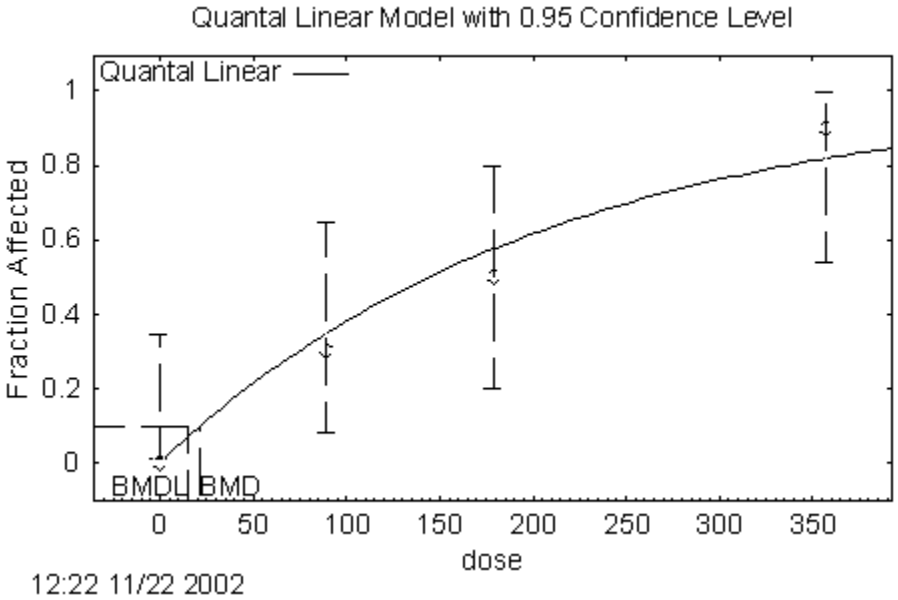

Figure B1-2. Observed incidences of liver lesions in female rats exposed to 1,2-dichlorobenzene for 13 weeks and incidences predicted by the Quantal-linear model.

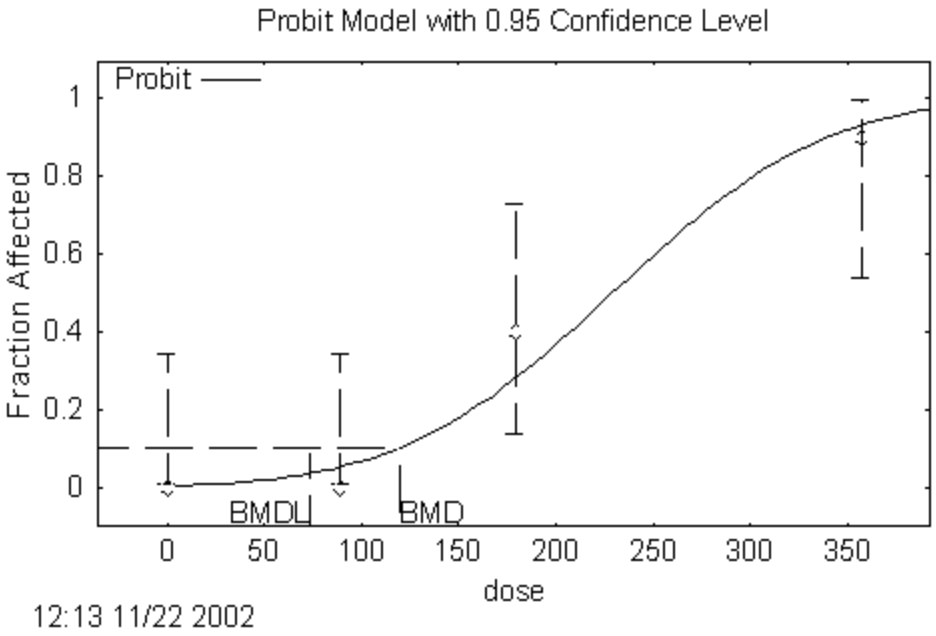

Figure B1-3. Observed incidences of liver lesions in male mice exposed to 1,2-dichlorobenzene for 13 weeks and incidences predicted by the Probit model.

APPENDIX B2

Benchmark dose modeling of incidence data for thyroid and pituitary lesions in rats orally exposed to 1,3-dichlorobenzene for 13 weeks.

All dichotomous models in the EPA Benchmark Dose Software (version 1.3.1) were fit to the male rat incidence data for: 1) reduced follicular colloidal density in the thyroid and 2) cytoplasmic vacuolation in the pars distalis of the pituitary shown in Table B2-1.

Table B2-1. Incidence of thyroid and pituitary lesions observed in male rats orally exposed to 1,3-dichlorobenzene for 90 days (McCauley et al., 1995)

Lesion	Dose (mg/kg-day)				
	0	9	37	147	588
thyroid, reduced follicular colloidal density	2/10	8/10†	10/10†	8/9†	8/8†
pituitary, cytoplasmic vacuolation in pars distalis	2/10	6/10	6/10	10/10†	7/7†
† Significantly ($p<0.05$) different from control; Fisher Exact Test performed by Syracuse Research Corporation.					

For each variable, Akaike's Information Criteria (AIC) was used to select the best fitting model from which benchmark doses (BMDs) and their lower 95% confidence limits (BMDLs) were calculated, using a benchmark response (BMR) of 10% extra risk.

For the thyroid incidence data, the Gamma, Multi-stage, Quantal-linear, and Weibull model runs obtained the same model (power parameters were restricted to be ≥ 1), which provided a better fit than the logistic, quantal-quadratic, or probit models (Table B2-2). The chi-square goodness-of-fit statistics for all of these models indicated poor statistical fits across all of the models ($p<0.1$), but a graph of the observed incidences of thyroid lesions and Gamma-model predicted incidences show a reasonable visual fit (Figure B2-1). Thus, the BMDL predicted from the Gamma model, 1.9 mg/kg-day, was selected as the best BMDL for thyroid lesions in male rats (Table B2-2).

For the pituitary cytoplasmic vacuolation incidence data, the Gamma, Quantal-linear, and Weibull model runs obtained the same model (power parameters restricted ≥ 1), which provided a nearly equivalent fit as the Probit model. The other models fit the data less well, using the AIC as the fit indicator (Table B2-2). The BMD and BMDL from the Gamma model were 4.08 and 2.10 mg/kg-day, whereas the BMD and BMDL from the Probit model were 7.79 and 4.46 mg/kg-day. Given the similarities of these BMDLs, their average, 3.3 mg/kg-day is selected as the BMDL for pituitary cytoplasmic vacuolation in male rats. A graph of the observed

incidences for pituitary lesions in male rats and incidences predicted by the Gamma model is shown in Figure B2-2.

Table B2-2. BMD modeling of incidence data for thyroid and pituitary lesions in male rats exposed to 1,3-dichlorobenzene (McCauley et al. 1995). BMDs and BMDLs were calculated based on a BMR of 10% extra risk for the lesion.

Model	AIC	Chi-square p-value	BMD (mg/kg-day)	BMDL (mg/kg-day)
thyroid, reduced follicular colloidal density				
Logistic	44.630	0.006	8.02	3.83
Gamma	42.974	0.002	4.09	1.90
Multi-stage (4 degree)	42.974	0.002	4.09	1.90
Probit	45.202	0.006	10.61	5.986
Quantal-linear	42.974	0.002	4.09	1.90
Quantal-quadratic	47.644	0.002	38.87	22.76
Weibull	42.974	0.002	4.09	1.90
pituitary, cytoplasmic vacuolation in pars distalis				
Gamma	43.466	0.4887	4.08	2.1
Logistic	43.58	0.4639	7.49	4.29
Multi-stage (4-degree)	45.056	0.3466	5.23	2.23
Probit	43.442	0.4823	7.79	4.46
Quantal-linear	43.466	0.4887	4.08	2.1
Quantal-quadratic	44.122	0.376	17.11	10.10
Weibull	43.466	0.4887	4.08	2.1

Since the BMDLs for thyroid lesions (1.9 mg/kg-day) and pituitary lesions (3.3 mg/kg-day) are similar, the point of departure for the RfD was selected as the rounded average of these values, 2.6 mg/kg-day.

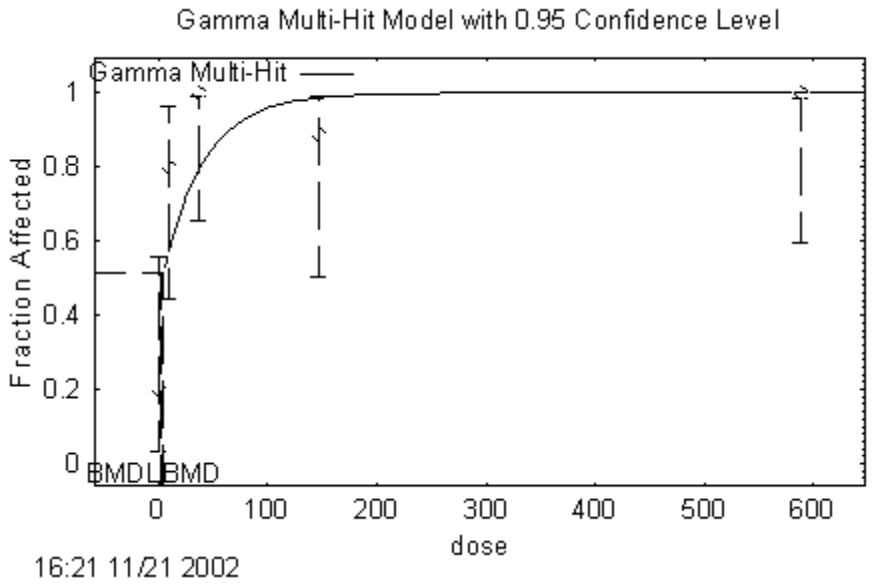

Figure B2-1. Observed Incidences of Thyroid Lesions in Male Rats and Gamma-model Predicted Incidences

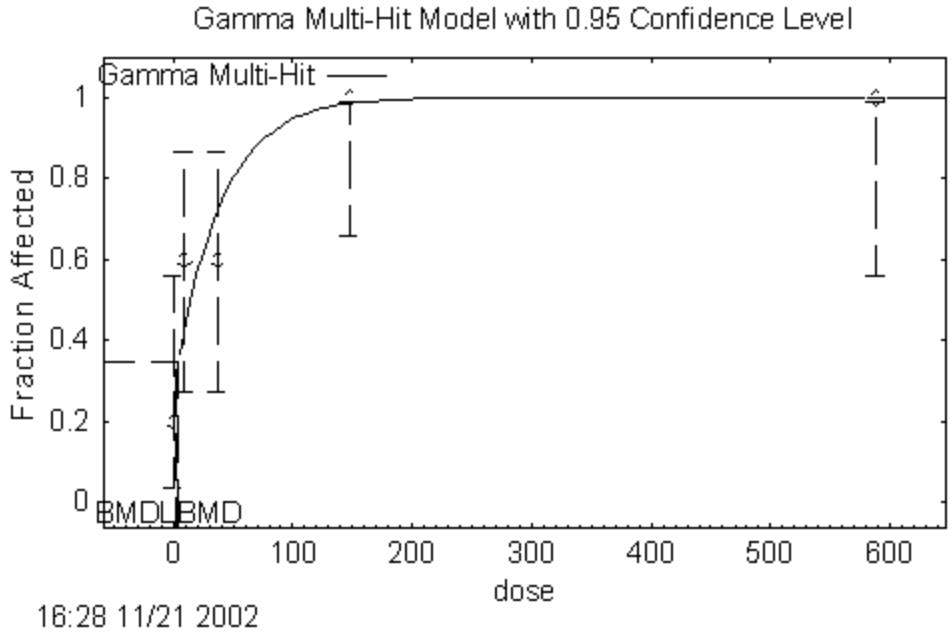

Figure B2-2. Observed Incidences for Pituitary Lesions in Male Rats and Incidences Predicted by the Gamma Model

APPENDIX B3

Benchmark dose modeling of incidence of liver lesions and absolute and relative liver, kidneys, adrenals and thyroid weights in male and female beagle dogs exposed orally to 1,4-dichlorobenzene.

Compound related liver lesions (diffuse hepatocellular hypertrophy, multifocal chronic inflammation, and multifocal hepatocyte pigment deposition in males and diffuse hepatocellular hypertrophy in females) in both male and female beagle dogs were analyzed by benchmark dose modeling because there was a statistically significant increase in liver lesions in the mid and high dose groups. All dichotomous models in the EPA Benchmark Dose Software (version 1.3.2) were fit to the incidence data for liver lesions in male and female beagle dogs (Table B3-1). Power parameters, when they occurred in the models, were restricted to values of ≥ 1. All models, except the Probit, Quantal-linear and Quantal-quadratic models (male beagle dogs) adequately ($p>0.1$) fit the data as assessed by the chi-square goodness-of-fit statistic (Table B3-1). Benchmark doses (BMDs) and their lower 95% confidence limits (BMDLs) were calculated for liver lesions, using a benchmark response (BMR) of 10% extra risk.(U.S. EPA, 2003). For the male liver lesion (multifocal chronic inflammation) analysis, the Gamma, Multistage, Linear, and Weibull models were a better fit to the data than the Log-logistic on the basis of the Akaike's Information Criterion (AIC), but the Log-logistic model was characterized by the closest match between predicted and observed response, as evidenced by the lowest chi-square value (Table B3-1 and Figure B3-1). In addition, in this instance, the Gamma, Multistage, and Weibull models were equivalent to the Linear models, as all but one of the model parameters for each of the Gamma, Multistage, and Weibull were constrained by their predefined lower bounds. The end result was that only one parameter needed to be estimated for the Gamma, Multistage, Linear models, and Weibull while two parameters were estimated for the Log-logistic model. Presumably, the Gamma, Multistage, and Weibull models would have yielded lower BMDLs had the parameter lower-bound constraints been removed. In general, a 2-parameter model would be superior to a 1-parameter model for fitting dose-response data. In this case, although the 1-parameter linear model appeared to fit the data slightly better than the 2-parameter Log-logistic model, the difference in goodness-of-fit was inconsequential. Therefore, the Log-logistic BMDL of 0.237 mg/kg-day for 10% extra risk of liver lesions in the male beagle dogs was chosen as the point-of-departure for the RfD because it was the most sensitive measure of toxicity and it arose from an unconstrained 2-parameter model and was more sensitive compared to the lesions in the female dogs (Table B3-2).

Table B3-1. BMD Modeling of Incidence Data for Liver Lesions in Male Beagle Dogs Exposed to 1,4-Dichlorobenzene (Monsanto Company, 1996). BMDs and BMDLs were calculated based on a BMR of 10% extra risk for the lesion.

Model	AIC	Chi-square p-value	BMD (mg/kg-day)	BMDL (mg/kg-day)
Diffuse Hepatocellular Hypertrophy				
Gamma	8.88452	0.08	24.234	6.09995
Log-Logistic	8.73462	0.00	31.1502	7.7263
Multistage-3 degrees	9.15306	0.25	16.6264	3.78861
Probit	10.7301	0.00	32.0714	7.47999
Quantal Quadratic	10.0978	0.81	10.766	7.68502
Weibull	10.7301	0.00	28.2718	6.05214
Multifocal Chronic Inflammation				
Gamma	24.8958	1.91	2.9798	1.29394
Log-Logistic	26.4232	1.43	1.16546	0.237025
Multistage-3 degrees	24.8958	1.91	2.97979	1.29394
Quantal Linear	24.8958	1.91	2.97971	1.29394
Weibull	24.8958	1.91	2.97971	1.29394
Multifocal Hepatocyte Pigment Deposition				
Gamma	18.045	0.51	18.0286	5.1137
Log-Logistic	18.0067	0.46	17.4673	3.64104
Multistage-3 degrees	16.2062	0.72	20.9665	5.00917
Probit	17.879	0.37	17.542	8.77067
Quantal Linear	16.3776	0.58	10.2144	4.90518
Quantal Quadratic	16.2062	0.72	20.9665	14.5079
Weibull	18.1169	0.55	17.0605	5.06598

Table B3-2. BMD Modeling of Incidence Date for Liver Lesions in Female Beagle Dogs Exposed to 1,4-Dichlorobenzene (Monsanto Company, 1996). BMDs and BMDLs were calculated based on a BMR of 10% extra risk for the lesion.

Model	AIC	Chi-square p-value	BMD (mg/kg-day)	BMDL (mg/kg-day)
Diffuse Hepatocellular Hypertrophy				
Gamma	15.7361	0.00	23.4038	4.10099
Log-Logistic	15.7387	0.00	24.1591	4.26188
Multistage-3 degrees	13.7758	0.02	21.6045	4.06118
Probit	15.7342	0.00	24.6023	6.4818
Quantal Linear	15.8457	1.44	5.60153	2.97246
Quantal Quadratic	14.1096	0.26	14.9645	10.8004
Weibull	15.7758	0.02	21.6108	4.06118

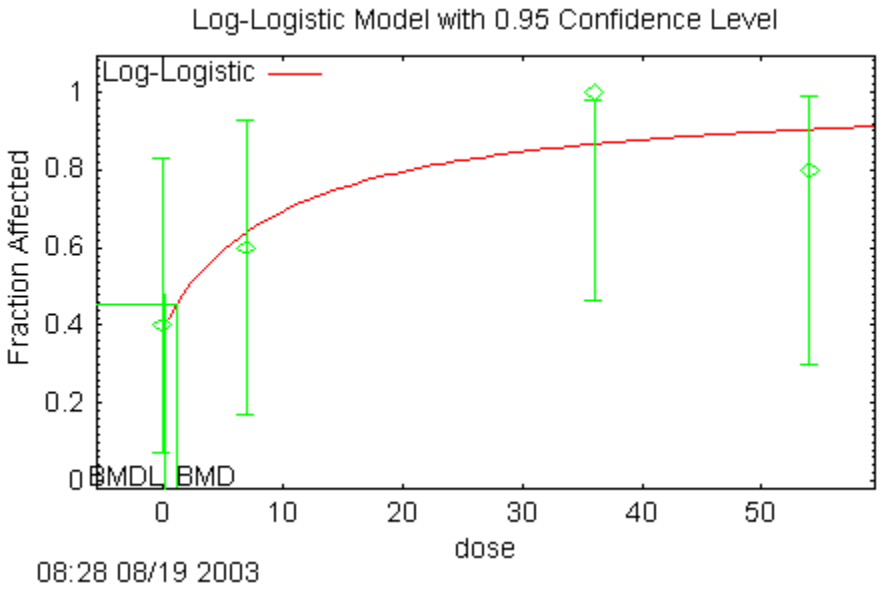

Figure B3-1. Observed Incidences for Liver Lesions in Male Beagle Dogs and Incidences Predicted by the Log-logistic Model

For comparison purposes, the mean relative organ weights for liver, kidneys, adrenals and thyroid were also analyzed using the benchmark dose approach. Liner models with a constant variance or a non-homogenous variance in the EPA Benchmark Dose Software (version 1.3.2) were fit to the mean relative liver, kidneys, adrenals and thyroid weight data in Table B3-3. Log-likelihood ratio tests for mean relative liver weights in male and female beagle dogs showed that the data were appropriate for modeling. Using the relative deviation at a BMR of 10%, the BMDs and BMDLs for liver, kidneys, adrenals and thyroid weights from the various continuous models ranged from 2.06063 to 38.5448 mg/kg-day and 0.917061 to 16.0017 mg/kg-day respectively in male dogs (Table B3-4; Figures B3-2, B3-3, and B3-4; plots for kidneys, adrenals and thyroid weights not shown). The BMDs and BMDLs for liver, kidneys, adrenals and thyroid weights from the various continuous models ranged from 1.81342 to 34.2563 mg/kg-day and 1.33343 to 20.4193 mg/kg-day respectively in female dogs (Table B3-5; Figures B3-5, and B3-6; plots for kidneys, adrenals and thyroid weights not shown). The BMDLs for relative organ weights were slightly to very much above the BMDL for 10% extra risk of liver lesions.

Table B3-3. Absolute and Relative Liver Weights of Female and Male Beagle Dogs Exposed to 1,4-Dichlorobenzene in Gelatin Capsules (Monsanto Company, 1996)

Effect	Dose (mg/kg-day)						
	0	7	% Control	36	% Control	54	% Control
Absolute Liver Weight (gm) Male	379.8	318.64	84	473.22	125	531.9[a]	140
Absolute Liver Weight (gm) Female	261.8	291.42	111	388.68	148	407.4[b]	156
Relative Liver Weight (%) Male	2.7738	2.8821	104	3.9663[b]	143	4.726[b]	170
Relative Liver Weight (%) Female	2.7078	3.0504	113	4.2028[b]	155	4.6040[b]	170

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

Table B3-4. BMD Modeling of Relative Organ Weights in Male Beagle Dogs Exposed to 1,4-Dichlorobenzene in Gelatin Capsules (Monsanto Company, 1996). BMDs and BMDLs were calculated based on a BMR of 10% relative risk for the relative organ weights.

Model	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Adrenals			
Polynomial - Linear	-181.092117	12.3959	6.79574
Polynomial - 2 Degrees	-179.668614	5.85674	2.27519
Polynomial - 3 Degrees	-179.931522	2.06063	0.917061
Power	-179.092117	12.3959	6.79574
Kidneys			
Polynomial Linear	-71.743215	29.3125	15.4671
Polynomial-2 Degrees	-70.087071	37.106	11.3648
Polynomial-3 degrees	-68.210219	36.4772	4.87982
Power	-70.073386	38.5448	16.0017
Liver			
Polynomial Linear	-9.585979	10.1584	7.65337
Polynomial-2 Degrees	-7.886115	13.4342	7.78095
Power	-3.991585	15.6199	7.80525

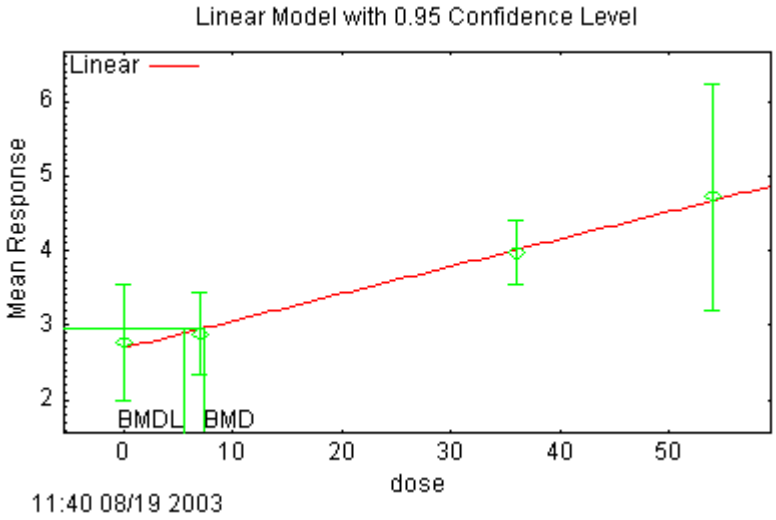

Figure B3-2. Observed Relative Weights for Liver in Male Beagle Dogs and Predicted Relative Liver Weights by the Linear Model

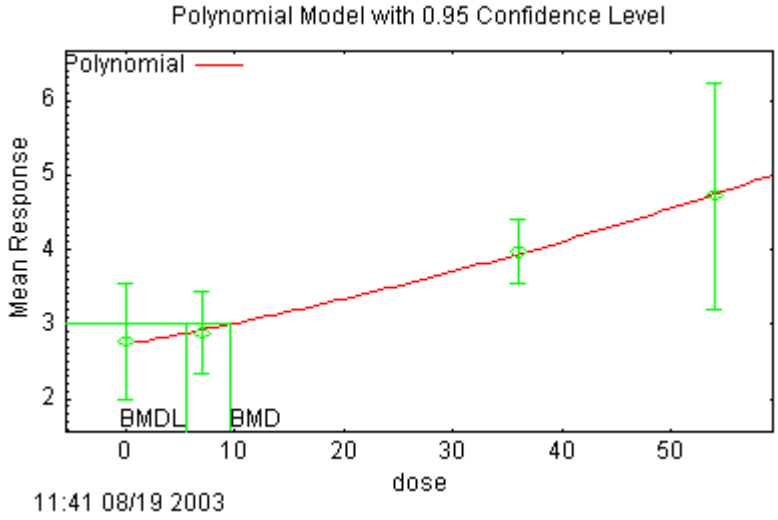

Figure B3-3. Observed Relative Weights for Liver in Male Beagle Dogs and Predicted Relative Liver Weights by the Polynomial (2-degrees) Model

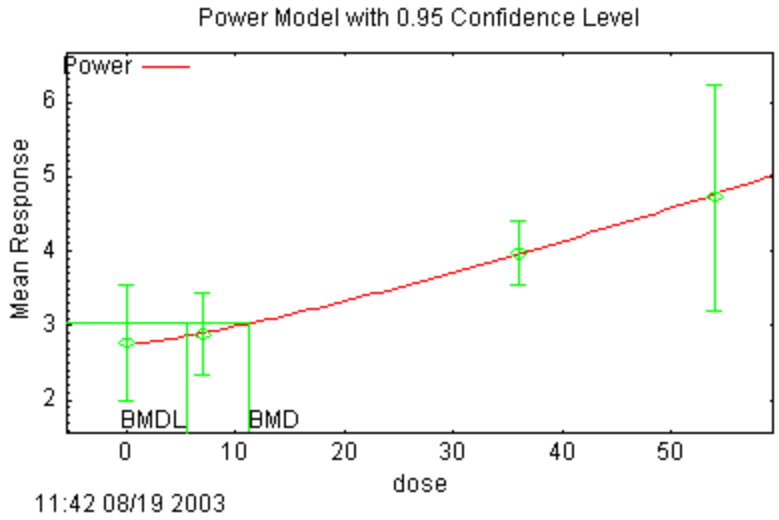

Figure B3-4. Observed Relative Weights for Liver in Male Beagle Dogs and Predicted Relative Liver Weights by the Power Model

Table B3-5. BMD Modeling of Relative Organ Weights in Female Beagle Dogs Exposed to 1,4-Dichlorobenzene in Gelatin Capsules (Monsanto Company, 1996). BMDs and BMDLs were calculated based on a BMR of 10% relative risk for the relative organ weights.

Model	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Adrenals			
Polynomial - Linear	-199.181598	9.46903	6.28068
Polynomial-2Degrees	-197.327998	12.5054	4.2259
Polynomial-3Degrees	-195.762595	17.769	3.67165
Power	-197.472186	13.8714	6.41574
Hill	-195.762601	18.8458	3.95206
Kidneys			
Polynomial Linear	-61.073320	12.8473	7.33834
Polynomial-2 Degree	-64.046524	34.2563	17.5607
Polynomial-3 degrees	-62.585796	33.8272	5.09919
Power	-64.570783	33.1619	20.4193
Liver			
Polynomial Linear	-5.550795	1.81342	1.33343
Power	-1.550795	1.81342	1.33343
Thyroid			
Polynomial Linear	-215.857074	17.387	10.5371
Power	-210.367329	16.5774	10.2044

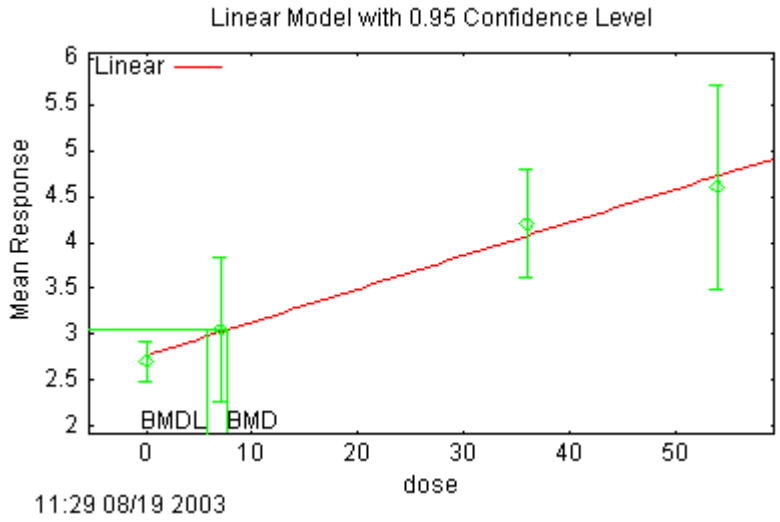

Figure B3-5. Observed Relative Weights for Liver in Female Beagle Dogs and Predicted Relative Liver Weights by the Linear Model

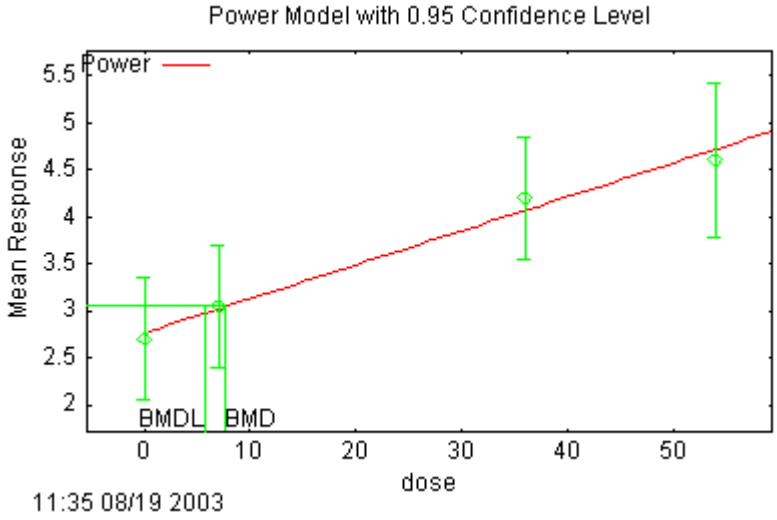

Figure B3-6. Observed Relative Weights for Liver in Female Beagle Dogs and Predicted Relative Liver Weights by the Power Model