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TOXICOLOGICAL REVIEW

OF

1,1-DICHLOROETHYLENE

(CAS No. 75-35-4)

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

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U.S. Environmental Protection Agency
Washington, DC

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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 1,1-dichloroethylene. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of 1,1-dichloroethylene.

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 301-345-2870.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

U.S. EPA Region 8 and the Office of Solid Waste and Emergency Response (OSWER) were responsible for preparing the IRIS toxicological review and summary documents. A comprehensive literature review was conducted in September 1999. The literature review was supplemented with additional references until May 2002.

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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 Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; OSWER; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

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A summary of the external peer reviewers' comments 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 the U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), an 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 1,1-dichloroethylene (DCE) 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 Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), and *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); Memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Policy for Risk Characterization; and *Science Policy Council Handbook, Risk Characterization* (U.S. EPA, 2000b).

Literature search strategies employed for this compound were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

EPA has previously reviewed 1,1-DCE (U.S. EPA, 1985a, b). This review replaces those assessments.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

1,1-DCE does not occur naturally. It is produced commercially by the dehydrochlorination of 1,1,2-trichloroethane in the presence of excess base. 1,1-DCE is used principally for the production of polyvinylidene chloride polymers (PVDC). PVDC is used principally in the food packaging industry as cast and extruded film (Saran and Velon wraps) and as a barrier coating for paper, cellulose, polypropylene, and other plastics. Extruded filaments of PVDC are also used in the textile industry for furniture and automobile upholstery, drapery fabric, and outdoor furniture. 1,1-DCE enters in the environment though release during its manufacture and use, from the breakdown of PVDC products, and from the biotic or abiotic breakdown of 1,1,1-trichloroethane, tetrachloroethylene, 1,1,2-trichoroethene, and 1,1-dichloroethane (ATSDR, 1994; IARC, 1999; and U.S. EPA, 1985 a, b).

The chemical and physical properties of 1,1-DCE (ATSDR, 1994; IARC, 1999) are presented below.

CAS name:	1,1-dichloroethene
CAS number:	75-35-4
IUPAC name:	1,1-dichloroethylene
Primary synonyms:	1,1-DCE; vinylidene chloride, vinylidene dichloride
Chemical formula:	$C_2H_2Cl_2$
Chemical structure:	$Cl_2C=CH_2$
Molecular weight:	96.94

Boiling point:	31.6 °C
Melting point:	-122.5 °C
Specific gravity:	1.218
Vapor pressure:	67 kPa at 20 °C
Solubility:	Practically insoluble in water; soluble in acetone, ethanol, and many organic solvents; very soluble in diethyl ether.
Odor:	Mild, sweet, resembling chloroform
Odor threshold:	500 ppm in air; no data in water
Partition coefficients:	
	Log K_{ow} 1.32
	Log K_{oc} 1.81
Flash point:	-19 °C, closed cup; -15 °C, open cup
Autoignition:	570 °C
Conversion factor:	1 ppm = 3.97 mg/m^3

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

1,1-DCE is rapidly absorbed following inhalation and oral exposures. Because of its low molecular weight and hydrophobic nature, dermal absorption is also likely, but no relevant data were found in the literature. In rats treated with 1,1-DCE by gavage in corn oil, complete gastrointestinal absorption was found to occur at ≤350 mg/kg (Jones and Hathway, 1978a, b; Putcha et al., 1986). 1,1-DCE is easily transported across the alveolar membrane. At constant ≤750 ppm concentration in the air, equilibrium or near steady-state is reached in the blood in rats in approximately 45 minutes (Dallas et al., 1983). Continued uptake in rats reflects to some extent continuing deposition in fatty tissues, but this is primarily a result of metabolism of 1,1-DCE.

The major route of excretion for unchanged 1,1-DCE is through the lung (Jones and Hathway, 1978a). However, the majority of 1,1-DCE is rapidly metabolized to nonvolatile compounds and covalently bound derivatives (McKenna et al., 1997, 1978a, b). Mice metabolize more 1,1-DCE than do rats. For example, when given 50 mg/kg by oral gavage in corn oil, mice excreted 6% and rats excreted 28% of the dose as unchanged 1,1-DCE through the

lungs (Jones and Hathway, 1978b). When exposed to 10 ppm for a single 6-hour episode, mice excreted 0.65% and rats excreted 1.63% of the absorbed dose as unchanged 1,1-DCE through the lungs (McKenna et al., 1977). Intraperitoneal (i.p.) administration of 125 mg/kg ^{14}C-1,1-DCE to mice resulted in the highest concentrations of covalent binding (based on protein content) in the kidney, lung, and liver (Okine et al., 1985; Okine and Gram, 1986a, b). The covalent binding and cellular damage in kidney, lung, and liver correlated with the high concentration of CYP2E1 in certain cell populations in these tissues.

The proposed metabolic pathways for 1,1-DCE are summarized in Figure 1. These pathways were determined from experimental studies in laboratory animals. It is not known whether the metabolism of 1,1-DCE is the same in humans, although in vitro microsomal preparations from human liver and lung form the same initial products (Dowsley et al., 1999). Oxidation of 1,1-DCE by CYP2E1 should produce three metabolites: DCE epoxide, 2-chloroacetyl chloride, and 2,2-dichloroacetaldehyde. All of these metabolites react with glutathione (GSH) and/or water. In the kidney, further metabolism of *S*-(2,2-dichloro-1-hydroxy)ethylglutathione could form another toxic compound, dicholorothioketene. The GSH conjugates formed are catabolized in the kidney to a variety of urinary excretion products. The epoxide, and perhaps to a lesser extent the chloroacetaldehyde, are believed to be associated with the tissue reactivity and toxic effects in tissues that ensue after significant depletion of GSH.

The primary metabolites of 1,1-DCE formed in rat hepatic microsomal incubations are DCE epoxide, 2,2-dichloroacetaldehyde, and 2-chloroacetyl chloride (Liebler et al., 1985, 1988; Costa and Ivanetich, 1982). These metabolites were also identified from mouse microsomal incubations (Dowsley et al., 1995). All these electrophilic metabolites undergo secondary reactions, including oxidation, conjugation with GSH, and hydrolysis. The major products formed are GSH conjugates, 2-(*S*-glutathionyl)acetyl glutathione [B], and 2-*S*-glutathionyl acetate [C], which are believed to be derived from the DCE epoxide (Fig. 1). *S*-(2,2-Dichloro-1-hydroxy ethyl glutathione [A], the GSH conjugate formed from reaction of GSH with 2,2-dichloroacetaldehyde, was not observed in rat liver microsomal incubations containing GSH (Dowsley et al., 1995). The acetal, together with chloroacetic acid and *S*-(2-chloroacetyl)-glutathione [D]—the hydrolysis and GSH-conjugated products of 2-chloroacetyl chloride, respectively—was detected at levels much lower than those for the DCE epoxide-derived conjugates [B] and [C].

In human liver and lung microsomal incubations, the DCE epoxide-derived GSH conjugates [B] and [C] were the major metabolites detected (Dowsley et al., 1999). 2,2-Dichloroacetaldehyde was detected at low levels. Liver microsomes from three out of five human samples metabolized 1,1-DCE to the epoxide-derived GSH conjugates at levels that were 2.5- to 3-fold higher than in mouse liver microsomes, based on milligrams of microsomal protein. These GSH conjugates were also the major products formed in lung microsomes from eight human samples; only low levels of 2,2-dichloroacetaldehyde were formed. The mean level

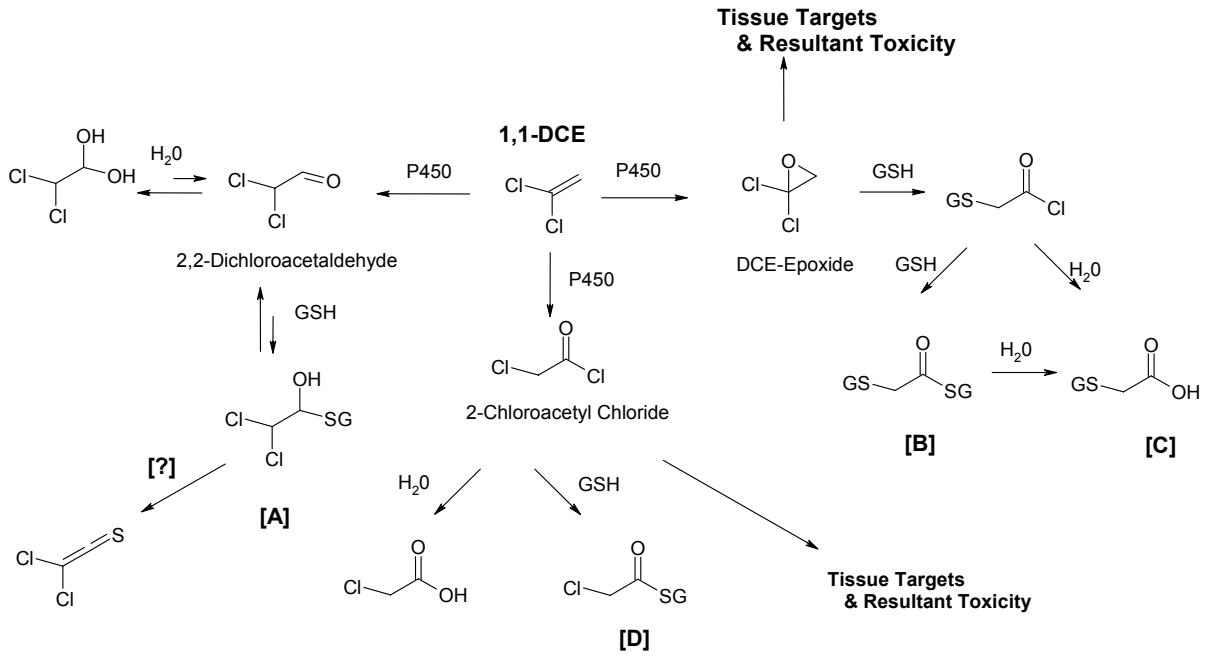

Figure 1: Proposed pathways for 1,1-DCE metabolism and toxicity.

Source: Adapted from Forkert, 1999a, b

in lung microsomes from humans was about 50% of the amount formed in lung microsomes from mice. In both animal and human tissues, cytochrome P450 CYP2E1 catalyzes the formation of the DCE epoxide (Dowsley et al., 1996).

The significance of the metabolic pathway in the liver involving 2,2-dichloroacetaldehyde is unclear. Existing evidence, however, suggests that this pathway is of minor toxicological importance. In addition to 2,2-dichloroacetaldehyde and the GSH conjugate, potential metabolites include the acetal (the hydration product of the aldehyde), dichloroacetic acid, and dichloroethanol. An initial study with rat liver microsomes found a trace level of 2,2-dichloroacetaldehyde but no detectable dichloroacetic acid (Costa and Ivanetich, 1982). A later report using isolated rat hepatocytes detected dichloroacetic acid and trace levels of 2,2-dichloroacetaldehyde, 2,2-dichloroethanol, and chloroacetic acid (Costa and Ivanetich, 1984). Forkert (1999a) and Forkert and Boyd (2001), using intact mice, found no acetal in liver cytosol; however, acetal was detected in the bile in the first study but was not mentioned as being found in the bile in the second study. In early studies on the metabolism of 1,1-DCE, none of the potential metabolites from this pathway were reported as being found in the urine of rodents using techniques that readily identified chloroacetic acid (Jones and Hathway, 1978a, b; McKenna et al., 1977, 1978a, b). A pharmacokinetic analysis showed that any dichloroacetic acid formed in the liver is rapidly metabolized in the liver to two carbon, nonchlorinated chemicals and carbon dioxide (Merdink et al., 1998).

The oxidative metabolism of 1,1-DCE has been found to reach saturation in rats at an oral exposure of 10–50 mg/kg and an inhalation exposure of 200 ppm (794 mg/m^3) (Andersen et al., 1979; D'Souza and Andersen, 1988; Dallas et al., 1983; McKenna et al., 1977).

Because 1,1-DCE is lipophilic and has a blood-to-air partition coefficient of 5 in rats (D'Souza and Andersen, 1988), any 1,1-DCE not metabolized following oral or inhalation exposure is rapidly exhaled unchanged when exposure is terminated. Because of its low octanol:water partition coefficient, 1,1-DCE will not bioaccumulate in tissues to a significant extent. The major metabolites found in urine of rodents include oxalic acid, thiodiglycolic acid, thioglycolic acid, dithioglycolic acid, N-acetyl-S-(2-carboxymethyl) cysteine, N-acetyl-S-(2-hydroxyethyl) cysteine, other –acetyl-S-cysteinyl derivatives, and methylthioacetylaminoethanol.

D'Souza and Andersen (1988) developed physiologically based pharmacokinetic (PBPK) models for 1,1-DCE in the rat for both oral and inhalation exposure. No validated model is available for humans. D'Souza and Andersen (1988) used allometric scaling to estimate comparative amounts of epoxide formed (mg/kg) in rats and humans. Cardiac output and pulmonary ventilation were scaled by (body weight)$^{0.7}$, Vmax was scaled by (body weight)$^{0.74}$, and body fat was estimated at 7% in the 200 g rat and 20% in the 70 kg human. When the oral exposure was less than 5 mg/kg, the estimated amount of epoxide formed was about the same in rats and humans. When the inhalation exposure was less than 100 ppm, the estimated amount of epoxide formed was fivefold lower in humans than in rats.

El-Masri et al. (1996a, b) used a combination of gas uptake experiments in Sprague-Dawley rats and PBPK modeling to assess the potential for interaction between 1,1-DCE and trichloroethylene. Both substrates are activated by CYP2E1. Thus, there is a potential for competitive inhibition when simultaneous exposure to both substrates occurs. The results of the gas uptake experiments confirmed a model based on competitive inhibition. There was, however, no evidence of competitive inhibition when exposure to both substrates was 100 ppm or less. As environmental exposures to these chemicals are expected to be less than 100 ppm, there is little potential for reduced toxicity from 1,1-DCE when individuals are also exposed to trichloroethylene.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY

Ott et al. (1976) investigated the health records of 138 employees who were occupationally exposed to 1,1-DCE in processes not involving vinyl chloride. The individuals included in the study had worked in experimental or pilot plant polymerization operations, in a monomer production process as tankcar loaders, or in a production plant manufacturing a monofilament fiber. Time-weighted average concentrations (8 hours) of 1,1-DCE in the workplace were estimated from job descriptions and the results of industrial hygiene sampling.

The subjects were grouped into three exposure categories: less than 10 ppm, 10–24 ppm, and greater than 25 ppm. The researchers estimated career exposure by taking into account average duration of employment. Results of the most recent health inventory for individuals in the exposed cohort were compared with findings for matched controls. An analysis of mortality in the cohort indicated no statistically significant differences. Overall, there were no significant differences in hematology and clinical chemistry parameters between the exposed cohort and the controls.

Three reports suggest an association between exposure to dichloroethylenes and birth defects. The California Department of Health Services (Swan et al., 1985) reported an increase in the number of cardiac congenital anomalies during 1980 and 1981 in an area served by a public water supply contaminated with 1,1,1-trichloroethane and dichloroethylene. The public water supply also contained chlorinated disinfection by products. Goldberg et al. (1990) reported an increase in congenital cardiac malformations between 1969 and 1987 in an area of Arizona where the drinking water was contaminated with trichloroethylene and dichloroethylene (isomer not specified). The dichloroethylene concentration in the drinking water was usually 5% to 10% of the trichloroethylene concentration. The paper does not specify whether the drinking water was chlorinated. Finally, Bove et al. (1995) reported increased odds ratio for oral cleft defects (1.71), for central nervous system defects (2.52), and for neural tube defects (2.60) associated with exposure to total dichloroethylenes of more than 2 μg/L from public drinking water supplies in an area of northern New Jersey. The period of time studied was 1985 to 1988. The drinking water also contained chlorinated disinfection by-products. It is not clear from the paper whether there was also co-exposure to other chlorinated solvents also reported on, including trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, carbon tetrachloride, and 1,2-dichloroethane. As all of these situations involved exposure to multiple contaminates, a cause-and-effect relationship between the reported birth defects and exposure to 1,1-DCE cannot be established.

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

4.2.1. Acute Exposure

Mice are more sensitive than rats to acute toxicity from 1,1-DCE. The National Toxicology Program (NTP) (NTP, 1982) conducted a study to determine lethality in five male and five female F344 rats and five male and five female B6C3F_1 mice (all animals 9 weeks old) after a single exposure to 1,1-DCE by gavage in corn oil at 0, 10, 50, 100, 500, or 1000 mg/kg.

By day 14 postexposure, mortality was 0/10, 1/10, 0/10, 0/10, 1/10, and 2/10 in the rats and 0/10, 0/10, 1/10, 0/10, 8/10, and 10/10 in the mice, respectively. Other representative lethality data are presented in Tables 1 and 2.

Table 1. Representative lethality (LD_{50}) from oral exposure to 1,1-DCE

Species	Dose (mg/kg)	Effect	Reference
Rat		LD_{50}	
male	1550		Jenkins et al., 1972
male	1800		Ponomarkov and Tomatis, 1980
male, adrenalectomized	84		Jenkins et al., 1972
female	1500		Ponomarkov and Tomatis, 1980
Mouse		LD_{50}	
male	217		Jones and Hathway, 1978b
female	194		

Table 2. Representative lethality (LC_{50}) or time for 50% lethality (LT_{50}) from inhalation exposure to 1,1-DCE

Species	Exposure (ppm)	Effect	Reference
Rat			
male, fed	6350 for 4 hr	LC_{50}	Siegal et al., 1971
male, fasted	200 for 4.1 hr 400 for 3.6 hr 500 for 3.0 hr 1000 for 2.4 hr 2000 for 1.4 hr	LT_{50}	Andersen et al., 1979
Mouse		LC_{50}	
male	98 for 22–23 hr		Short et al, 1977a
female	105 for 22–23 hr		

Toxicity is enhanced by fasting (Andersen and Jenkins, 1977; Chieco et al., 1981; Jaeger et al., 1974, 1975, 1977a, b; McKenna et al., 1978a, b; Moslen et al., 1985), by GSH depletion (Andersen et al., 1980; Jaeger et al., 1974, 1977a, b; Kanz et al., 1988; Moussa and Forkert, 1992), and by administration in oil vehicles compared to administration in aqueous Tween (Chieco et al., 1981). Toxicity is decreased by agents that decrease metabolism by the P450 system (Andersen et al., 1978; Moslen et al., 1989) or by hypothyroidism, which increases intracellular GSH (Kanz et al., 1991).

The target organs for toxicity after acute oral or inhalation exposure are the liver, the kidney, and the Clara cells of the lung. The effects in the liver include an increase in liver enzymes in the serum (Jenkins et al., 1972; Jaeger, 1977a, b; Short et al., 1977a; Jenkins and Andersen, 1978; Reynolds et al., 1980); severe histopathological damage, including disruption of bile canaliculi, cytoplasmic vacuolization, and hemorrhagic necrosis (Short et al., 1977a; Kanz and Reynolds, 1986; Reynolds et al., 1984); an increase in covalent binding of 1,1-DCE (Forkert and Moussa, 1991, 1993; Jaeger et al., 1977a, b); and a decrease in GSH (Forkert and Moussa, 1991, 1993; Kanz et al., 1988; Reichert et al., 1978, 1979) mediated by CYP2E1 metabolism of 1,1-DCE to intermediates that react with GSH (Kainz et al., 1993; Lee and Forkert, 1994).

Several researchers have investigated the hepatotoxicity of 1,1-DCE. In a study by Jenkins and Andersen (1978), four female Sprague-Dawley rats (body weight, 223 g) received a single oral exposure by gavage in corn oil at 400 mg/kg. Four to 8 hours after exposure, there was a significant increase in aspartate aminotransferase (approximately 75-fold), alanine aminotransferase (approximately 70-fold), lactate dehydrogenase (approximately 110-fold), and sorbitol dehydrogenase (approximately 320-fold). The serum enzymes returned to approximately normal values within 82 hours after exposure.

Reynolds et al. (1984) administered a single oral exposure by gavage in mineral oil at 200 mg/kg 1,1-DCE to fasted male Sprague-Dawley rats (body weight, 225–375 g). Within 2 hours after exposure, the livers showed evidence of dilatation and disruption of bile canaliculi, plasma membrane invagination and loss of microvilli, cytoplasmic vacuolization, and loss of density in mitochondrial matrices. One hour after a single inhalation exposure at 250 ppm 1,1-DCE for 4 hours, Sprague-Dawley rats showed a significant decrease ($p<0.05$) in GSH concentration in the liver (Jaeger, 1977). Four hours after exposure there was an increase in the serum concentration of sorbitol dehydrogenase (approximately 230-fold) and ornithine carbamoyl transferase (approximately 380-fold).

Short et al. (1977a) studied CD-1 male mice (Charles River) and CD male rats (Charles River) exposed by inhalation for 22–23 hrs/day for 1–5 days at 0, 15, 30, or 60 ppm 1,1-DCE (mice) or for 1–3 days at 0 or 60 ppm (rats). In male mice exposed to ≥15 ppm, serum enzymes (alanine aminotransferase and aspartate aminotransferase) were significantly increased (four- to sixfold), and hepatocellular degeneration was observed in one of five mice after the first exposure. In two of five male rats exposed to 60 ppm, mild centrilobular degeneration and/or necrosis was observed after the first exposure, but serum enzymes (alanine aminotransferase and

aspartate aminotransferase) were not significantly increased (four- to sixfold) until after the second exposure.

Reynolds et al. (1980) found that after a single 4-hour exposure by inhalation at 200 ppm 1,1-DCE, the liver of fasted male Sprague-Dawley rats (body weight, 150–200 g) showed catastrophic morphological alterations of the parenchymal cells, including retraction and central rarefaction of nuclei with peripheral displacement of chromatin to nuclear margins, progressing to frank hemorrhagic centrilobular necrosis. GSH concentrations were also depleted. After the extensive hepatocellular damage, cytochrome P450 and oxidative –demethylase were deactivated.

Toxic effects of 1,1-DCE exposure in the kidney include increased kidney weight, increased blood urea nitrogen and creatinine (Jackson and Conolly, 1985; Jenkins and Andersen, 1978), and histopathological changes, including vacuolization, tubular dilatation, and nephrosis and necrosis of the proximal tubules (Short et al., 1977a; Jackson and Conolly, 1985; Jenkins and Andersen, 1978). These changes were correlated with metabolic activation of 1,1-DCE by CYP2E1 in the proximal tubules, decreased GSH concentration, increased covalent binding of 1,1-DCE, and the presence of a relatively high concentration of β-lyase activity in rodent kidney tissue (Brittebo et al., 1993; Dekant et al., 1989; Dekant, 1996). In addition, renal toxicity can be inhibited by pretreatment of mice and rats with aminooxyacetic acid, an inhibitor of renal cysteinyl-ß-lyase (Ban et al., 1995; Cavelier et al., 1996).

Jenkins and Andersen (1978) investigated the nephrotoxicity of 1,1-DCE in Sprague-Dawley rats after a single oral exposure by gavage in corn oil. Fasted male rats (two to six per group; body weight, 300 g) were administered 0, 50, 100, 200, 400, or 600 mg/kg. At 600 mg/kg, there was a fivefold increase in blood urea nitrogen. Histopathological examination was not conducted in animals treated at 600 mg/kg. In male rats at 400 mg/kg, there was a statistically significant increase ($p<0.05$) in blood urea nitrogen (fourfold) and in creatinine (threefold). The increases became apparent 8 hours after exposure, reached a peak 24 hours after exposure, and returned to normal 96–144 hours after exposure. In male rats at 400 mg/kg, there was also a twofold increase in relative kidney weight 48 hours after exposure. The relative kidney weight had nearly returned to normal 144 hours after exposure. In female rats at 400 mg/kg, there was no substantial increase in blood urea nitrogen, creatinine, or relative kidney weight. Histopathological lesions (tubular dilatation and tubular necrosis) were observed in both sexes at 400 mg/kg. No significant effects were seen at 200 mg/kg and below.

Short et al. (1977a) studied CD-1 male mice (Charles River) after inhalation exposure for 22–23 hrs/day for 1–5 days at 0, 15, 30, or 60 ppm 1,1-DCE. Tubular nephrosis was observed at ≥15 ppm after the first exposure. Jackson and Conolly (1985) reported that in male Sprague-Dawley rats (body weight, 225–275 g) exposed continuously for 4 hours to 0, 200, 250, 300, 375, or 400 ppm, mortality was 0/22, 1/4, 1/16, 3/14, 3/12, and 3/6, respectively. At ≥250 ppm there were significant increases ($p<0.05$) in kidney-to-body weight ratios (approximately 1.4-fold), serum urea nitrogen (approximately fourfold) and creatinine (approximately threefold).

Histopathological examination revealed severe tubular necrosis with calcium deposits at ≥300 ppm.

Using autoradiographic methods, Brittebo et al. (1993) investigated the mechanism of nephrotoxicity in C57BL6 mice (body weight, 18–22 g) following i.p. injection of 0.4 mg/kg of ^{14}C-labeled 1,1-DCE. Selective covalent binding of radioactivity occurred in the proximal tubules, in the midzonal parts of the liver lobules, and in the mucosa of the upper and lower respiratory tract. Treatment with buthionine sulphoximine (BSO), an irreversible inhibitor of γ-glutamylcysteine synthetase and a GSH-depleting agent, caused a threefold increase in covalent binding of 1,1-DCE. Histopathological examination of kidneys in BSO-pretreated male mice given single i.p. injections of 25 and 50 mg/kg 1,1-DCE showed necrosis in the proximal tubules (S_1 and S_2 segments). In mice given 1,1-DCE only, no significant lesions in the kidneys were observed. The authors concluded that the severe renal toxicity of 1,1-DCE in BSO-pretreated mice is related to metabolic activation of 1,1-DCE in the proximal tubules, resulting in GSH depletion and covalent binding.

The effects in the Clara cells of the lung in mice include extensive histopathological changes (Forkert and Reynolds, 1982; Forkert et al., 1985, 1990), repair of damage through cell proliferation (Forkert et al., 1985), depletion of GSH, and covalent binding of 1,1-DCE mediated through the formation of DCE epoxide by CYP2E1 (Dowsley et al., 1996; Forkert and Mousa, 1991; Forkert, 1999b; Lee and Forkert, 1994; Moussa and Forkert, 1992). No studies are available showing similar effects in the lungs of rats.

Forkert and Reynolds (1982) investigated the ability of 1,1-DCE administered orally to induce pulmonary injury. Male C57BL6 mice (three to five per group) were administered a single dose of 1,1-DCE by gavage in mineral oil at 0, 100, or 200 mg/kg. At 100 mg/kg, Clara cells showed extensive dilatation of cisternae and degeneration of the endoplasmic reticulum. The bronchiolar epithelium showed a few vacuolated cells 12 hours after exposure. By 24 hours the Clara cells showed prominent cytoplasmic vacuoles, but ciliated cells were not affected. By 48 hours, complete recovery had occurred. At 200 mg/kg, both ciliated and Clara cells showed necrosis of the bronchiolar epithelium. By 24 hours, the lesion had increased in severity and areas of bronchioles were denuded of epithelium. Peribronchial and perivascular edema, hemorrhage, and focal atelectasis were also present. Complete recovery occurred by 7 days.

A subsequent study (Forkert et al., 1985) examined regeneration of the damaged epithelium by cellular proliferation. Male C57BL6 mice were administered a single dose of 1,1-DCE by gavage in mineral oil at 200 mg/kg followed by a single pulse of ^{3}H-thymidine. Changes in cellular proliferation were calculated from measurement of radioactivity incorporated into total pulmonary DNA. Incorporation of radioactivity was significantly inhibited 1 day after treatment and thereafter increased. The peak incorporation of radioactivity occurred between 3 and 5 days after treatment and returned to baseline by day 7. The majority of the radioactivity was taken up by the nonciliated bronchiolar epithelial cells.

4.2.2. Longer-Term Exposure

4.2.2.1. *Oral*

4.2.2.1.1. ***Rats.*** NTP (1982) conducted a 14-day study of 1,1-DCE in male and female F344 rats (five animals of each sex, 9 weeks old) by gavage in corn oil at 0, 10, 50, 100, 500, or 1,000 mg/kg. Survival was 10/10, 10/10, 10/10, 10/10, 7/10, and 3/10 mg/kg, respectively. Mean body weight was significantly depressed at ≥500 mg/kg. Hemorrhagic necrosis in the liver was observed in all of the rats that died at 500 and 1,000 mg/kg.

In the same study, male and female F344 rats (10 of each sex, 9 weeks old) were administered 1,1-DCE by gavage in corn oil at 0, 5, 15, 40, 100, or 250 mg/kg five times per week for 13 weeks. Representative tissues from rats receiving 250 mg/kg and from control rats were examined microscopically. Livers from all groups were examined. Three female rats receiving 250 mg/kg died during the first week of the study. No other rats died. Mean body weight was depressed 13% for male rats receiving 250 mg/kg as compared with controls. Mean body weight in other groups was comparable. Only the liver showed effects attributed to 1,1-DCE. At 250 mg/kg, the three female rats that died showed severe centrilobular necrosis. Minimal to moderate hepatocytomegaly was seen in the rest of the rats at 250 mg/kg. Minimal to mild hepatocytomegaly was seen in 6/10 male rats and 3/10 female rats that received 100 mg/kg. No biologically significant changes were observed in rats that received 40 mg/kg or less. The no-observed-adverse-effect level (NOAEL) in this study is 40 mg/kg (equivalent to 28.5 mg/kg-day); the lowest-observed-adverse-effect level (LOAEL) is 100 mg/kg (equivalent to 71.4 mg/kg-day).

4.2.2.1.2. ***Mice.*** NTP (1982) conducted a 14-day study in male and female B6C3F_1 mice (five of each sex, 9 weeks old) administered 1,1-DCE by gavage in corn oil at 0, 10, 50, 100, 500, or 1,000 mg/kg. Survival was 10/10 in all groups except the 1000 mg/kg group, where survival was 0/10. Hemorrhagic necrosis in the liver was observed in all mice at 1,000 mg/kg.

In the same study, male and female B6C3F_1 mice (10 of each sex, 9 weeks old) were administered 1,1-DCE by gavage in corn oil at 0, 5, 15, 40, 100, or 250 mg/kg five times per week for 13 weeks. Representative tissues from mice receiving 100 and 250 mg/kg and from control mice were examined microscopically. Livers from all groups were also examined. Survival was 20/20, 19/20, 19/20, 19/20, 15/20, and 1/20 at 0, 5, 15, 40, 100, and 250 mg/kg, respectively. At 100 mg/kg, there was a decrease in mean body weight in males (14%) but not in females. No change in mean body weight was observed at lower exposures. Only the liver showed effects attributed to 1,1-DCE. Centrilobular necrosis of the liver was observed in 5/10 males and 5/10 females that received 250 mg/kg and 2/10 males and 2/10 females that received 100 mg/kg. No biologically significant changes in the liver occurred in mice receiving 40 mg/kg or below. The NOAEL in this study is 40 mg/kg (adjusted to a continuous daily exposure of 28.6 mg/kg-day); the LOAEL is 100 mg/kg (adjusted to a continuous daily exposure of 71.4 mg/kg-day).

4.2.2.1.3. ***Dogs.*** Quast et al. (1983) conducted a study in beagle dogs (four per group, 8 months old) administered 1,1-DCE by gavage in peanut oil at 0, 6.25, 12.5, or 25 mg/kg-day for 97 days.

There were no significant differences among the groups in appearance and demeanor, mortality, body weight, food consumption, hematology, urinalysis, clinical chemistry determinations, organ weights, and organ-to-body weight ratios. No exposure-related gross or histopathological changes were present in tissues. There was no depletion of the nonprotein sulfhydryl levels in the liver or kidneys. The NOAEL in this study is 25 mg/kg-day (the highest exposure tested).

4.2.2.2. *Inhalation*

Gage (1970) exposed four male and four female Alderly Park rats (body weight 200 g) to 200 ppm or 500 ppm 1,1-DCE 6 hrs/day for 20 days. At 200 ppm there was slight nasal irritation (not further described). At necropsy all organs appeared normal. At 500 ppm there was nasal irritation (not further specified), retarded weight gain (data not reported), and liver cell degeneration (not further defined).

Plummer et al. (1990) exposed black hooded Wistar rats to 50 ppm 1,1-DCE (18 males and 18 females, age not specified) continuously for 4 weeks (except for two 1.5-hour periods per week) or to 250 ppm (six males and six females, age not specified) for 6 hrs/day, 5 days/wk for 4 weeks. The total exposure (concentration x time) was the same for the two profiles (33,533 ppm/hr for the continuous exposure and 32,200 ppm/hr for the intermittent exposure). Rats in the intermittent exposure group showed signs of early coagulative necrosis in the liver (incidence not reported). Eleven of the 12 rats in the continuous-exposure group showed less severe injury, including fatty changes in variable numbers of hepatocytes and only very occasional focal liver cell necrosis. The LOAEL in this study is 50 ppm.

Prendergast et al. (1967) evaluated the toxicity of 1,1-DCE in Long-Evans and Sprague-Dawley rats, Hartley guinea pigs, beagle dogs, New Zealand albino rabbits, and squirrel monkeys. One set of test animals (15 rats/group, 15 guinea pigs/group, 3 rabbits/group, 2 dogs/group, or 3 monkeys/group) was exposed to 1,1-DCE vapors for 8 hrs/day, 5 days/wk, for a total of 30 exposures at 395 ± 32 mg/m^3. The age of the animals was not specified. The exposed animals were evaluated for visible signs of toxicity, mortality, and hematologic, biochemical, pathologic, and body weight changes. In this study there were no deaths, no visible signs of toxicity, and no histopathological changes. The NOAEL in this study is 395 mg/m^3 (the highest exposure tested), equivalent to an adjusted NOAEL based on continuous exposure of 94 mg/m^3.

Another set of test animals (15 rats/group, 15 guinea pigs/group, 3 rabbits/group, 2 dogs/group, or 3 or 9 monkeys/group) was exposed continuously for 90 days to 1,1-DCE vapors at 189 ± 6.2, 101 ± 4.4, 61 ± 5.7, or 20 ± 2.1 mg/m^3. The concurrent controls included 304 rats, 314 guinea pigs, 48 rabbits, 34 dogs, and 57 monkeys. The age of the animals was not specified. The exposed animals were evaluated for visible signs of toxicity, mortality, and hematologic, biochemical, pathologic, and body weight changes. There was apparent exposure-related mortality in guinea pigs and monkeys. In the 0, 20, 61, 101, or 189 mg/m^3 exposure groups, guinea pig mortality was 2/314, 2/45, 3/15, 3/15, and 7/15, and monkey mortality was 1/57, 1/21, 0/9, 2/3, and 3/9, respectively. The guinea pigs died between days 3 to 9 of exposure;

the monkeys died on days 26, 39, 47, 60, and 64 of exposure. There were no visible signs of toxicity in any surviving animals. At the highest exposure in monkeys, but not in guinea pigs, there was some histopathological evidence of liver damage (see below). In guinea pigs at the highest exposure, there was an increase in serum glutamic-pyruvic transaminase and liver alkaline transaminase (see below). Because visible signs of toxicity were not observed, and only minor liver damage was apparent in this study, the mortality data in guinea pigs and monkeys are given no weight.

Varying degrees of growth depression were found in all exposures, but were significant in all species only at 189 mg/m^3. The test animals exhibited no significant hematologic alterations, and serum urea nitrogen levels were within control limits in all exposures in which determinations were made. Significant elevations of serum glutamic-pyruvic transaminase and liver alkaline phosphatase activities were found in rats (a threefold and 1.75-fold increase, respectively) and guinea pigs (a sevenfold and 2.4-fold increase, respectively) exposed to 189 mg/m^3 (other species not tested) but not at 20 mg/m^3 (enzyme levels at intermediate exposures not tested). Histopathological examination of liver from dogs, monkeys, and rats revealed damage at 189 mg/m^3 (other species not examined). The effects observed included fatty metamorphosis, focal necrosis, hemosiderosis deposition, lymphocytic infiltration, bile duct proliferation, and fibrosis. The changes were most severe in dogs. Sections of kidney from all rats showed nuclear hypertrophy of the tubular epithelium. No detectable liver or kidney damage was observed in any species exposed to 101 mg/m^3 or less. The NOAEL in this study is 101 mg/m^3 (equivalent to 25 ppm); the LOAEL is 189 mg/m^3 (equivalent to 47 ppm).

4.2.3. Chronic Studies and Cancer Bioassays

4.2.3.1. *Oral*

4.2.3.1.1. ***Rats.*** Ponomarkov and Tomatis (1980) treated 24 female BD IV rats by gavage with 1,1-DCE dissolved in olive oil (150 mg/kg body weight) on gestation day (GD) 17. Their offspring (81 males and 80 females) were treated weekly with 1,1-DCE at 50 mg/kg body weight by gavage in olive oil from the time of weaning for 120 weeks or until the animal was moribund. A control group of offspring (49 males and 47 females) received only olive oil. Liver and meningeal tumors were more frequently observed in treated than in untreated animals, but the difference was not statistically significant. The total number of tumor-bearing animals was not statistically different between the treated and untreated groups.

NTP (1982) conducted chronic toxicity and carcinogenicity studies for 104 weeks in male and female F344 rats (50 of each sex in each group, 9 weeks old) by gavage in corn oil at 0, 1, or 5 mg 1,1-DCE/kg-day. There were no significant differences in survival, clinical signs, or body weight between test animals and controls for any group, suggesting that the maximum tolerated dose was not achieved. The results of histopathological examination indicated chronic renal inflammation in male rats (26/50, 24/48, 43/48) and female rats (3/49, 6/49, 9/44). The increase was statistically significant only in males at the highest exposure. As this lesion commonly occurs in aged male albino rats (Kluwe et al., 1984, Kluwe, 1990), it is not

considered to be biologically significant in this study. All of the increased tumor incidences that were statistically significant by the Fisher exact test or by the Cochran-Armitage linear trend test (adrenal pheochromocytoma, pancreatic islet cell adenoma or carcinoma, and subcutaneous fibroma in males and pituitary adenoma in females) were not significant when life-table analyses were used. This difference occurs because life table analyses adjust for intercurrent mortality and thus minimize the impact of animals dying before the onset of late-appearing tumor. This adjustment was particularly critical for the analyses of tumor incidences in male rats because 12 controls and 10 low-dose animals were accidentally killed during week 82 of the study. Accordingly, NTP concluded that no increased incidence of tumors was found at any site in these bioassays. Under the conditions of this bioassay, 1,1-DCE administered by gavage was not carcinogenic for F344 rats. The NOAEL in this study is 5 mg/kg-day (the highest exposure tested).

Quast et al. (1983) conducted a 2-year chronic toxicity and carcinogenicity study of 1,1-DCE in Sprague-Dawley rats (6–7 weeks old). There were 80 rats of each sex in the control group and 48 rats of each sex in each exposed group. The 1,1-DCE was incorporated in the drinking water of the rats at nominal concentrations of 0, 50, 100, or 200 ppm. The time-weighted average exposure over the 2-year period was 7, 10, or 20 mg/kg-day for males and 9, 14, or 30 mg/kg-day for females. Rampy et al. (1977) also reported some of the data; Humiston et al. (1978) reported more detailed data. No significant differences were found between the groups in appearance and demeanor, mortality, body weight, food consumption, water consumption, hematology, urinalysis, clinical chemistry determinations, organ weights, or organ-to-body weight ratios. After 1 year of study, no depletion of the nonprotein sulfhydryl levels in the liver or the kidneys was observed (Rampy et al., 1977).

The only treatment-related effect observed was minimal hepatocellular midzonal fatty change and hepatocellular swelling. At the termination of the study, male rats showed an increased incidence of minimal heptocellular fatty change (control, 14/80; 50 ppm, 5/48; 100 ppm, 13/48; 200 ppm, 19/47) and minimal hepatocellular swelling (control, 0/80; 50 ppm, 1/48; 100 ppm, 2/48; 200 ppm, 3/47). The changes were statistically significant ($p<0.05$) only in the 200 ppm group. Female rats also showed an increased incidence of minimal hepatocellular fatty change (control, 10/80; 50 ppm, 12/48; 100 ppm, 14/48; 200 ppm, 22/48; statistically significant [$p<0.05$] at 100 and 200 ppm) and minimal hepatocellular swelling (control, 3/80; 50 ppm, 7/48; 100 ppm, 11/48; 200 ppm, 20/48; statistically significant [$p<0.05$] in all groups). No exposure-related neoplastic changes occurred at any exposure. No hepatocellular necrosis was evident at any exposure.

On the basis of the minimal nature of the hepatocellular swelling reported by the authors and no change in liver weight, no change in clinical chemistry measurements diagnostic for liver damage, and no other indication of abnormal liver function, the hepatocellular swelling is not considered to be biologically significant or an adverse effect in this study. The statistically significant hepatocellular midzonal fatty change, however, is considered a minimal adverse effect in this study. Accordingly, the NOAEL in male rats is 10 mg/kg-day and the LOAEL is 20 mg/kg-day; the NOAEL in female rats is 9 mg/kg-day and the LOAEL is 14 mg/kg-day. A

benchmark dose (BMD) analysis was conducted for the results in female rats (Appendix B). In female rats, the BMD_{10} (the dose that gives a 10% response) is 6.6 mg/kg-day and the $BMDL_{10}$ (the lower 95% confidence limit on the BMD_{10}) is 4.6 mg/kg-day.

Maltoni et al. (1985) conducted a carcinogenicity and toxicity study of 1,1-DCE in Sprague-Dawley rats. Animals (9 or 10 weeks old) were exposed by gavage in olive oil to 0, 0.5, 5, 10, or 20 mg/kg, 4–5 days/wk for 52 weeks. There were two control groups, one with 150 animals (75 of each sex) and the other with 200 animals (100 of each sex). The exposed groups had 100 animals (50 of each sex). Following the 52-week exposure, animals were observed until spontaneous death (total duration 147 weeks). Body weight was measured every 2 weeks during the 52-week exposure and every 8 weeks thereafter. Full necropsy and histopathological examination were performed. No biologically significant changes were observed in mortality or body weight, and no biologically significant noncancer or cancer effects were found in any organ.

4.2.3.1.2. ***Mice***. NTP (1982) conducted chronic toxicity and carcinogenicity studies for 104 weeks of 1,1-DCE in male and female $B6C3F_1$ mice (50 of each sex in each group, 9 weeks old) by gavage in corn oil at 0, 2, or 10 mg/kg. No significant differences were observed in survival, clinical signs, or body weight in any group, and there was evidence of only slight toxicity in the liver, suggesting that the maximum tolerated dose was not achieved. The only noncancer effect observed by histopathological examination was necrosis of the liver (male: 1/46, 3/46, 7/49; female: 0/47, 4/49, 1/49). The effect was not statistically significant at either exposure ($p = 0.6$ and 0.06 at the mid- and high-exposure levels in males using a two-tailed test, respectively). The only observed significant increase ($p<0.05$) in tumor incidence occurred in low-dose females for lymphoma (2/48, 9/49, 6/50) and for lymphoma or leukemia (7/48, 15/49, 7/50). These increases were not considered to be related to 1,1-DCE administration because similar effects were not found in the high-dose females or in males. Under the conditions of this bioassay, 1,1-DCE administered by gavage was not carcinogenic for $B6C3F_1$ mice. In male and female mice the NOAEL is 10 mg/kg-day (the highest exposure tested). The BMD_{10} is 7.8 mg/kg-day and the $BMDL_{10}$ is 4.1 mg/kg-day.

4.2.3.1.3. ***Trout.*** Hendricks et al. (1995) conducted an 18-month carcinogenicity study of 1,1-DCE in rainbow trout (8 weeks old) at 4 mg/kg-day. Tissues examined for neoplasms included liver, kidney, spleen, gill, gonads, thymus, thyroid, heart, stomach, pyloric ceca, duodenum, rectum, pancreas, and swimbladder. 1,1-DCE produced no neoplasms at the exposure levels used and no increase in liver weight. There was no evidence of any other chronic toxic effects.

4.2.3.2. *Inhalation*

4.2.3.2.1. ***Rats.*** Lee et al. (1977, 1978) exposed 2-month-old Charles River CD rats (36 males and 35 females) to 55 ppm 1,1-DCE for 6 hrs/day, 5 days/wk for 12 months. No significant changes were observed in survival, body weight, hematology, clinical blood chemistry, pulmonary macrophage count, cytogenetic analysis of bone marrow, x-ray examination of extremities, collagen contents in liver and lung, serum aminolevulinic acid (ALA) synthetase, urinary ALA level, or serum alpha-fetoprotein. A mild to markedly severe focal, disseminated vacuolization was observed in livers of most of the rats. No hemangiosarcomas were found in the liver or lung. The incidence of hemangiosarcomas in mesenteric lymph node or subcutaneous tissue was 2/36 in males and 0/35 in females.

Viola and Caputo (1977) exposed 2-month-old Sprague-Dawley rats (30 males and 30 females per group) to 0, 75, or 100 ppm 1,1-DCE for 22–24 months (hours of daily exposure not reported). The incidence of tumors observed at necropsy (males and females combined) was 15/60, 10/36, and 20/60, respectively. The tumors observed were classified as subcutaneous fibromas or abdominal lymphomas. The histopathological results from this study have not been published. No other data were reported.

In the same study, 2-month-old albino Wistar rats (37 males and 37 females) were exposed to 1,1-DCE for 4 hrs/day, 5 days/wk for 12 months. Exposures were 200 ppm for the first 6 months and 100 ppm for the rest of the study. A control group of 30 males and 30 females received air only. The incidence of tumors (described as reticulum cell sarcomas of a nonsincytial type, primarily in the abdominal cavity) was 15/60 and 17/74 in control and exposed rats, respectively. No other data were reported.

Hong et al. (1981) evaluated mortality and tumor incidence in groups of 2-month-old CD rats of both sexes exposed to 0 or 55 ppm 1,1-DCE 6 hrs/day, 4 days/wk for 1 month (4 of each sex), 3 months (4 of each sex), 6 months (4 of each sex), or 10 months (16 of each sex). Following exposure, all animals were observed for an additional 12 months. In rats exposed for 10 months, there was an increase in mortality following the 12-month observation period (67% in exposed; 41% in controls). There was no significant increase in tumors at any site for any exposure period.

Maltoni et al. (1985) conducted a carcinogenicity and toxicity study of 1,1-DCE in Sprague-Dawley rats. Animals (16 weeks old) were exposed by inhalation to 0, 10, 25, 50, 100, or 150 ppm for 4 hrs/day, 4–5 days/wk for 52 weeks. The control group had 200 animals (100 of each sex); the 10, 25, 50, and 100 ppm groups had 60 animals (30 of each sex), and the 150 ppm group had 120 animals (60 of each sex). Following the 52-week exposure, animals were observed until spontaneous death (total duration 137 weeks). Body weight was measured every 2 weeks during the 52-week exposure and every 8 weeks thereafter. Full necropsy and histopathological examination were performed. No biologically significant changes in mortality or body weight were observed, and there were no biologically significant noncancer effects in any organ in either sex or an increase in tumors in males at any site. There was a statistically

significant increase ($p<0.05$) in each treatment group as compared with the control group in the number of females with mammary fibromas and fibroadenomas. The incidence was 44/56 (78.6%), 24/24 (100%), 20/20 (100%), 21/22 (95.4%), 21/23 (91.3%), and 38/43 (88.4%) in the control, 10, 25, 50, 100, and 150 ppm groups, respectively. The latency time and the number of tumors per tumor-bearing animal were similar among all groups. The incidence of mammary carcinoma in the exposed groups was consistently less than that of controls—16/56 (28.6%), 5/24 (20.8%), 4/20 (20%), 1/21 (4.5%), 3/21 (13.0%), and 9/38 (20.9%) in the control, 10, 25, 50, 100, and 150 ppm groups, respectively. This study provides no evidence that 1,1-DCE is carcinogenic in male and female Sprague-Dawley rats.

Quast et al. (1986) and Rampy et al. (1977) reported results from studies in which male and female Sprague-Dawley rats (Spartan substrain, 86 animals/group) were exposed to 1,1-DCE by inhalation 6 hrs/day, 5 days/wk for up to 18 months. Interim sacrifices occurred at 1, 6, and 12 months. Rats were exposed to 1,1-DCE concentrations of 10 ppm and 40 ppm for the first 5 weeks of the study. Because of the absence of observable treatment-related effects among rats sacrificed after 1 month of exposure, the concentrations were increased to 25 and 75 ppm. Exposures were continued at these concentrations through the 18th month of the study. The surviving animals were then held without exposure to 1,1-DCE until 24 months. Cytogenetic evaluations were performed on a separate group of animals (four/sex) exposed to 0, 25, or 75 ppm for 6 months.

A separate 90-day study using 20 rats/sex/treatment group was conducted at 0, 25, and 75 ppm, with an interim sacrifice of 8 rats/group at 30 days. No exposure-related changes in mortality, appearance and demeanor, body weight, clinical chemistry determinations, hematologic evaluations, urinalysis, or cytogenetic evaluation of bone marrow preparations were observed. Minimal hepatocellular fatty change in the midzonal region of the hepatic lobule was observed in both male and female rats in the 25 ppm and 75 ppm groups at the 6-month interim sacrifice (male: control, 0/5; 25 ppm, 1/5; 75 ppm, 4/5; female: control, 0/5; 25 ppm, 2/5; 75 ppm, 4/5). The fatty change was also observed at the 12-month sacrifice, but there was no indication of progression of severity (male: control, 0/5; 25 ppm, 3/5; 75 ppm, 5/5; female: control, 0/5; 25 ppm, 5/5; 75 ppm, 5/5). At the 18-month sacrifice the incidence of this change was no longer increased in male rats (control, 0/27; 25 ppm, 0/25; 75 ppm, 1/27). However, the change persisted in female rats (control, 0/16; 25 ppm, 6/29; 75 ppm, 7/20). In female rats the fatty change was statistically significant ($p<0.05$) only at the higher exposures. During the last 6 months of the study, after exposure had been discontinued, this effect was no longer discernible (male: control, 0/46; 25 ppm, 1/47; 75 ppm, 0/51; female: control, 0/49; 25 ppm, 0/46; 75 ppm, 1/48).

Although the incidence of several tumors and/or tumor types was found to be statistically increased or decreased as compared to controls, none of these differences were judged to be attributable to 1,1-DCE. The tumor incidence data for both control and treated rats in this study was comparable to historical control data for the Sprague-Dawley rats (Spartan substrain) used by this laboratory for several studies of similar design and duration. Although the minimal hepatocellular midzonal fatty change is reversible, did not result in altered organ weight, clinical

chemistry changes diagnostic for liver damage, or any obvious decrement in liver function, the fatty change in liver is considered a minimal adverse effect. Accordingly, the NOAEL in male rats in this study is 75 ppm (the highest exposure tested). The NOAEL for female rats is 25 ppm; the LOAEL is 75 ppm. A BMD analysis was conducted (Appendix B). In female rats the BMC_{10} (the concentration that gives a 10% response) is 15.1 ppm and the $BMCL_{10}$ (the lower 95% confidence limit on BMC_{10}) is 9.8 ppm, equivalent to 1.8 ppm adjusted for continuous exposure (9.8 ppm × 6/24 × 5/7).

Cotti et al. (1988) exposed Sprague-Dawley rats to 1,1-DCE at 0 or 100 ppm for 4–7 hrs/day, 5 days/wk. The exposures were to 13-week-old females for 104 weeks (60 control animals and 54 exposed animals) and to the offspring of pregnant rats exposed from GD 12 and for 15 or 104 weeks after birth (158 males and 149 females as controls, 60 males and 60 females exposed for 15 weeks, and 62 males and 61 females exposed for 104 weeks). Animals were observed until spontaneous death. In males and females exposed for 104 weeks and in male offspring exposed for 15 weeks, a slight decrease in body weight was observed (data not reported). An increased percentage of rats bearing malignant tumors (30.9 vs. 17.3% in controls) and an increased number of malignant tumors per 100 animals (34.1 vs. 17.9% in controls) were observed in male and female offspring exposed for 104 weeks (statistical analysis not presented). An increase in leukemia that appeared to be related to length of exposure was also observed in offspring (4.2% for controls, and 8.3% and 11.4% for exposure of 15 and 104 weeks, respectively). Tumors at other sites (total benign and malignant tumors, total benign and malignant mammary tumors, malignant mammary tumors, pheochromocytomas) showed no change or a decreased incidence. Data from this study are also reported in Maltoni et al. (1985).

4.2.3.2.2. ***Mice.*** Lee et al. (1977, 1978) exposed 2-month-old CD-1 mice (18 males and 18 females) to 0 or 55 ppm 1,1-DCE for 6 hrs/day, 5 days/wk, for up to 12 months. No deaths occurred in the control or exposed groups. Weight gain was comparable between groups. No changes in hematology, clinical blood chemistry, cytogenetic analysis of bone marrow, x-ray examination of extremities, or serum alpha-fetoprotein were observed. The livers showed no increase in mitotic figures using ^{14}C-thymidine incorporation. Animals exposed for 6 to 12 months had several changes in the liver, including enlarged and basophilic hepatocytes with enlarged nuclei, mitotic figures or polyploidy, microfoci of mononuclear cells, focal degeneration, and necrosis. The incidence and severity of these lesions progressed with length of exposure (data not reported). The incidence of bronchioalveolar adenoma (males and females combined) for 1–3 months, 4–6 months, 7–9 months, and 10–12 months of exposure was 0/24, 1/8, 2/10, and 3/28, respectively. The incidence of hemangiosarcomas in liver (males and females combined) for 6 months, 7–9 months, and 10–12 months of exposure was 0/16, 1/10, and 2/28, respectively. No hemangiosarcomas were found in other tissues.

Hong et al. (1981) evaluated mortality and tumor incidence rates in mice exposed to 1,1-DCE. Groups of 2-month-old albino CD-1 mice of both sexes were exposed to 0 or 55 ppm for 6 hrs/day, 4 days/wk for 1 month (8 of each sex), 3 months (8 of each sex), or 6 months (12 of each sex). Following exposure, all animals were observed for an additional 12 months. In mice exposed for 6 months there was a slight increase in mortality following the 12-month

observation period (46% in exposed, 39% in controls). There was no significant increase in tumors at any site for any exposure period.

Maltoni et al. (1985) conducted a carcinogenicity and toxicity study of 1,1-DCE in Swiss mice. Animals (9 or 16 weeks old) were exposed by inhalation to 0, 10, or 25 ppm for 4 hrs/day, 4–5 days/wk, for 52 weeks. Groups of animals exposed to ≥50 ppm showed extreme toxicity after only a few exposures, causing termination of this portion of the bioassay. There were two control groups, one with 180 animals (90 of each sex) and the other with 200 animals (100 of each sex). The 10 ppm group had 60 animals (30 of each sex). Two groups were exposed to 25 ppm: one group consisted of 60 animals (30 of each sex) and the other of 240 animals (120 of each sex). Following the 52-week exposure, animals were observed until spontaneous death (total duration 126 weeks). Body weight was measured every 2 weeks during the 52-week exposure and every 8 weeks thereafter. Full necropsy and histopathological examination were performed.

No biologically significant changes in body weight were seen. The exposed animals had a somewhat higher survival than did controls. No biologically significant noncancer effects where observed in any organ, except for a marginal increase in regressive changes in the kidney (presumably necrosis and proliferation of the cortical tubules) and a marginal increase in kidney abscesses and nephritis. In males the incidence of regressive changes was 103/190 (54%), 23/30 (77%), and 102/150 (68%), and the incidence of kidney abscesses and nephritis was 45/190 (24%), 13/30 (43%), and 58/150 (39%) in the control, 10 ppm, and 25 ppm exposure groups, respectively. The results in male mice were statistically significant ($p<0.05$) for both effects at both exposures. In females the incidence of regressive changes was 93/190 (49%), 19/30 (63%), and 97/150 (65%), and the incidence of kidney abscesses and nephritis was 52/190 (27%), 8/30 (27%), and 50/150 (33%) in the control, 10 ppm, and 25 ppm exposure groups, respectively. The results in female mice were statistically significant ($p<0.05$) only for regressive changes at the higher exposure. There was a statistically significant increase ($p<0.01$) over controls in kidney adenocarcinomas in male mice at 25 ppm, but not in male mice at 10 ppm or in female mice at either exposure. The incidence was 0/126 (0%), 0/25 (0%), and 28/119 (23.5%) in male mice in the combined control, 10 ppm, and combined 25 ppm groups, respectively.

A statistically significant increase ($p<0.01$) over controls was seen in mammary carcinomas in female mice at both exposures, but there was no clear exposure-response relationship. The incidence was 3/185 (1.6%), 6/30 (20%), and 16/148 (11%) in females in the combined control, 10 ppm, and combined 25 ppm groups, respectively. There was also a statistically significant increase ($p<0.01$) over controls in pulmonary adenomas in both exposed groups, but there was no clear exposure-response relationship. The incidence was 12/331 (3.6%), 14/58 (24.1%), and 41/288 (14.2%) in male and female mice combined in the combined control, 10 ppm, and combined 25 ppm groups, respectively. No pulmonary carcinomas were observed in any mice. The incidence data are reported as the number of tumor-bearing animals compared to the number of animals alive when the first tumor was observed in that organ (kidney adenocarcinoma, 55 weeks; mammary tumor, 27 weeks; pulmonary adenoma, 36 weeks).

4.2.3.2.3. ***Hamsters.*** Maltoni et al. (1985) conducted a carcinogenicity and toxicity study of 1,1-DCE in Chinese hamsters. Animals (28 weeks old) were exposed by inhalation to 0 or 25 ppm for 4 hrs/day, 4–5 days/wk for 52 weeks. The control group had 35 animals (18 male and 17 female); the 25-ppm group had 60 animals (30 of each sex). Following the 52-week exposure, animals were observed until spontaneous death (total duration 157 weeks). Body weight was measured every 2 weeks during the 52-week exposure and every 8 weeks thereafter. Full necropsy and histopathological examination were performed. No biologically significant changes were seen in mortality or body weight, and there were no biologically significant noncancer or tumor effects in any organ.

4.2.3.3. *Dermal*

Van Duuren et al. (1979) evaluated the carcinogenicity of 1,1-DCE in male and female non-inbred Ha:ICR Swiss mice. Carcinogenicity was assessed in three types of tests: a dermal initiation-promotion assay, a repeated dermal application assay, and a subcutaneous injection assay. Vehicle, no-treatment, and positive control groups were included in the tests. In the initiation-promotion assay, 1,1-DCE was tested as a tumor-initiating agent with phorbol myristate acetate as the promoter. Thirty female mice were treated with 121 mg 1,1-DCE. A significant increase ($p<0.005$) was observed in skin papillomas (nine in eight mice). In the repeated dermal application assay, exposures of 40 and 121 mg/mouse were used. 1,1-DCE was applied to the back of the shaved animals (30 females/dose). No sarcomas were observed at the site of treatment. No statistically significant increase in tumors was observed at any site remote from the site of treatment. In the subcutaneous injection assay, the test animals were given weekly injections of 2 mg of 1,1-DCE. After 548 days on test, none of the animals had developed sarcomas at the injection site. 1,1-DCE showed initiating activity in the two-stage carcinogenesis experiments but was inactive as a whole-mouse dermal carcinogen and after subcutaneous injection.

4.3. REPRODUCTIVE AND DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Direct Infusion

Dawson et al. (1990) conducted studies in Sprague-Dawley rats using direct infusion of a solution of 1.5 or 150 ppm 1,1-DCE to the gravid uterus during the period of organ differentiation and development. The delivery rate of the test solution was 0.5 μL/hour beginning at GD 7 and continuing for 2 weeks. On GD 22 the pregnant rats were killed and the gravid uterus was removed for examination. The only effect noted was an increase in a variety of congenital heart changes (atrial septal, pulmonary valve, aortic valve, and membranous ventricular septal changes). The incidence of total cardiac changes was 3% in the control group and 12.5% and 21% in the 1.5 and 150 ppm groups, respectively. The increase was statistically significant ($p<0.05$) at both exposures; however, the statistical analysis was based on total occurrence, not on numbers of litters affected or fetuses per litter affected.

Goldberg et al. (1992) conducted studies on chick embryos to determine whether 1,1-DCE was a cardiac teratogen. On day 3 of incubation, fertilized White Leghorn chick eggs (N = 418) were inoculated just above the embryo with 30 μL of a test solution of 1,1-DCE in mineral oil at 5 μM (N = 76), 20 μM (N = 62), or 25 μM (N = 76). Two control groups were also tested using normal saline (N = 96) or mineral oil (N = 108). Chicks were terminated on day 18 of incubation. No change was seen in mortality among groups. Cardiac changes included atrial and ventricular septal changes, malformations of all valves, and great vessel changes. Cardiac and great vessel changes occurred in 4% of each of the two control groups and in 17, 19, and 2% of the low-, mid-, and high-dose groups, respectively.

4.3.2. Oral

Nitschke et al. (1983) evaluated the reproductive and developmental toxicity of 1,1-DCE in Sprague-Dawley rats. Three generations of the test animals were exposed to drinking water containing nominal 1,1-DCE concentrations of 0 (initially 15 males and 30 females), 50, 100, or 200 ppm (initially 10 males and 20 females at each exposure). The authors provided no information on water consumption. This study was a companion study to Quast et al. (1983) and used the same concentrations of 1,1-DCE in drinking water. In the Quast et al. study the average exposure to females was 9, 14, or 30 mg/kg-day. After 100 days of exposure, the rats were mated. In the Nitschke et al. three-generation study, there were no biologically significant changes in fertility index, in average number of pups per litter, in average body weight of pups, or in pup survival at any exposure. Neonatal survival was decreased from concurrent control values in the f_2 and f_{3a} litters of dams ingesting 1,1-DCE from drinking water. The survival indices, however, were within the range of control values for this strain of rats in this laboratory. The authors attributed the decreased survival index in f_2 to increased litter size at birth in dams exposed to 1,1-DCE. The apparent effect seen in the f_{3a} litters was not repeated in subsequent matings of the same adults to produce either the f_{3b} or the f_{3c} litters. The authors attributed the decreased survival in the f_{3a} litters as being due to chance.

Histopathological examination of tissues of rats exposed to 1,1-DCE in the drinking water in utero, during lactation, and postweaning revealed slight hepatocellular fatty change and an accentuated hepatic lobular pattern of a reversible nature in the adult rats (data not reported, but the observation is consistent with that reported by Quast et al. [1983] in a chronic bioassay). These effects were observed in the 100 and 200 ppm groups in the F_1 generation and in all groups of the F_2 generation. The authors did not present incidence data and did not report statistical analysis. Exposure to 1,1-DCE in drinking water at concentrations causing mild, dose-related changes in the liver did not affect the reproductive capacity of rats through three generations that produced six sets of litters. The NOAEL for reproductive and developmental toxicity in this study is 200 ppm for exposure to 1,1-DCE in drinking water (the highest exposure tested and about 30 mg/kg-day).

Murray et al. (1979) evaluated the developmental toxicity of 1,1-DCE administered in drinking water at 0 (27 animals) or 200 ppm (26 animals) to pregnant Sprague-Dawley rats (body weight 250 g). Rats were exposed on GDs 6–15 at 40 mg/kg-day. Using standard

techniques for soft and hard tissue examination, no teratogenic effects were seen in the embryos, and there was no evidence of toxicity to the dams or their offspring. The NOAEL for developmental toxicity in this study is 40 mg/kg-day (the highest exposure tested).

Dawson et al. (1993) evaluated the ability of 1,1-DCE administered in drinking water at 110 ppm or 0.15 ppm to female Sprague-Dawley rats (body weight 250 g) to induce fetal cardiac changes. Rats were administered 110 ppm 1,1-DCE for 61 days before mating or for 48 days before mating and for 20 days during gestation. Other rats were administered 0.15 ppm 1,1-DCE for 82 days before mating or for 56 days before mating and for 20 days during gestation. The dams were killed on GD 22 and the gravid uterus was removed and examined. No effect was seen on maternal weight gain, average resorption sites (sites where development began but resorption later occurred), or average implantation sites (sites that did not appear to develop beyond implantation and contained a metrial gland only). There was no increase in the incidence of cardiac changes when dams were exposed only before mating. There was, however, a statistically significant increase ($p<0.01$) in the percent of fetuses with cardiac changes (atrial septal, mitral valve, and aortic valve changes) when the dams were exposed before mating and during gestation. The incidence was control, 7/232 (3%); 0.15 ppm, 14/121 (12%); and 110 ppm, 24/184 (13%). This statistical analysis was based on total occurrence of affected fetuses. Because the exposure was to the dam and not to individual fetuses, a nested statistical analysis is preferred. Such an analysis takes into account the correlation among fetuses within a litter and the possible nesting of effects within litters. This analysis has not been conducted because all the necessary data are not available.

The author provided additional data (letter from B. Dawson, University of Auckland, New Zealand, to R. Benson, U.S. EPA, January 24, 2001) to resolve typographical errors in the exposure information for each group and to clarify the number of affected litters and number of fetuses per litter affected. The exposure to dams before and during pregnancy was 0, 0.02, or 18 mg/kg-day in the control, 0.15 ppm, and 110 ppm groups, respectively. The number of affected litters was 5/21 (24%), 8/11 (73%), and 13/17 (76%). The mean number of affected fetuses per litter for affected litters only was 1.40 (13% of the fetuses in the litter), 1.75 (16% of the fetuses in the litter), and 1.85 (17% of the fetuses in the litter). The mean number of affected fetuses per litter all litters was 0.33 (3% of the fetuses in the litter), 1.27 (12% of the fetuses in the litter), and 1.41 (13% of the fetuses in the litter).

These investigators did a much more thorough evaluation of alterations in cardiac development than is done in standard developmental toxicity testing protocols. There is no experience with the background rates or the functional significance of such alterations from other studies or laboratories. The incidence of alterations in control fetuses (3% of all fetuses, 24% of all litters, and 1.40 affected fetuses per affected litter) suggests a high background incidence. The authors report that examinations were done blind to the treatment group, so the data are presumed to be unaffected by observer bias.

No demonstrated exposure-response relationship was found in the Dawson et al. (1993) study. A 900-fold increase in exposure did not produce a significant increase in response in any

measure of effect. The observed cardiac changes are of questionable biological significance, as there were no biologically significant effects reported on growth and survival in the three-generation study (Nitschke et al., 1983). No cardiac effects were reported in a prenatal developmental study (Murray et al., 1979); however, in this study exposure to 1,1-DCE did not occur throughout pregnancy. The pharmacokinetics of 1,1-DCE make it biologically implausible that the observed cardiac changes were causally associated with exposure to 1,1-DCE. The exposures used in Dawson et al. (1993) were below the level of saturation of CYP2E1 in the rat liver. Essentially all of the 1,1-DCE administered to the dams would have been metabolized in the liver and would have reacted with GSH or macromolecules in the liver. See the discussion and references in section 3. Therefore, it is extremely unlikely that any significant amount of 1,1-DCE or any toxic metabolite would have been present in the fetal compartment. CYP2E1 is not expressed in fetal liver but begins to be expressed shortly after birth (Cresteil, 1998). EPA is not aware of any information on the expression of CYP2E1 in fetal cardiac tissue. Cardiac tissue, however, is not generally considered to be a tissue with significant potential for metabolism of xenobiotics. For these reasons EPA cannot conclude that the observed cardiac changes were caused by exposure to 1,1-DCE.

4.3.3. Inhalation

Short et al. (1977b) evaluated developmental toxicity of 1,1-DCE administered by inhalation to pregnant CD-1 rats (Charles River). Animals were exposed to 0 (58 animals), 15 ppm (18 animals), 57 ppm (20 animals), 300 ppm (18 animals), or 449 ppm (18 animals) for 22–23 hrs/day on GDs 6–16. Dams were sacrificed on GD 20. Maternal toxicity was indicated by severe maternal weight loss (> 28 g/dam) at ≥15 ppm and by maternal mortality at ≥57 ppm. There was a statistically significant increase in the mean number of fetuses per litter, with hydrocephalus at 15 and 57 ppm, malaligned sternebrae at 15 ppm, and unossified sternebrae at 57 ppm. Because of the severe maternal toxicity at ≥15 ppm (≥ 60 mg/m^3), this study is not useful for evaluating developmental toxicity.

In the same study, pregnant CD-1 mice (Charles River) were exposed by inhalation to 1,1-DCE at 0 (65 animals), 15 ppm (23 animals), 30 ppm (19 animals), 57 ppm (21 animals), 144 ppm (18 animals), or 300 ppm (15 animals) for 22–23 hrs/day on GDs 6–16. Dams were sacrificed on GD 17. Maternal toxicity occurred at ≥ 30 ppm, as shown by statistically significant decreases in maternal weight gain. At 144 and 300 ppm there was an increase in maternal mortality. At 30 ppm and higher there was severe fetal toxicity with complete early resorption of the litters. At 15 ppm there was no evidence of maternal toxicity, no decrease in fetal body weight, and no decrease in the percentage of viable fetuses. At 15 ppm, there was an increase in the mean number of fetuses per litter with hydrocephalus, occluded nasal passages, microphthalmia, cleft palate, small liver, and hydronephrosis. None of these changes, however, were statistically significant when compared to controls. Also at 15 ppm there was a statistically significant increase in the mean number of fetuses with an unossified incus and with incompletely ossified sternebrae. This study provides evidence of fetal toxicity at 15 ppm, the only exposure without significant maternal toxicity. In this study the LOAEL for developmental toxicity is 15 ppm (60 mg/m^3), the lowest exposure tested.

Murray et al. (1979) evaluated developmental toxicity of 1,1-DCE administered by inhalation to pregnant Sprague-Dawley rats (body weight 250 g). Animals were exposed to 0 (20 or 47 animals), 20 ppm (44 animals), 80 ppm (30 animals), or 160 ppm (30 animals) for 7 hours/day on GDs 6–15. At 20 ppm there was no maternal toxicity and no effect on embryonal or fetal development. At 80 and 160 ppm, there was toxicity to the dams (statistically significant depression in weight gain at GDs 6–9, more severe at 160 ppm). At 80 and 160 ppm, there was also a statistically significant increased incidence of wavy ribs and delayed ossification of the skull, which was regarded as an embryotoxic effect. Both effects were more severe at 160 ppm. No teratogenic effects were seen at any exposure. The NOAEL for developmental toxicity in this study is 20 ppm (80 mg/m^3); the LOAEL is 80 ppm (320 mg/m^3). Under the guidelines for developmental toxicity (U.S. EPA, 1991), these values are not adjusted to continuous exposure.

Murray et al. (1979) evaluated the developmental toxicity of 1,1-DCE administered by inhalation to New Zealand white rabbits (body weight 3.4–4.7 kg). Animals were exposed to 0 (16 animals), 80 ppm (22 animals), or 160 ppm (18 animals) for 7 hrs/day on GDs 6–18. No maternal toxicity or effect on embryonal or fetal development was observed at 80 ppm. Toxicity to both the dams and their developing embryos was observed at 160 ppm, as indicated by a marked increase in the incidence of resorptions per litter (0.3 ± 0.6 vs. 2.7 ± 3.9) and a significant change in the incidence of several minor skeletal variations in their offspring, including an increase in the occurrence of 13 pairs of ribs and an increased incidence of delayed ossification of the fifth sternebra (data not reported). No teratogenic effects were seen at any exposure. The NOAEL for developmental toxicity in this study is 80 ppm (320 mg/m^3); the LOAEL is 160 ppm (640 mg/m^3). Under the guidelines for developmental toxicity (U.S. EPA, 1991), these values are not adjusted to continuous exposure.

4.4. OTHER STUDIES

4.4.1. Developmental Neurotoxicity

Short et al. (1977b) evaluated developmental neurotoxicity of 1,1-DCE administered by inhalation to CD-1 rats (Charles River). Pregnant rats were exposed to 0 (24 animals), 56 ppm (20 animals), or 283 ppm (19 animals) for 22–23 hrs/day on GDs 8–20. Maternal toxicity was seen at both exposures, as shown by weight loss of 7 g per dam at 56 ppm and 15 g per dam at 283 ppm. There was complete resorption of three litters at 283 ppm. A statistically significant decrease in average pup weight as compared to controls was noted at both exposures on post-natal day 1. The difference in pup weight between control and exposed groups decreased with time and disappeared by postnatal day 21. There was no evidence of developmental neurotoxicity at either exposure in pups tested at various times from postnatal day 1 to day 21 in a battery of behavioral tasks, including surface righting, pivoting, auditory startle, bar holding, righting in air, visual placing, swimming ability, physical maturation, and activity. This study showed evidence of maternal and fetal toxicity at both exposures, but no evidence of developmental neurotoxicity at either exposure. Accordingly, the NOAEL for developmental neurotoxicity in this study is 283 ppm (1124 mg/m^3), the highest exposure tested.

4.4.2. Cardiac Sensitization

Siletchnik and Carlson (1974) investigated the effects of epinephrine on cardiac sensitization by exposure to 1,1-DCE in male albino rats. The test animals (body weight, 250–400 g) were exposed to 1,1-DCE at 0 or 25,600 ± 600 ppm and the dose of epinephrine was titrated to determine the minimum concentration needed to produce arrhythmias. A dose of 4 μg/kg of epinephrine failed to induce cardiac arrhythmias in air-exposed animals. However, the dose necessary to produce life-threatening arrhythmias was 2.0 μg/kg following 58 to 61 minutes of exposure to 1,1-DCE, 1.0 μg/kg following 64 minutes of exposure to 1,1-DCE, and 0.5 μg/kg following 67 to 80 minutes of exposure. The cardiac sensitization was found to be completely reversible upon discontinuance of exposure.

4.4.3. Species Specificity

Speerschneider and Dekant (1995) investigated the metabolic basis for the species- and sex-specific nephrotoxicity and tumorigenicity of 1,1-DCE. In kidney microsomes from Swiss-Webster male mice, the rate of oxidation of 1,1-DCE depended on the hormonal status of the animals. Oxidation of 1,1-DCE was decreased by castration and restored when the castrate was supplemented with exogenous testosterone. In kidney microsomes from naive female mice, the rate of oxidation of 1,1-DCE was significantly lower than in males but could be increased by administration of exogenous testosterone. Using an antibody to rat liver CYP2E1, the researchers showed expression of a cross-reacting protein in male mouse kidney microsomes that was regulated by testosterone and correlated with the ability to oxidize 1,1-DCE and other substrates for CYP2E1 (e.g., p-nitrophenol and chlorozoxazone). The researchers also showed that different strains of mice express different levels of CYP2E1. The strains most sensitive to the effects of 1,1-DCE expressed greater levels of CYP2E1. Nephrotoxicity in Swiss-Webster mice after inhalation of 1,1-DCE was observed in males and in females treated with exogenous testosterone, but not in naive females. In kidney microsomes obtained from both sexes of rats and in six samples of human kidney from male donors, no p-nitrophenol oxidase activity was detected. Other research groups have also reported the absence of detectable CYP2E1 in human kidney tissue (Amet et al., 1997; Cummings et al., 2000).

4.4.4. Genetic Toxicity

Reitz et al. (1980) investigated the ability of 1,1-DCE to cause DNA alkylation, DNA repair, DNA replication, and tissue damage in liver and kidney of rats and mice. Male Sprague-Dawley rats (body weight, 200–250 g) and male CD-1 mice (body weight, 18–20 g) were exposed by inhalation for 6 hours. Rats were exposed to 0 or 10 ppm; mice were exposed to 0, 10, or 50 ppm. In rats at 10 ppm, there was only a minimal increase in DNA alkylation and a small increase in DNA replication (twofold increase in ^{3}H-thymidine incorporation) in the kidney but no increase in liver. In mice at 10 and 50 ppm, there was only a minimal increase in DNA alkylation. In mice DNA repair was not increased in liver or kidney at 10 ppm or in liver at

50 ppm, but was increased in kidney at 50 ppm. In kidney of mice, there was an eightfold increase in DNA replication at 10 ppm and a 25-fold increase at 50 ppm, as measured by ^{3}H-thymidine incorporation. There was a corresponding increase in mitotic figures. No histopathological damage or increased DNA replication in the liver of mice was observed at 10 or 50 ppm. In mice at 10 ppm, there was slight dilation and swelling and variable amounts of nephrosis in the kidney, but no effect in the liver. At 50 ppm, mice showed toxic nephrosis in the kidney and slight centrilobular swelling in the liver.

1,1-DCE induced mutations in *Salmonella typhimurium* and *Escherichia coli* in the presence of an exogenous metabolic system. In *Saccharomyces cerevisiae,* 1,1-DCE induced reverse mutation and mitotic gene conversion in vitro and in a host-mediated assay in mice. In a single study in *Saccharomyces cerevisiae*, it induced aneuploidy in the presence and absence of metabolic activation. In vitro, gene mutations were increased in mouse lymphoma cells but not in Chinese hamster lung cells, with or without an exogenous metabolic system. In a single study, 1,1-DCE induced sister chromatid exchanges in Chinese hamster lung cells in the presence of an exogenous metabolic system but not in its absence. In single studies in vivo, 1,1-DCE did not induce micronuclei or chromosomal aberrations in bone marrow or in fetal erythrocytes of mice or dominant lethal mutations in mice or rats.

1,1-DCE causes gene mutations in microorganisms in the presence of an exogenous activation system. Although most tests with mammalian cells show no evidence of genetic toxicity, the test battery is incomplete, as it lacks an in vivo assessment of chromosomal damage in the mouse lymphoma assay, a test EPA considers an important component of a genotoxicity battery. Data on the genetic and related effects of 1,1-DCE are summarized in Table 3.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION

There are no useful epidemiological studies or case reports in humans characterizing the noncancer health effects of 1,1-DCE.

Table 3. Genetic and related effects of 1,1-DCE

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without	With		
S. typhimurium BA13/BAL13, forward mutation	–	+	500	Roldan-Arjona et al., 1991

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without	With		
S. typhimurium TA 100, reverse mutation	NT	+	2% in air	Malaveille et al., 1997
	NT	+	5% in air	Jones and Hathway, 1978c
	–	+	5% in air	Simmon and Tardiff, 1978
	+	+	5% in air	Waskell, 1978
	NT	+	2% in air	Bartsch et al., 1979
	–	+	375 ppm in air	Oesch et al., 1983
	(+)	+	125	Strobel and Grummt, 1987
S. typhimurium TA 104, reverse mutation	–	–	500	Strobel and Grummt, 1987
S. typhimurium TA 1535, reverse mutation	–	+	3% in air	Baden et al., 1977
	NT	+	5% in air	Jones and Hathway, 1978c
	–	+	375 ppm in air	Oesch et al., 1983
S. typhimurium TA 1537, reverse mutation	–	(+)	375 ppm in air	Oesch et al., 1983
S. typhimurium TA 98, reverse mutation	–	+	375 ppm in air	Oesch et al., 1983
	–	(+)	125	Strobel and Grummt, 1987
S. typhimurium TA 92, reverse mutation	–	(+)	375 ppm in air	Oesch et al., 1983
S. typhimurium TA 97, reverse mutation	–	+	5	Strobel and Grummt, 1987
E. coli K12, forward or reverse mutation	–	(+)	242	Oesch et al., 1983

Table 3. Genetic and related effects of 1,1-DCE (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without	With		
E. coli WP2 uvrA, reverse mutation	–	+	375 ppm in air	Oesch et al., 1983
S. cerevisiae D7, gene conversion	–	+	2910	Bronzetti et al., 1983
S. cerevisiae D7, mitotic gene conversion	+[c]	–	7300	Koch et al., 1988
S. cerevisiae D7, reverse mutation	–	+	2910	Bronzetti et al., 1983
	+[c]	+	4876	Koch et al., 1988
S. cerevisiae D61.M, aneuploidy	+	+	2435	Koch et al., 1988
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus in vitro	–	–	10% in air	Drevon and Kuroki, 1979
Gene mutation, Chinese hamster lung V79 cells, ouabain resistance in vitro	–	–	10% in air	Drevon and Kuroki, 1979
Gene mutation, mouse lymphoma L5178Y cells, *tk* locus in vitro	?	+	0.16% in air	McGregor et al., 1991
Sister chromatid exchange, Chinese hamster lung in vitro	–	+	75	Sawada et al., 1987
Chromosomal aberrations, Chinese hamster DON-6 cells in vitro	–	NT	2910	Sasaki et al., 1980
Chromosomal aberrations, Chinese hamster fibroblast CHL cells in vitro	–	NT	2000	Ishidate (ed.), 1983

Table 3. Genetic and related effects of 1,1-DCE (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without	With		
Chromosomal aberrations, Chinese hamster lung cells in vitro	–	+	250	Sawada et al., 1987
Host-mediated assay, *S. cerevisiae* D7 in CD mouse hosts	+	NT	100 po × 23	Bronzetti et al., 1981
	+	NT	400 po × 1	Bronzetti et al., 1981
Micronucleus test, mouse bone marrow in vivo	–	–	200 po × 1	Sawada et al., 1987
Micronucleus test, mouse fetal erythrocytes in vivo	–	–	100 po × 1	Sawada et al., 1987
Chromosomal aberrations, Sprague-Dawley rat bone marrow in vivo	–	–	6 hrs/day, 3 days/wk, 2 yrs	Rampy et al., 1977
Dominant lethal test, male CD-1 mice	–	–	50 ppm inh, 6 hrs/day, 5 days	Anderson et al., 1977
Dominant lethal test, CD rats	–	–	55 ppm inh 6 hrs/day, 5 days/wk, 11 wks	Short et al., 1977c

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive.
[b] LED, lowest effective dose; HID, highest ineffective dose. In vitro tests, µg/ml; in vivo tests, mg/kg body weight; po, orally; inh, inhalation
[c] Positive in cells grown in logarithmic phase

In laboratory animals 1,1-DCE is rapidly absorbed following oral and inhalation exposure. Most of the free 1,1-DCE, its metabolites, and covalently bound derivatives are found in the liver and kidney. 1,1-DCE is rapidly oxidized by CYP2E1 to 1,1-DCE epoxide, which can be transformed to 2-chloroacetyl-chloride and 2,2-dichloroacetaldehyde (Figure 1). It is not known whether the metabolism of 1,1-DCE is the same in humans, although in vitro microsomal preparations from human liver and lung form the same initial products.

Following acute exposure by the oral or the inhalation route, the target organs are the liver, the kidney, and the Clara cells of the lung.

Following longer term and chronic exposure at less than an acutely toxic exposure, the liver is the major target in rats following oral or inhalation exposure. The minimal fatty change observed in the liver of rats following long-term exposure—the critical effect—occurs primarily in mid-zonal hepatocytes, but the change is not restricted to the centrilobular region. The minimal fatty change in the liver also occurs in the absence of significant depletion of cellular GSH. It is not known whether this reversible effect is the consequence of covalent binding of 1,1-DCE derivatives formed in situ by CYP2E1 or of disruption of phospholipid synthesis in the cells. Although the minimal fatty change might not be considered adverse—as there is no evidence of a functional change in the liver in rats exposed at this level, and GSH levels are not reduced—it is defined as the critical effect from both oral and inhalation exposure because limiting exposure to this level will protect the liver from more serious damage (for example, fatty liver or necrosis) that could compromise liver function.

The kidney is the major target organ in mice following inhalation exposure. The effects in the kidney appear to be related to a gender-specific expression of CYP2E1 in male mice, the presence of higher amount of β-lyase in kidney tissue of mice relative to other species, and the general pharmacokinetic principle that more 1,1-DCE will be delivered to the kidneys following inhalation exposure relative to oral exposure.

There is no evidence that toxicity occurs in the respiratory tract following exposure to 1,1-DCE at levels that cause minimal toxicity in the liver of rats and in the kidney of mice. However, regional responses in olfactory epithelium or bronchiolar changes in Clara cells might have been missed by the methods used in the toxicological studies to evaluate these regions.

As shown in a three-generation study, there is no evidence that reproductive toxicity is a critical effect for 1,1-DCE. No reproductive or developmental toxicity was observed at an exposure that caused minimal toxicity in the liver of the dams. There is also no evidence that teratogenicity is a critical effect. Some evidence was found of developmental variations in the heart following direct infusion of 1,1-DCE into the uterus of pregnant rats and fertilized chicken eggs and ingestion of 1,1-DCE by pregnant rats from drinking water, but it is not clear whether these effects were directly caused by exposure to 1,1-DCE. The biological significance of these cardiac structural variations is unclear. There is no indication that the structural variations have functional consequences in the animals. However, animals known to have the structural variations have not been tested under conditions of stress.

There are no focused studies on neurotoxicity, but no indication from chronic, reproductive, and developmental bioassays in rats and mice by oral or inhalation exposure that neurotoxicity is an important toxic endpoint. No long-term studies have evaluated immunotoxicity in laboratory animals by any route of exposure; however, the existing bioassays provide no suggestion that immunotoxicity is a critical effect.

These various observations on toxicity and metabolism of 1,1-DCE indicate that cytotoxicity is associated with cytochrome P450-catalyzed metabolic activation of 1,1-DCE to reactive intermediates that bind covalently to cellular macromolecules. The extent of binding is inversely related to loss of GSH, so that severities of tissue damage parallel the decline in GSH (Forkert and Moussa, 1991; Moussa and Forkert, 1992). Hepatotoxicity is also exacerbated by treatments that diminish GSH (McKenna et al., 1978b; Andersen et al., 1980; Jaeger et al., 1973, 1974). Thus, the responses to 1,1-DCE at low doses, which cause little depletion of GSH, are expected to be very different from the responses at high doses, which cause substantial GSH depletion. The targets of toxicity are centrilobular hepatocytes and bronchiolar Clara cells (Forkert et al., 1986), cell types that are rich in CYP2E1 (Forkert et al., 1991; Forkert, 1995). Immunohistochemical studies showed formation of DCE epoxide-cysteine protein adducts within the centrilobular hepatocytes and Clara cells (Forkert et al., 1999a, b). In combination, these findings indicate that DCE-induced toxicity is associated with formation and reactivity of the DCE epoxide within the target centrilobular hepatocytes and Clara cells.

In the absence of specific information on the toxicity of 1,1-DCE in humans, the most scientifically appropriate way of conducting a risk assessment would be to use a PBPK model to calculate the concentration of the toxic metabolite in the target tissue. The model would incorporate the appropriate physiological variables for laboratory animals and humans and what is known about the mode of action for 1,1-DCE. As discussed above, the toxicity of 1,1-DCE is attributed to its metabolites, not to the parent compound. Intracellular GSH provides a mechanism for detoxification of the metabolites. Toxicity is attributed to the amount of metabolite that escapes conjugation in the liver. The model would thus also incorporate information on the rate of metabolism of 1,1-DCE in the liver, the initial amount of GSH in the liver, the rate of conjugation of GSH with the reactive metabolites, and the rate of regeneration of GSH.

Such a model is available for vinyl chloride (Clewell et al., 1999a, b). In fact, the vinyl chloride model was developed using a simpler model developed by D'Souza and Andersen (1988) for 1,1-DCE. The vinyl chloride model has been validated for humans by successfully predicting the concentration of vinyl chloride in volunteers. Application of the model for oral exposure to vinyl chloride shows that the human equivalent dose is equal to the dose to rats divided by approximately 1.4; similarly, for inhalation exposure the human equivalent concentration is equal to the inhalation exposure to rats divided by approximately 5. These factors are not significantly different from those determined for 1,1-DCE in the original model developed by D'Souza and Andersen (1988).

EPA does not believe that it is appropriate to apply the vinyl chloride model to this assessment for 1,1-DCE at this time. For dose estimates in liver, the original 1,1-DCE model needs to be updated to include the more current understanding of the metabolism of 1,1-DCE. In addition, it also appears necessary for the model to estimate dose in other target tissues, namely, the lung and kidney. For EPA to provide such analysis is beyond the scope of effort for an assessment for the IRIS program. EPA also does not believe that there is adequate information to apply the vinyl chloride model to 1,1-DCE using a simpler parallelogram approach (Jarabek et

al., 1994; Williams et al., 1996). EPA may, however, modify this assessment when a more complete PBPK model is available.

In the absence of a suitable PBPK model, EPA used its default procedure to determine the RfD and the default procedure for a category 3 gas to determine the RfC. EPA recognizes the scientific limitations of this approach for determining the RfC. The default procedure for a category 3 gas was developed with the assumption that the parent gas, not a metabolite, is the toxic substance. As discussed above, that is not the case for 1,1-DCE. At an exposure much less than the point of saturation of the oxidative pathway for 1,1-DCE (approximately 200 ppm), essentially all of the absorbed 1,1-DCE is metabolized in the liver. Under these conditions there will be a constant ratio between the concentration of 1,1-DCE in the ambient air and the concentration of the toxic metabolite in the liver. Therefore, the category 3 gas default procedure will provide a reasonable approximation of the exposure-response relationship.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Under the 1986 cancer guidelines (U.S. EPA, 1986a), 1,1-DCE is assigned to Group C, possible human carcinogen.

Under the draft revised guidelines for carcinogen risk assessment (U.S. EPA, 1999), EPA concludes 1,1-DCE exhibits *suggestive evidence* of carcinogenicity but not sufficient evidence to assess human carcinogenic potential following inhalation exposure in studies in rodents. Male mice developed kidney tumors at one exposure in a lifetime bioassay, a finding tempered by the absence of similar results in female mice or male or female rats and by the enzymatic differences (i.e., CYP2E1) between male mice and female mice, male and female rats, and human kidney cells. Limited evidence of genotoxicity has been reported in bacterial systems with metabolic activation. The data for 1,1-DCE are *inadequate* for an assessment of human carcinogenic potential by the oral route, based on the absence of statistically or biologically significant tumors in limited bioassays in rats and mice balanced against the suggestive evidence in male mice in a single bioassay by inhalation and the limited evidence of genotoxicity. The human epidemiological results on the carcinogenicity of 1,1-DCE are too limited to draw useful conclusions. EPA concludes that the results of kidney tumors in one sex and one exposure in a single species of rodents are too limited to support an exposure-response assessment.

Bioassays for cancer by the oral route of exposure have been conducted in rats (Maltoni et al., 1985; NTP, 1982; Ponomarkov and Tomatis, 1980; Quast et al., 1983), mice (NTP, 1982), and trout (Hendricks et al., 1995). Some of these bioassays were conducted at an exposure below the maximum tolerated dose. The bioassay conducted by Maltoni et al. (1985) exposed the animals for only 1 year. The bioassay conducted in rats by Quast et al. (1983) and the bioassay conducted in mice by NTP (1982) were well conducted, and both showed some toxicity in the liver at the highest exposure. Neither of these bioassays provides any significant evidence that 1,1-DCE is a carcinogen by the oral route of exposure. The genotoxicity studies are incomplete, but most studies in mammalian cells indicate a lack of genotoxicity.

This assessment of carcinogenicity by the oral route of exposure under the draft revised guidelines for carcinogen risk assessment (U.S. EPA, 1999) differs from the previous EPA evaluation (U.S. EPA, 1985a, b, 1987). The previous evaluation derived an oral slope factor from the highest of four slope factors calculated from two studies (NTP, 1982; Quast et al., 1983) that did not show statistically significant increases in tumor incidence attributable to oral exposure. The highest slope factor was based on the adrenal pheochromocytomas in male rats (NTP, 1982). Following the 1999 draft revised guidelines for carcinogen risk assessment, EPA emphasizes the importance of using data that show a statistically significant increase in tumor incidence for calculating a slope factor. As there is no statistically or biologically significant increase in tumor incidence at any site in the relevant oral bioassays, the present evaluation characterizes the weight of evidence as *inadequate* and, accordingly, does not derive an oral slope factor. This conclusion is consistent with the evaluation by the International Agency for Research on Cancer (IARC, 1999).

Bioassays for cancer by the inhalation route of exposure have been conducted in rats (Lee et al., 1977, 1978; Viola and Caputo, 1977; Hong et al., 1981; Maltoni et al., 1985; Quast et al., 1986; Cotti et al., 1988), mice (Lee et al., 1977, 1978; Hong et al., 1981; Maltoni et al., 1985), and hamsters (Maltoni et al., 1985). None of these bioassays was conducted by a protocol that meets current standards. The major defects in most of these bioassays include exposure of the animals for 1 year and exposure at less than the maximum tolerated dose. The only bioassay showing some evidence of carcinogenicity was the study in Swiss-Webster mice (Maltoni et al., 1985). This study was conducted at or near the maximum tolerated dose, as animals exposed at 50 ppm died after a few exposures. Although the animals were exposed for only 1 year and then observed until natural death, this study showed an increased incidence of kidney adenocarcinomas in male mice at 25 ppm but not at 10 ppm. The incidence of mammary carcinomas in female mice and pulmonary adenomas in male and female mice did not increase with increased exposure. The responses were actually lower at 25 ppm than at 10 ppm, but survival and other toxicities were comparable.

There is evidence that the induction of kidney adenocarcinomas is a sex- and species-specific response related to the expression of CYP2E1 in the kidney of male mice (Speerschneider and Dekant, 1995; Amet et al., 1997; Cummings et al., 2000). However, the data presented by these researchers are not sufficient to justify a conclusion that the kidney tumors in male mice have no relevance for a human health risk assessment. This conclusion is made with the knowledge that compounds similar in structure to 1,1-DCE (e.g., tetrachloroethylene, trichloroethylene, and 1,2-dichloroethylene) produce varying degrees of kidney tumors in animal bioassays. The genotoxicity studies are incomplete, but most studies in mammalian cells indicate a lack of genotoxicity. Accordingly, EPA concludes that the data on the increased incidence of kidney adenocarcinomas in male mice (Maltoni et al., 1985) provide *suggestive evidence* of carcinogenicity by the inhalation route of exposure. EPA also concludes, considering the evidence of a potential sex- and species-specific response, that the results of this bioassay showing an increase in tumors in one sex and one exposure in a single species of rodents are too limited to support an exposure-response assessment.

This assessment of carcinogenicity by the inhalation route of exposure under the draft revised guidelines for carcinogen risk assessment (U.S. EPA, 1999) differs from the previous EPA evaluation (U.S. EPA, 1985a, b, 1987). EPA's previous evaluation considered the incidence of kidney adenocarcinomas (Maltoni et al., 1985) as providing sufficient evidence of carcinogenicity to justify deriving an inhalation unit risk for quantifying the potential human cancer risk. As noted in the paragraph above and in Section 4.4.3, the new data suggesting that the kidney adenocarcinomas could be a sex- and species-specific response reduce the weight of evidence for carcinogenicity by the inhalation route of exposure. Accordingly, the present evaluation does not derive an inhalation unit risk. This conclusion is consistent with the evaluation by IARC (1999).

1,1-DCE causes gene mutations in microorganisms in the presence of an exogenous activation system. Although most tests with mammalian cells have shown no evidence of genetic toxicity, the test battery is incomplete because it lacks an in vivo test for chromosomal damage in the mouse lymphoma system.

A number of uncertainties exist in the assessment of the carcinogenicity of 1,1-DCE. As noted above, many of the bioassays by the inhalation route of exposure were not conducted at the maximum tolerated dose or for the full lifetime of the animals. EPA has acknowledged this uncertainty in the weight of evidence classification. In addition, our knowledge of the metabolic pathways for 1,1-DCE in the human is incomplete. Although it is likely that the initial oxidation of 1,1-DCE in humans occurs via CYP2E1, there could be other CYP isoforms that could activate 1,1-DCE. Thus, there is some potential for a species-specific carcinogenic response in humans similar to the apparent sex- and species-specific response observed by Maltoni et al. (1985) in the kidney of male mice.

4.7. SUSCEPTIBLE POPULATIONS

There are no adequate epidemiological studies or case reports in humans directly demonstrating a susceptible human population. However, because of the role of CYP2E1 and GSH in the expression of toxicity of 1,1-DCE, individuals with high levels of CYP2E1 (e.g., abusers of ethanol and individuals routinely exposed to ketones and heterocyclic compounds and other inducers of CYP2E1) could be more sensitive to the adverse effects of 1,1-DCE. There is some evidence, however, that the rate of hepatic blood flow is an important limiting factor in the metabolism of 1,1-DCE (Kedderis, 1997). This effect would reduce the importance of the variability among individuals in concentration of CYP2E1 in the liver as a determinant of susceptibility to the adverse effects of 1,1-DCE. Individuals at risk from exposure to 1,1-DCE would also include those who have an extremely low level of GSH, for example, individuals who are malnourished or fasting or who are poisoned from acetaminophen (Wright and Moore, 1991).

4.7.1. Possible Childhood Susceptibility

Although there are many drugs that exhibit a higher systemic clearance in children than in adults, no studies in laboratory animals or epidemiological studies or case reports in humans

have demonstrated increased susceptibility (i.e., greater response at the same exposure) of children to 1,1-DCE. The major determinants of the liver toxicity of 1,1-DCE are the CYP2E1 and the GSH content of the liver, cardiac output, and liver volume. CYP2E1 was not detectable in fetal liver samples from humans, but it increased dramatically within hours after birth and nearly reached levels found in adults by 1 year of age (Cresteil, 1998). Fetal CYP2E1 may be inducible by exposure to CYP2E1 substrates such as ethanol (Carpenter et al., 1996). No significant difference between children and adults was found in the activity of CYP2E1 using a nonspecific substrate, ethoxycoumarin (Blanco et al., 1999). On the basis of these observations, it does not seem likely that children will exhibit increased susceptibility to the adverse liver effects of 1,1-DCE. The variability between children and adults in the GSH content of the liver, cardiac output, and liver volume are likely to be within the intraspecies uncertainty factor (UF) of 10 used to derive the RfD and the RfC.

As noted in section 4.3.2, some data in laboratory animals studies suggest an increased incidence of cardiac changes following exposure to 1,1-DCE (Dawson et al., 1990; Goldberg et al., 1992; Dawson et al., 1993). It would be helpful if more definitive studies with a greater range of exposures were conducted to determine the cause and biological significance of the cardiac changes apparently associated with exposure to 1,1-DCE during the period of cardiac organogenesis.

4.7.2. Possible Gender Differences

Some data suggest that nephrotoxicity might be a specific response in male mice, and there is some indication that female rats might be more sensitive than male rats to hepatotoxicity, as the fatty change—the critical effect—appeared at a slightly lower exposure in female rats as compared to male rats. There are no epidemiological studies or case reports in humans suggesting gender specificity for any target tissue.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

The candidate oral chronic or long-term studies for deriving the RfD included the studies by Maltoni et al. (1985) and Quast et al. (1983) in Sprague-Dawley rats, the three-generation reproduction study by Nitschke et al. (1983) in Sprague-Dawley rats, the study by NTP (1982) in F344 rats, and the study by NTP (1982) in C57Bl6 mice. The Maltoni et al. (1985) study was rejected because the animals were exposed for only 1 year and there was no evaluation of endpoints at the termination of exposure. The Nitschke et al. (1983) study provides evidence of minimal liver toxicity at exposures comparable to those reported in Quast et al. (1983); however, Nitschke et al. did not provide information on the actual exposure of the animals the number of animals responding at each exposure or a statistical analysis of the results. The NTP (1982)

study in rats did not show any toxicity at the highest exposure tested (5 mg/kg-day) but did show a nonstatistically significant increase in liver necrosis in male mice at 10 mg/kg-day following gavage dosing. This study was not used because the gavage route of exposure affects the pharmacokinetics of 1,1-DCE and the exposure-response relationship.

The Quast et al. (1983) study exposed animals for 2 years to 1,1-DCE in drinking water and provided exposure-response data for minimal toxicity in the liver (hepatocellular midzonal fatty change and hepatocellular swelling). These data were used to derive the RfD. The incidence of hepatocellular swelling was statistically significant at all exposures in female rats. On the basis of the minimal nature of this effect, as reported by the authors, this response is not considered to be biologically significant in this study. Nonetheless, BMD modeling was conducted on the exposure-response data for this effect and revealed a BMD_{10} of 7.7 mg/kg-day and a $BMDL_{10}$ of 4.7 mg/kg-day (analysis not presented). The critical effect is hepatocellular midzonal fatty change in female rats. The NOAEL for this effect is 9 mg/kg-day, the LOAEL is 14 mg/kg-day, the BMD_{10} is 6.6 mg/kg-day, and the $BMDL_{10}$ is 4.6 mg/kg-day (Appendix B). Although this minimal effect might not be considered adverse—as there is no evidence of a functional change in the liver in rats exposed at this level, and GSH levels are not reduced—the $BMDL_{10}$ is used to derive the RfD, as limiting exposure to the $BMDL_{10}$ will protect the liver from more serious damage (for example, fatty liver or necrosis) that could compromise liver function.

5.1.2. Methods of Analysis

BMD modeling using data from Quast et al. (1983) for the critical effect was used to determine the BMD_{10} of 6.6 mg/kg-day and the $BMDL_{10}$ of 4.6 mg/kg-day.

As discussed in section 4.5, no validated pharmacokinetic model was available for this assessment. Accordingly, EPA used its default procedure for determining the RfD.

5.1.3. RfD Derivation

The RfD of 0.05 mg/kg-day was calculated from the $BMDL_{10}$ of 4.6 mg/kg-day and a total UF of 100 and a modifying factor (MF) of 1 (4.6 mg/kg-day × 1/100 = 0.046, rounded to 0.05 mg/kg-day). Individual UFs of 10 each were used for interspecies extrapolation and intraspecies variability because there were no applicable data to justify departing from the default values. Derivation of the RfD from the $BMDL_{10}$ for the minimal fatty change in the liver does not require an effect-level extrapolation. This conclusion is based on the minimal nature of the fatty change and its questionable biological significance because of the absence of any observable functional deficit in the liver. A subchronic-to-chronic extrapolation factor was not applied because the Quast et al. (1983) study exposed the animals for 2 years. A database UF was not applied because the database is considered complete.

A number of long-term bioassays in rodents exposed by the oral or inhalation route show that liver toxicity is the critical effect. There is no chronic bioassay in a nonrodent mammal;

however, 90-day bioassays in several species (rats, mice, dogs, guinea pigs, rabbits, and monkeys) suggest similar exposure-response relationships across species. Therefore, the lack of a chronic bioassay in a nonrodent mammal is not considered a data gap. No focused studies of 90 days or longer exist for evaluating neurotoxicity or immunotoxicity. EPA does not consider these data gaps compelling enough to require application of a database UF.

This RfD differs from the previous EPA value of 0.009 mg/kg-day. The previous EPA evaluation used the same study but considered the lowest exposure of 9 mg/kg-day in female rats as a LOAEL for minimal hepatocellular fatty change and minimal hepatocellular swelling and applied a total UF of 1000 (10 for LOAEL-to-NOAEL extrapolation, 10 for interspecies extrapolation, and 10 for human variability). As noted above, EPA no longer considers hepatocellular swelling in the absence of other effects, such as increased liver enzymes in the serum, as biologically significant in this bioassay. The increased incidence of midzonal fatty change at 9 mg/kg-day in female rats is not statistically significant. The NOAEL in this bioassay in 9 mg/kg-day. In addition, the present evaluation uses BMD methodology and calculates $BMDL_{10}$ for midzonal fatty change in female rats.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

The candidate studies for deriving the RfC included the studies by Maltoni et al. (1985) in Sprague-Dawley rats and Swiss-Webster mice and by Quast et al. (1986) in Sprague-Dawley rats. The Maltoni et al. (1985) study was rejected because the animals were exposed for only 1 year, and there was no evaluation of endpoints at the termination of exposure. Thus the true incidence of the effect due to exposure to 1,1-DCE cannot be determined. The Quast et al. (1986) study exposed the animals for 18 months and provided exposure-response information for minimal toxicity in the liver. The critical effect is minimal hepatocellular midzonal fatty change in female Sprague-Dawley rats. The NOAEL for this effect in female Sprague-Dawley rats is 25 ppm, the LOAEL is 75 ppm, the BMC_{10} is 15.1 ppm, and the $BMCL_{10}$ is 9.8 ppm (Appendix B). The $BMCL_{10}$ adjusted to continuous exposure ($BMCL_{ADJ}$) is 1.75 ppm (6.9 mg/m^3) (9.8 ppm x 6/24 x 5/7 = 1.75 ppm, 1.75 ppm x 3.97 mg/m^3 per ppm = 6.9 mg/m^3). Although this minimal effect might not be considered adverse—as there is no evidence of a functional change in the liver in rats exposed at this level and GSH levels are not reduced—the $BMCL_{10}$ is used to derive the RfC, as limiting exposure to the $BMCL_{10}$ will protect the liver from more serious damage (fatty liver or necrosis) that could compromise liver function.

5.2.2. Methods of Analysis

As discussed in section 4.5, no validated PBPK model is available for interspecies extrapolation. Accordingly, EPA used its default procedure for a category 3 gas (a gas that is relatively insoluble and unreactive in the extrathoracic and tracheobroncial liquid and tissue [U.S. EPA, 1994b]) to determine the RfC. BMD analysis was used to determine a BMC_{10} of 15.1 ppm and a $BMCL_{10}$ of 9.8 ppm. The $BMCL_{ADJ}$ is 6.9 mg/m^3. The human equivalent

concentration for the $BMCL_{10}$ ($BMCL_{HEC}$) is calculated using inhalation dosimetry for a category 3 gas:

$$BMCL_{HEC} = BMCL_{ADJ} \times (H_{b/g})_A/(H_{b/g})_H$$

The blood:air partition coefficient in rats $[(H_{b/g})_A]$ is 5 (D'Souza and Andersen, 1988). No published data are available to determine the blood:air partition coefficient in humans $[(H_{b/g})_H)]$. Unpublished data from a single measurement in one person (verbal statement by M. Andersen, Colorado State University, to R. Benson, U.S. EPA, Aug. 7, 2001) suggest a value for the blood:air partition coefficient of 1.75. EPA does not consider this observation sufficiently robust for deriving the RfC. In addition, EPA has made a policy decision that a ratio for the blood:air partition coefficient greater than 1 will not be used to derive the RfC (U.S. EPA, 1994b). Therefore, the default value of 1 is used for $(H_{b/g})_A/(H_{b/g})_H$. The $BMCL_{HEC}$ is 6.9 mg/m^3.

5.2.3. RfC Derivation

The RfC of 0.2 mg/m^3 is calculated from the $BMCL_{HEC}$ of 6.9 mg/m^3 in a chronic bioassay using a total UF of 30 and an MF of 1 (6.9 mg/m^3 × 1/30 = 0.23, rounded to 0.2 mg/m^3). A UF of 3 is used for interspecies extrapolation because a dosimetric adjustment was used. There is some suggestion that effects in the kidney of mice may occur at an exposure lower than the level that causes effects in the liver of rats. Thus, there is some uncertainty as to whether the most sensitive species has been used to derive the RfC. As noted above, however, the long-term study in mice (Maltoni et al., 1985) is not suitable for deriving the RfC. A UF of 10 is used for intraspecies variability because there were no applicable data to justify departure from the default value. Derivation of the RfC from the $BMCL_{10}$ for the minimal fatty change in the liver does not require an effect-level extrapolation. This conclusion is based on the minimal nature of the fatty change and its questionable biological significance because of the absence of any observable functional deficit in the liver. Although the rats in Quast et al. (1986) were exposed for 18 months rather than for their full lifetime, there was no indication that the fatty change was progressing. In contrast, the evidence indicated that the fatty change was decreasing in incidence with continued exposure. EPA, therefore, did not apply a subchronic-to-chronic extrapolation factor. A database UF was not applied because the database is considered complete.

A number of long-term bioassays in rodents exposed by the oral or inhalation route show that liver toxicity is the critical effect. There is no chronic bioassay in a nonrodent mammal; however, 90-day bioassays in several species (rats, mice, dogs, guinea pigs, rabbits, and monkeys) suggest similar exposure-response relationships across species. Therefore, the lack of a chronic bioassay in a nonrodent mammal is not considered a data gap. No studies of 90 days or longer exist for evaluating neurotoxicity or immunotoxicity. EPA does not consider these data gaps compelling enough to require application of a database UF.

The previous EPA evaluation did not derive an RfC.

5.3. CANCER ASSESSMENT

None of the bioassays by the oral route of exposure provide sufficient evidence that 1,1-DCE is a carcinogen. Accordingly, EPA did not derive an oral slope factor. This differs from EPA's previous evaluation (U.S. EPA, 1987), which relied on studies that did not show a statistically significant increase in tumor incidence attributable to oral exposure to 1,1-DCE.

One bioassay by the inhalation route of exposure showed suggestive evidence of carcinogenicity for humans. There is evidence suggesting that the tumor response in male mice is a sex- and species-specific response. While the previous EPA evaluation relied on these data, EPA does not currently believe that the suggestive evidence of a tumor response provides sufficient weight of evidence to justify deriving an inhalation unit risk.

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

6.1. HUMAN HAZARD POTENTIAL

1,1-DCE does not occur naturally. It is used mainly in the production of PVDC, which is used principally in food packaging. 1,1-DCE can be found in the environment from release during its manufacture and use, from the breakdown of products containing its polymers, and from breakdown of other chlorinated ethanes and ethenes.

1,1-DCE is rapidly absorbed following oral and inhalation exposure. It is rapidly oxidized by CYP2E1 to reactive intermediates that bind covalently with tissue macromolecules, or it can be conjugated with tissue GSH. The GSH status of the exposed animal is a major determinant in the expression of cellular toxicity. In addition, the presence of renal CYP2E1 and renal β-lyase activity seem to be major determinants in the expression of nephrotoxicity in mice. As there is evidence that human kidney does not contain CYP2E1, the kidney is unlikely to be a target tissue in humans.

There are no useful epidemiological studies or case reports in humans showing adverse health effects. The target organs for noncancer effects in laboratory animals are the liver, the kidney, and the Clara cells of the lung. A number of bioassays show that 1,1-DCE is a not carcinogen by the oral or dermal route of exposure. One bioassay in male mice shows suggestive evidence that 1,1-DCE is a carcinogen by the inhalation route of exposure. However, the weight of evidence is not sufficient to conclude that carcinogenesis is the critical effect by the inhalation route of exposure. No useful epidemiological studies or case reports exist that directly demonstrate a susceptible human population or increased susceptibility of children to the adverse effects of 1,1-DCE. Some data demonstrate gender specificity in mice to the increased incidence of renal adenocarcinomas, but no useful epidemiological studies or case reports in humans suggest gender specificity for any target tissue.

6.2. DOSE RESPONSE

The RfD of 0.05 mg/kg-day was calculated from the $BMDL_{10}$ for liver toxicity of 4.6 mg/kg-day in a chronic bioassay in rats using a total UF of 100 and an MF of 1. Individual UFs of 10 each were used for interspecies extrapolation and intraspecies variability.

The RfC of 0.2 mg/m^3 was calculated from the $BMCL_{HEC}$ for liver toxicity of 6.9 mg/m^3 in a chronic bioassay in rats using a total UF of 30 and an MF of 1. A UF of 3 was used for interspecies extrapolation, as a dosimetric adjustment was used. A UF of 10 was used for intraspecies variability.

Data showing equivocal carcinogenicity by the oral route of exposure are not sufficient to justify calculating an oral slope factor under the draft revised cancer guidelines (U.S. EPA, 1999). The suggestive data showing carcinogenicity by the inhalation route of exposure are not considered of sufficient weight to justify calculating an inhalation unit risk.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW COMMENTS AND DISPOSITION

At the Peer Review Workshop held on August 7, 2001, in Washington, DC, the Peer Review Panel addressed each of the General Questions and Chemical-Specific Questions in its charge. The questions and a summary of the Panel's responses follow. EPA also received scientific comments from the public. These comments are included in a separate section. EPA provides a response only if the recommendation differs significantly from what EPA included in the final assessment or if additional explanation was necessary.

Scientific Comments from the Peer Review Panel

General Question 1: Are you aware of any other data/studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of the adverse health effects, both cancer and noncancer, of this chemical?

The Panel was not aware of any other primary toxicity studies with 1,1-DCE that need to be considered. One panelist provided copies of recent mechanistic studies completed by the panelist's research group on the relationship between metabolism and toxicity in mice. A continuing theme throughout the comments from several panelists was the strong recommendation to emphasize the very-well-developed understanding of the mode of action of 1,1-DCE, including the mechanistic basis of tissue toxicity caused by 1,1-DCE metabolites in lung, liver, and kidney in rodents.

General Question 2: (a) For the RfD and the RfC, has the most appropriate critical effect been chosen (i.e., that adverse effect appearing first in a dose-response continuum)? (b) For the cancer assessment, are the tumors observed biologically significant? Are the tumors observed relevant to human health? Points relevant to this determination include whether the choice follows from the dose-response assessment, whether the effect is considered adverse, and whether the effect (including tumors observed in the cancer assessment) and the species in which it is observed is a valid model for humans.

The Panel agreed that the liver fatty changes in Quast et al. (1983, 1986) were the appropriate critical effects, although the Panel was divided on the question of whether these minimal, reversible fatty changes were adverse. Two panelists called the response adverse. Because these alterations appeared to have no impact on organ function or the health of the animals, other panelists believed that they should be regarded as adaptive rather than adverse changes.

In the 1986 IRIS documentation for 1,1-DCE, the exposure of 9 mg/kg-day from the oral study (Quast et al., 1983) was considered the LOAEL and was used as the point of departure for determining the RfD. The 2001 documents regard this same exposure from Quast et al. (1983) as a NOAEL and calculate a BMD ($BMDL_{10}$) as the point of departure for subsequent analysis. EPA should include a justification explaining why 9 mg/kg-day was previously considered a

LOAEL and is currently considered a NOAEL. At least one of the panelists was opposed to using BMD methods when good quality data were available to estimate NOAELs.

The Panel regarded the kidney tumors in Maltoni et al. (1985) as biologically significant, that is, they were directly related to the 1,1-DCE exposures and were increased in incidence significantly as compared to controls at the 25 ppm exposure concentration. However, this increased incidence was found only in a single study, it was found only at the highest concentration, and it was species-, strain- and gender-specific. The tumors might have relevance as indicators of potential carcinogenic responses for humans at very high exposures. However, the mode of action, including metabolism by an enzyme (CYP2E1) present in the mouse kidney at very much higher activity than in the human kidney, and the intrinsic nephrotoxicity of 1,1-DCE are not expected to lead to carcinogenic potential at much lower environmental exposures in humans. The enzyme required to bioactivate the S-(2,2-dichloro-1-hydroxy)ethylglutathione directly in kidney, that is, cysteine-beta-lyase, is also present at much lower activities in human compared to mouse kidneys. Thus, the high-exposure carcinogenic responses in mice were not considered a relevant model for human cancer risk at environmental exposures. Again, one panelist indicated that these differences in bioactivating enzyme activities between mice and humans were so large that 1,1-DCE should be not be regarded as a potential human carcinogen at all.

EPA has added wording to indicate that the fatty change might not be considered adverse, but it is being used to derive the RfD and RfC, as limiting exposure to this level will protect the liver from more serious damage that might compromise liver function.

EPA believes that BMD analysis has several advantages because it uses more of the experimental data from the study and allows comparison of different studies using the same incidence for the effect. For example, in Quast et al. (1983) the response at the lowest exposure is not statistically different from that of controls, but the response is still elevated (i.e., 25% vs. 13%) and is not much different from the response at the mid-exposure level (29%) that was statistically different from the control. Similarly the response for necrosis in male mice (NTP, 1982) at the highest exposure was 14%, but this change was not statistically significant (p=0.06) when a two-tailed test was used.

General Question 3: For the RfD and the RfC, have the appropriate studies been chosen as "principal"? The principal study should present the critical effect in the clearest dose-response relationship. If not, what other study (or studies) should be chosen and why?

The Panel unanimously agreed that Quast et al. (1983, 1986) were the appropriate studies for the RfC and RfD evaluations. The Panel also discussed the Dawson et al. (1993) developmental study, which suggested an increased incidence of cardiac malformations in neonatal rats after exposure of dams to 1,1-DCE in drinking water before mating and throughout gestation. This study was discussed both to assert why the Quast et al. (1983, 1986) studies were used and why the panel did not recommend use of the Dawson et al. (1993) developmental study as the principal study.

Although their reasons differed, the panelists unanimously believed that the Dawson et al. (1993) developmental toxicity study should not be considered as the principal study or considered to represent a potential developmental hazard from 1,1-DCE exposure. The reasons included concerns for the high positive responses on a litter basis in the controls, the lack of increased response between the two exposures that varied by 900-fold, and quality control issues identified in a 1996 Agency for Toxic Substances and Disease Registry review of other developmental toxicity studies with trichloroethylene (TCE) conducted by these investigators. Quality control issues, including lack of analytical confirmation of the concentrations in the drinking water in the TCE studies, were brought to the attention of the Panel by one panelist on the basis of his participation in an earlier review of these studies. Finally, other studies by Fisher et al., 2001 were cited as failing to replicate developmental cardiac changes with TCE.

Before the discussion of the deficiencies in the developmental toxicity drinking water studies, no panel member felt that Dawson et al. (1993) study should be used as the principal study. Interestingly, the panelists were against using the Dawson et al. (1993) study because it does not provide confidence that the effects were exposure-related and associated with DCE exposures, not because the changes were variations in cardiac morphology.

General Question 4: Studies included in the RfD and RfC under the heading "Supporting/Additional Studies" are meant to lend scientific justification for the designation of critical effect by including any relevant pathogenesis in humans, any applicable mechanistic information, and any evidence corroborative of the critical effect or to establish the comprehensiveness of the database with respect to various endpoints (such as reproductive/developmental toxicity studies). Should other studies be included under the "Supporting/Additional" category? Should some studies be removed?

In terms of supporting/additional studies, the Panel once more stressed the need to include (1) information on mode of action of 1,1-DCE regarding its metabolism to toxic intermediates, (2) the role of GSH in limiting the reactivity of the metabolites with tissue targets, and (3) the nephrotoxicity of 1,1-DCE as a precursor in development of the mouse tumors. The toxicity of 1,1-DCE increases markedly in fasted rats and in rats treated to deplete GSH stores. The protective role of GSH also means that responses at high exposures, where GSH is significantly depleted, cannot be extrapolated directly to lower exposure levels without considering the role of GSH in detoxifying metabolites before they react to initiate toxicity. Some early quantitative attempts were made to assess the protection afforded by GSH against liver toxicity in rats (Andersen et al., 1980). In general, the Panel noted that quantitative information related to pharmacokinetics and metabolism of 1,1-DCE has not been optimally utilized for evaluating the RfC and RfD for this compound.

One panelist suggested including in the IRIS Summary the Short et al. (1977c) paper on dominant lethality in male rats after vinyl chloride or vinylidene chloride exposures. The Toxicological Review includes this reference. Suggestions were also make to include specific references on susceptible populations and children's health issues—two concerns that need to be addressed in evaluating risks posed by 1,1-DCE to diverse human populations.

General Question 5: (a) Are there other data that should be considered in developing the UFs or the MF? (b) Do you consider that the data support use of different values than those proposed?

The Panel agreed that the UFs applied to derive the RfD were acceptable. EPA based the RfD on conventional risk assessment methods and, as a result, developed a conservative estimate of toxicity. EPA should include text about mode of action data and the rationale for the use of a BMD. The Panel agreed that the UFs applied to derive the RfC were acceptable. However, the panelists suggested that EPA justify the use of the default values in deriving the RfC and expand the discussions of mode-of-action data. One panelist also suggested that EPA include text describing how the RfC would ideally be derived if appropriate mode of action data were available. This panelist also suggested that EPA consider using the pharmacokinetic models developed for vinyl chloride or chloroform in the 1,1-DCE assessment. Language clarifying the text in Section 5.1.2 of the Toxicological Review was also necessary.

General Question 6: Do the confidence statements and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the principal study, the relevancy of the critical effect to humans, and the comprehensiveness of the database? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments? If not, what needs to be added?

The Panel agreed that the document was well organized and clearly stated the reasons for selecting the two major studies for the exposure-response analysis. The weight of evidence for carcinogenic potential was also clearly articulated. The rationale for using the factor of 10 for intra-individual differences, which would include both knowledge of metabolic parameters and the role of these parameters together with blood flow in controlling amount metabolized, needs to be strengthened. In addition, the decision for not using Dawson et al. (1993) as a critical study needs to be strengthened in relation to the quality and reproducibility of the study rather than questioning the nature of the changes as normal variation or potentially adverse alterations in structure. In regard to the carcinogenic response in the high-concentration male mice in the single positive study, both the mode-of-action discussion and the nephrotoxicity of 1,1-DCE deserve to be more heavily emphasized.

Chemical-Specific Question 1: Do you agree that the minimal hepatocellular swelling (Quast et al., 1983) is not an adverse response but that the minimal hepatocellular fatty change in the midzonal region (Quast et al., 1983, 1986) is adverse response?

The Panel unanimously agreed that the hepatocellular swelling is not an adverse response. The Panel also unanimously agreed that the fatty changes in the oral and inhalation studies should be used for the exposure-response assessment, although the Panel was not unanimous in calling these changes adverse. Several panelists believed that these changes are transient adaptive responses that clarify upon cessation of exposure. However, the Panel did not believe that calling the responses adverse was imperative for using them for the exposure-response analysis.

EPA has added wording to indicate that the fatty change might not be considered adverse, but it is being used to derive the RfD and RfC, as limiting exposure to this level will protect the liver from more serious damage that might compromise liver function.

Chemical-Specific Question 2: Do you agree that the cardiac changes (Dawson et al., 1993) are properly characterized as variations in cardiac morphology and should not be considered adverse effects?

The Panel's determination that the Dawson et al. (1993) study was unusable was not because the changes were acceptable variations in cardiac morphology. Instead, the unanimous opinion of the Panel was that the study does not provide confidence that the effects were exposure-related and causally associated with DCE exposures. The Panel did not formally address the question of whether these valvular and septal changes should be regarded as simply variations in cardiac morphology. However, several panelists stated that these changes would be considered adverse and suitable for a exposure-response assessment if they were actually related to 1,1-DCE exposures. Due to the concerns with the study noted in General Question 3, the Panel unanimously believed that this study should not be used for exposure-response assessment and believed that there was no convincing evidence that these changes were actually related to 1,1-DCE in the drinking water.

Chemical-Specific Question 3: Is the weight of evidence for cancer from both oral and inhalation exposure assigned at the appropriate level?

The Panel agreed that the weight of evidence assigned for oral exposures is appropriate, that is, the available data do not indicate any cancer risks via this route of administration. For inhalation exposures, the weight of evidence under the new cancer guidelines (U.S. EPA, 1999) should be used. The Panel felt that the renal tumors observed in the Maltoni et al. (1985) study are biologically significant, that is, tumor incidence is causally related to exposure to 1,1-DCE. However, the renal tumors were observed only at the highest exposure level of 25 ppm in male mice. Such tumors were not observed in any other study regardless of the species, strain, exposure, or exposure route. On the basis of published studies on the lack of activity of CYP2E1 in human kidneys and the much lower activity of beta-lyase in human kidney, the Panel felt that the renal tumors observed in the mouse study are of questionable relevance to humans exposed at environmental levels. Further, the Panel agreed that 1,1-DCE is likely to be carcinogenic only at exposures at which GSH is depleted and cytotoxicity is expressed. One panelist believed that 1,1-DCE should be regarded as not likely to be carcinogenic to humans. Without dissent, the Panel unanimously agreed that derivation of an inhalation unit risk (IUR) from the renal tumor incidence in mice was inappropriate.

The Panel recommended that EPA include the following narrative (from the 1999 draft EPA guidance document for cancer risk assessment) for 1,1-DCE cancer risk assessment:

> Suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential: This descriptor is applied when carcinogenicity data are

suggestive but inconclusive. For example, this descriptor would be applicable in situations where increased tumor incidence is marginal or is observed only in a single study. According to EPA guidelines, a cancer dose-response assessment is not indicated for chemicals with this descriptor.

On the basis of the above narrative, neither a quantitative cancer assessment nor an IUR derivation is warranted for 1,1-DCE.

Chemical-Specific Question 4: Do you agree that an inhalation unit risk should not be derived from the data on kidney adenocarcinomas in Swiss mice (Maltoni et al., 1985)?

The Panel agreed unanimously that it was inappropriate to use the Maltoni et al. (1985) study with the high-exposure-level increased incidence of adenocarcinoma in male mice to derive an IUR. However, differences of opinion existed about whether the cancer endpoint should be evaluated using a margin-of-exposure approach from the proposed revisions to the EPA carcinogen risk assessment guidelines.

The Panel noted in Chemical-Specific Question 3 that the 1999 EPA guidelines would not pursue a cancer risk assessment for the descriptor: Suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential.

However, additional discussions among the panelists led to the question of the benefit of conducting an nonlinear cancer risk assessment for 1,1-DCE using a margin-of-exposure approach with appropriate UFs. Three panelists felt that, based on the in-depth knowledge of the mode of action, the weight of evidence for 1,1-DCE carcinogenicity, and the toxicity of 1,1-DCE to mouse kidneys, the use of a margin-of-exposure approach for 1,1-DCE is not warranted at this time. However, two panelists felt that the Swiss mice data on renal tumor incidence should be analyzed according to the margin-of-exposure approach, as available quantitative data on 1,1-DCE metabolism (the relative enzyme levels and GSH levels) in Swiss mice and human kidneys are not sufficient to ignore the concern of possible renal tumor incidence in humans at high exposures. One panelist felt that a margin-of-exposure cancer risk approach could be used, although the UFs applied should be no larger than those recommended for the RfC derivation with the critical effects in the liver.

EPA does not believe that a margin-of-exposure approach for a cancer risk assessment for 1,1-DCE is warranted.

Additional Comments

The Panel also provided other comments to improve the scientific quality of the document. The Panel emphasized adding materials on susceptibility, interactions with other compounds, specific risks to children and neonates, and influences of lifestyle such as smoking in altering susceptibility or risk. The Panel strongly suggested a revision of Figure 1 and careful development of mode-of-action arguments in the text. The Panel provided a suggested revision

to Figure 1 and a rewrite of the mode-of-action section based on current knowledge of the toxicology of 1,1-DCE.

Scientific Comments from the Public

One public commentor wanted EPA to include additional references to LC_{50} studies.

EPA has incorporated several references to LC_{50} studies. Several references to LC_{50} studies listed by the commentor were not included, as they are unpublished reports from BASF and are not available to EPA.

One public commentor thought that the RfC should be lower than the value selected by EPA because 1,1-DCE shows acute and developmental toxicity in the 10–25 ppm range, only slightly higher than the $BMCL_{10}$ calculated from the results of Quast et al. (1986). The commentor was concerned that EPA had not used the most sensitive studies and endpoints.

EPA has reevaluated the results of the studies cited. The acute study by Reitz et al. (1980) shows evidence of slight damage to the kidney of mice at 10 ppm following a single 6-hour exposure. The exposure is equivalent to a continuous exposure of 2.5 ppm. The developmental study of Short et al. (1977b) in mice shows effects at 15 ppm, the only exposure not showing severe maternal toxicity. Exposure in this study was for 22-23 hours/day on GDs 6 to 16. The effects in these studies occurred at an exposure higher than the 1.8 ppm calculated continuous exposure in Quast et al. (1986) and are not used to derive the RfC. The long-term study by Maltoni et al. (1985) in male mice showed effects in the kidney at 10 ppm. Exposure in this study was for 4 hrs/day, 4.5 days/wk, for 52 weeks, which is equivalent to a continuous exposure of 1.1 ppm. Animals were then held without exposure until spontaneous death (total duration 126 weeks). Because there was no investigation of the effects at the termination of the 1-year exposure, this study cannot be used to derive the RfC. EPA has used the default interspecies UF of 3 because there is some concern that the most sensitive species was not used to derive the RfC.

One public commentor, although concurring with EPA's decision not to use the cardiac changes observed by Dawson et al., (1993) to derive the RfD, wanted a more extensive discussion of the uncertainty raised by this observation. The commentor suggested that this uncertainty does not support EPA's decision to assign the database to the "medium" confidence category in Section I.A.5 of the IRIS summary. The commentor was concerned about the reports of cardiac abnormalities associated with exposure to chlorinated solvents from drinking water in human epidemiological studies. The commentor was also concerned that, because there is no test of cardiac function in stressed animals, the functional consequences of the morphological changes might not be observable in the developmental studies that have been conducted. The commentor cited the results of Siletchnik and Carlson (1974) on cardiac sensitization in support of that latter concern.

Public commentors were concerned about EPA's withdrawal of the IUR for 1,1-DCE. These commentors wanted a stronger justification for EPA's position that the IUR should not be derived from Maltoni et al. (1985) and a more extensive discussion of the uncertainties and data gaps. One commentor expressed the view that a full discussion of the uncertainties and data gaps should lead EPA to a different conclusion on the need for providing an IUR in this assessment. The commentors advanced a number of scientific reasons for their concern about the lack of an IUR in this assessment. The most important reasons included (1) the poor quality of the bioassays on 1,1-DCE and the suspicious results in some of these bioassays; (2) the structural correlation between 1,1-DCE and vinyl chloride, the fact that vinyl chloride seems to express its full carcinogenic potential from short-term exposure to young animals, and the lack of comparable testing on 1,1-DCE; (3) limitations in the range of metabolites investigated by Speerschneider and Dekant (1995) that argue against using this study as the primary rationale for not quantifying the cancer risk; (4) the lack of detailed knowledge about the metabolism of 1,1-DCE by humans; (5) the possibility that CYP isoforms other than CYP2E1 could activate 1,1-DCE in humans; and (6) EPA's unstated assumption of concordance in tumor site between mice and humans.

EPA has added additional discussion of the uncertainties in the cancer assessment to Section 4.6 of the Toxicological Review. However, EPA does not believe it is appropriate to increase the weight of evidence in the cancer assessment based on uncertainty. With regard to the testing of 1,1-DCE for carcinogenicity in immature animals, the only relevant bioassay was reported by Cotti et al. (1988). This bioassay exposed pregnant Sprague-Dawley rats from GD 12 to parturition and the subsequent offspring for 13 or 104 weeks. The results of this bioassay provide no convincing evidence that 1,1-DCE is carcinogenic.

Additional References

EPA added the following references to the toxicological review: Blanco et al. (1999); Clewell et al. (1995a); Costa and Ivanetich (1984); Cresteil (1998); El-Masri et al. (1996a); El-Masri et al. (1996b); Fisher et al. (2001); Forkert (1995); Forkert (1999a); Forkert et al. (1986); Forkert et al. (1991); Jaeger et al. (1973); Jarabek et al. (1994); Liebler et al. (1985); Liebler et al. (1988); Short et al. (1977a); Short et al. (1977c); Williams et al. (1996); and Wright and Moore (1991).

APPENDIX B. BENCHMARK DOSE (BMD) ANALYSIS

B.1. ORAL

Data on fatty change in the liver from Quast et al. (1983) were analyzed using EPA's BMD software. Each of the seven models gave an adequate fit (p>0.2). The gamma, logistic, multistage, quanta-linear, and Waybill models showed the best visual fit to the data points. The gamma, multistage, quanta-linear, and Waybill models showed identical Acacia's Information Criterion (AID) values and identical BMD and BMDLs. The results from the gamma model are presented.

The form of the probability function is:

P[response] = background+(1-background)*CumGamma[slope*dose,power]

where CumGamma(.) is the cumulative Gamma distribution function

Default initial (and specified) parameter values:

Background = 0.12963
Slope = 0.0192007
Power = 1.12817

Asymptotic correlation matrix of parameter estimates:

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

	Background	Slope
Background	1	-0.54
Slope	-0.54	1

Parameter estimates:

Variable	Estimate	SE
Background	0.125627	0.0350171
Slope	0.0158781	0.00405428
Power	1	NA

NA indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of deviance table:

Model	Log(likelihood)	Deviance Test	DF	*p*-value
Full model	-119.212			
Fitted model	-119.229	0.0326243	2	0.9838
Reduced model	-128.113	17.8011	3	0.0004834

AIC = 242.458

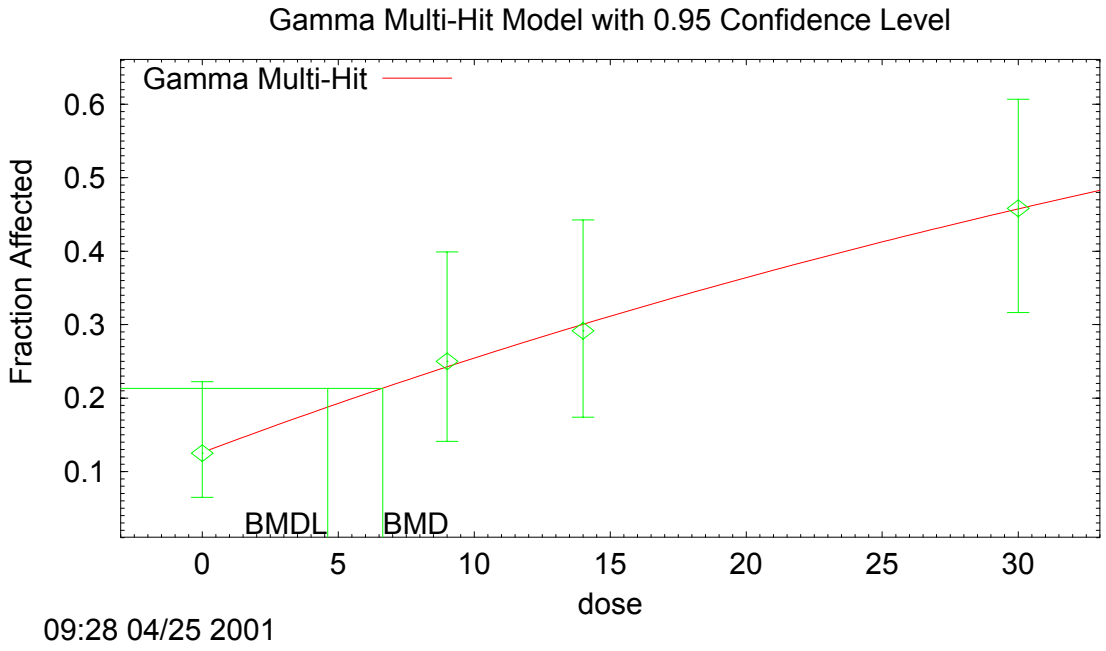
Goodness of Fit:

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0.1256	10.050	10	80	-0.01693
9	0.2421	11.619	12	48	0.1284
14	0.2999	14.396	14	48	-0.1246
30	0.4570	21.935	22	48	0.01895

Chi-square = 0.03 DF = 2 *p*-value = 0.9838

BMD computation:

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 6.63557
BMDL = 4.61215



B.2 INHALATION

Data on fatty change in the liver from Quast et al. (1986) were analyzed using EPA's BMD software. The gamma, multistage, and quantal-linear models gave an adequate fit (p>0.2). These models also gave an adequate visual fit to the data points. The quantal-linear model gave the lowest AIC value. The results from this model are presented.

The form of the probability function is:

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

Default initial (and specified) parameter values:

Background = 0.0294118
Slope = 0.00549306
Power = 1 Specified

Asymptotic correlation matrix of parameter estimates:

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

Slope = 1

Parameter estimates:

Variable	Estimate	SE
Background	0	NA
Slope	0.00697979	0.00194885

NA indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of deviance table:

Model	Log(likelihood)	Deviance Test	DF	p-value
Full model	-27.7336			
Fitted model	-28.0929	0.718624	2	0.6982
Reduced model	-32.5262	9.58514	2	0.008291

AIC = 58.1858

Goodness of Fit:

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0		0.0000	0.000	0	16 0
25	0.1601	4.643	6	29	0.6869
75	0.4075	8.151	7	20	-0.5237

Chi-square = 0.75 DF = 2 *p*-value = 0.6886

BMD computation:

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMC = 15.0951
BMCL = 9.84365