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TOXICOLOGICAL REVIEW

OF

cis-1,2-DICHLOROETHYLENE
and
trans-1,2-DICHLOROETHYLENE

(CAS Nos. cis: 156-59-2; trans: 156-60-5; mixture: 540-59-0)

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

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NOTICE

U.S. Environmental Protection Agency
Washington, DC

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LIST OF ACRONYMS

AAP	4-aminoantipyrine
ACGIH	American Conference of Governmental Industrial Hygienists
ADH	alcohol dehydrogenase
AFC	antibody-forming cell
AH	aniline hydroxylation
AIC	Akaike Information Criteria
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substance and Disease Registry
BMD	benchmark dose
BMR	benchmark response
BUN	blood urea nitrogen
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CI	confidence interval
CNS	central nervous system
Con A	concanavalin A
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DCA	dichloroacetic acid
DCE	dichloroethylene
DTH	delayed-type hypersensitivity
EC_{10}	concentration causing 10% change in effect
ED_{50}	median effective dose
EN-D	ethylmorphine N-demethylation
EPA	Environmental Protection Agency
G-6-Pase	glucose-6-phosphatase
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
GD	gestation day
GSH	reduced glutathione
GST	glutathione S-transferase
GSTZ	glutathione S-transferase zeta
HEC	human equivalent concentration
ID_{50}	concentration to achieve 50% decrease in immobility
i.p.	intraperitoneal or intraperitoneally
IRIS	Integrated Risk Information System
KM	Michaelis constant
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
LPS	lipopolysaccharide
MTD	maximum tolerated dose
NOAEL	no-observed-adverse-effect level

NLM	National Library of Medicine
POD	point of departure
PBTK	physiologically based toxicokinetic
PSP	phenolsulfonephthalein
QSAR	quantitative structure-activity relationship
RAM	rate of metabolism
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RVMT	rate of change of inhibitable metabolism
S9	supernatant fraction
SDH	sorbitol dehydrogenase
SGOT	glutamate oxaloacetate transaminase (now called AST)
SGPT	glutamate pyruvate transaminase (now called ALT)
sRBC	sheep red blood cell
TBARS	thiobarbituric acid-reactive substances
TLV	threshold limit value
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency
V_{max}	maximum substrate turnover velocity
VOC	volatile organic compound

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 cis- and trans-1,2-dichloroethylene. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of cis- and trans-1,2-dichloroethylene.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response,* is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing, the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

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

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Audrey Galizia, Dr. PH
Office of Research and Development
U.S. Environmental Protection Agency
Edison, NJ

AUTHORS

Audrey Galizia, Dr. PH
Office of Research and Development
U.S. Environmental Protection Agency
Edison, NJ

D. Charles Thompson, R.Ph., Ph.D., DABT
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

CONTRACTOR SUPPORT

C. Clifford Conaway, Ph.D., DABT
Consulting Toxicologist
Mahopac, NY

Janusz Z. Byczkowski, Ph.D., DABT
Toxicology Consultant
Fairborn, OH

Susan Goldhaber, M.S.
Toxicology Consultant
Raleigh, NC

George Holdsworth, Ph.D.
Oak Ridge Institute for Science and Education
Oak Ridge, TN

REVIEWERS

This document has been reviewed by EPA scientists and interagency reviewers from other federal agencies.

INTERNAL EPA REVIEWERS

Andrew Rooney, Ph.D.
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

Channa Keshava, Ph.D.
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

Allan Marcus, Ph.D.
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

Karen Hogan, M.S.
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Lynn Flowers, Ph.D., DABT
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summaries of the hazard and dose-response assessments of cis- and trans-1,2-dichloroethylene (cis- and trans-1,2-DCE). Toxicological assessment of mixtures of cis- and trans- 1,2-DCE is beyond the scope of this document. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute ($\leq$24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. 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 may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a plausible inhalation unit risk is an upper bound on the estimate of risk per μg/m^3 air breathed.

Development of these hazard identification and dose-response assessments for cis- and trans-1,2-DCE has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk*

Assessment (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for these compounds was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through January 2009.

2. CHEMICAL AND PHYSICAL INFORMATION

There are two isomers of 1,2-DCE, the cis-isomer and the trans-isomer. The cis-isomer is configured with the chlorine atoms on the same side of the C=C double bond, while in the trans-isomer they are on opposite sides, resulting in different physical, chemical, and biological properties (Figure 2-1). The trans-isomer is more widely used in industry than either the cis-isomer or the commercial mixture, which typically consists of a 60/40% ratio of the cis-isomer and trans-isomer, respectively (American Conference of Governmental Industrial Hygienists [ACGIH], 2001). 1,2-DCE has been used as a solvent for waxes, resins, and acetylcellulose, in the extraction of rubber, and as a coolant in refrigeration plants (National Library of Medicine [NLM], 2006).

Figure 2-1. Chemical structures of cis- and trans-1,2-dichloroethylene.

Some relevant chemical and physical properties of cis-1,2-DCE, trans-1,2-DCE, and a mixture of both isomers are listed in Table 2-1 (NLM, 2006; Agency for Toxic Substance and Disease Registry [ATSDR], 1996). Exposure to the 1,2-DCEs may occur after the chemicals are released to the environment from industrial emissions, leaching from landfills, or evaporation from wastewater streams. The estimated half-lives of cis- and trans-1,2-DCE in air are 12 and 5 days, respectively. Volatilization is the major fate process when the chemicals are released to surface water, with an estimated half-life of about 3–6 hours. In soil, 1,2-DCE may leach through the subsurface and contaminate groundwater. The chemical may also be found in groundwater due to anaerobic degradation of more highly chlorinated chemicals, such as trichloroethylene and tetrachloroethylene (ATSDR, 1996). Although no degradation occurs in sterile microcosms, anaerobic biodegradation of the cis-isomer to chloroethane and vinyl chloride and biodegradation of the trans-isomer to vinyl chloride alone have been reported (Barrio-Lage et al., 1986, as reported in ATSDR, 1996). The cis-isomer is degraded more readily than the trans-isomer (Barrio-Lage et al., 1986, as reported in ATSDR, 1996). The rates

of degradation of both isomers are dependent on the availability of an electron donor and the presence of active anaerobes.

Table 2-1. Properties of the 1,2-dichloroethylene isomers and their mixture

Descriptor	cis-Isomer	trans-Isomer	Mixture
CAS name	cis-1,2-Dichloroethylene	trans-1,2-Dichloroethylene	1,2-Dichloroethylene
CAS number	156-59-2	156-60-5	540-59-0
Primary synonyms	cis-1,2-Dichloroethene, 1,2-cis-dichloroethylene, cis-acetylene dichloride, cis-1,2-DCE	trans-1,2-Dichloroethene, 1,2-trans-dichloroethylene, trans-acetylene dichloride, trans-1,2-DCE	1,2-Dichloroethene, acetylene dichloride, 1,2-DCE
Chemical formula	$C_2H_2Cl_2$		
Molecular weight	96.95		
Boiling point	60.1°C at 760 mm Hg	48.7°C at 760 mm Hg	Approximately 55°C
Melting point	–80°C	–49.8°C	–50°C
Specific gravity	1.2837 @ 20°C/4°C	1.2565 @ 20°C/4°C	Approximately 1.28
Vapor pressure	2.00×10^2 mm Hg @ 25°C	3.31×10^2 mm Hg @ 25°C	2.01×10^2 mm Hg @ 25°C
Solubility	Miscible with alcohol, ether, acetone, benzene, chloroform; solubility in water = 6.41 g/L @ 25°C	Miscible with alcohol, ether, acetone, benzene, chloroform; solubility in water = 4.52 g/L @ 25°C	Miscible with alcohol, ether, acetone, benzene, chloroform; solubility in water = 3.5 g/L @ 25°C
Odor	Ethereal, slightly acrid, sweet, pleasant		
Odor threshold (air)	NA[a]	0.085 ppm	NA[a]
Partition coefficients: Log K_{ow} Log K_{oc}	1.86 1.69 (estimated)	2.06 1.56 (estimated)	2.00 NA
Henry's law constant	4.08×10^{-3} atm-m^3/mol @ 24°C	9.28×10^{-3} atm-m^3/mol @ 24°C	4.08×10^{-3} atm-m^3/mol @ 25°C
Flash point	2–4°C	2°C	2°C
Conversion factor	1 mg/m^3 = 0.252 ppm; 1 ppm = 3.97 mg/m^3		

[a]NA = not available.

Sources: NLM (2006); ATSDR (1996).

The 1,2-DCEs are highly flammable; the vapors may explode when heated or exposed to an open flame. Combustion by-products of 1,2-DCE include hydrogen chloride and phosgene (NLM, 2006).

3. TOXICOKINETICS

3.1. ABSORPTION

3.1.1. Oral

No studies were identified that examined oral absorption of either cis- or trans-1,2-DCE.

3.1.2. Inhalation

Considerable work has been done in this area to determine parameters applicable to physiologically based toxicokinetic (PBTK) modeling. Filser and Bolt (1979) studied the uptake of cis- and trans-1,2-DCE in male Wistar rats (250 g). The animals were exposed to various initial concentrations of the substances in a closed chamber, and the decline of the substance with time was monitored by gas-liquid chromatography. The authors did not report initial airborne concentrations, but, judging from time zero in their graphs (Filser and Bolt, 1979), they ranged from about 20 to 1,000 ppm. Plots by the authors of chamber concentration vs. time displayed two or three phases, depending on the initial concentration. A first phase of rapid decline of gas concentration in the chamber represented the initial uptake of gas and its equilibration with the chamber atmosphere that lasted about 2 hours for cis-1,2-DCE and 1.5 hours for trans-1,2-DCE. The second phase was typical of a first-order metabolic disappearance of the substance when initial gas concentrations were sufficiently low but took on the characteristics of zero-order elimination with high gas concentrations, saturating the metabolic capacity of the animals in the chamber. A third phase was seen in cases with high initial gas concentration, where, with time, the concentration in the chamber fell low enough to no longer saturate the metabolic enzymes, displaying first-order disappearance characteristics from there on.

Filser and Bolt (1979) analyzed the disappearance curves mathematically and established saturation points for both cis- and trans-1,2-DCE (i.e., gas concentrations at which the metabolic capacities of the experimental animals became saturated and gas disappearance from the chamber changed from first to zero order). These values were given as 20 ppm for cis-1,2-DCE and 15 ppm for trans-1,2-DCE. The shorter equilibration time and lower saturation point concentration for trans-1,2-DCE were interpreted by the authors to indicate slower metabolic removal of trans-1,2-DCE, as compared with cis-1,2-DCE.

In vitro gas/blood distribution data indicate that trans-1,2-DCE is less soluble in blood than cis-1,2-DCE, which would suggest that inhalation uptake of trans-1,2-DCE is less than that of cis-1,2-DCE. Eger et al. (2001) conducted experiments with rats and found that the alveolar concentration of trans-1,2-DCE required to induce anesthesia in 50% of the animals was about twice as high as that of cis-1,2-DCE. Gargas et al. (1989, 1988) published blood:air partition coefficients of 21.6 and 9.58 in the rat and 9.85 and 6.04 in humans, for cis- and trans-1,2-DCE, respectively. Sato and Nakajima (1987) also reported values of 9.2 and 5.8 for cis- and

trans-1,2-DCE, respectively, but the species in which these values were obtained was not specified. A comparison of values given by Gargas et al. (1989) for humans, rats, and, to a lesser extent, mice indicated that, for most chlorinated aliphatics examined, human blood had only about one-half the affinity of that measured in rat and mouse blood. Equilibrium constants for inhalation uptake over exhalation elimination calculated by Filser and Bolt (1979) showed the same approximate 2:1 ratio (i.e., 20 and 11.5 for cis- and trans-1,2-DCE, respectively). Therefore, several studies support the conclusion that cis- and trans-DCE are absorbed relatively quickly by the lungs and in a ratio of 2:1.

Andersen et al. (1980) used male F344 rats (180–280 g) to conduct inhalation experiments with trans-1,2-DCE, using a closed chamber system with gas recirculation. The results were similar to those obtained by Filser and Bolt (1979) in that the uptake of trans-1,2-DCE leveled off after about 2 hours, with about 40–60% of the gas remaining in the chamber at exposure concentrations of 10,000, 1,000, and 30 ppm. Using a model developed earlier (Ramsey and Andersen, 1984), Gargas et al. (1988) calculated the maximum substrate turnover velocity (V_{max}) values for pulmonary uptake of both cis- and trans-1,2-DCE of 30.9 µmol/kg-hour (3 mg/kg-hour) for rats (likely this value is true only for trans-1,2-DCE, as estimated by Andersen et al. [1980]). Both Andersen et al. (1980) and Filser and Bolt (1979) pointed out that results obtained in a given rat strain could not be extrapolated to another strain. In addition, Gargas et al. (1990) pointed out that the uptake of gaseous cis- or trans-1,2-DCE could be approximated only by using a model that corrected for suicide inhibition of the cytochrome P450 (CYP450) enzymes that metabolize these agents.

In an experiment using isolated perfused liver from female Wistar rats and exposing the perfusate to cis- or trans-1,2-DCE in the gas phase, Bonse et al. (1975) found that, at a given concentration in the gas phase, trans-1,2-DCE attained less than one-half the concentration of cis-1,2-DCE in liver, which was attributed in part to inhibition of CYP450 by the trans-isomer.

3.1.3. Dermal

No studies were located that investigated the dermal uptake of cis- or trans-1,2-DCE either as a liquid or from the vapor phase. Pleil and Lindstrom (1997) conducted experiments with human volunteers who were exposed to cis-1,2-DCE via showering with contaminated water (informed consent from the volunteers and institutional approval were obtained). Appearance of the substance in exhaled air, collected as single breaths of 1 L volume, was monitored by gas chromatography/mass spectrometry (GC/MS). Samples of microenvironmental air from the exposure area and control samples of inspired air after the exposure were also collected and analyzed using the same equipment. In two separate experiments, two volunteers were exposed under a shower for 10 minutes each to an environment with 125 and 83.9 µg/m^3 cis-1,2-DCE in the air and 28.4 and 20.4 µg/L in the water, respectively. Samples of exhaled air and blood were collected for 30 minutes after

exposure. Samples of air from the exposure area and control samples of inspired air after the exposure were also collected and analyzed. The authors calculated total exposure doses of 1.19 and 2.34 µg, while the corresponding maximum blood concentrations were 0.25 and 0.18 µg/L, respectively. The authors considered these values as indicative of efficient absorption of cis-1,2-DCE with mixed inhalation and dermal exposure.

The interim report *Dermal Exposure Assessment* (U.S. EPA, 1992) gives a dermal permeability coefficient, K_p, of 1.0×10^{-2} cm/hour for human skin. This value references uptake from aqueous solution, but there is uncertainty in whether it refers to cis-1,2-DCE, trans-1,2-DCE, or mixed isomers. By using a formula for dermal absorption of liquids proposed by Potts and Guy (1992):

$$\log K_p = -2.7 + 0.71 \times \log K_{ow} - (0.0061 \times \text{molecular weight})$$

K_p values of 1.07×10^{-2} and 1.55×10^{-2} cm/hour are obtained for cis- and trans-1,2-DCE, respectively. Such values indicate efficient dermal absorption, comparable to that of lipophilic aromatics, such as cresols, chlorophenols, or hexanol (U.S. EPA, 1992). These values also suggest that the higher lipophilicity of trans-1,2-DCE may affect its dermal absorption.

3.2. DISTRIBUTION

No in vivo studies pertaining to organ and/or tissue distribution of cis- or trans-1,2-DCE have been reported in the literature. However, Bonse et al. (1975) reported that in an experiment using isolated perfused liver from female Wistar rats, at equimolar concentrations in the perfusate, uptake for cis-1,2-DCE was about 3 times faster than for trans-1,2-DCE. Gargas et al. (1988) reported tissue:air partition coefficients (at 37°C) for rat tissues in vitro that are compiled in Table 3-1. These data provide further support, albeit indirectly, that the trans-isomer is likely to be taken up less efficiently by mammalian tissues than the cis-isomer. Furthermore, if the previously discussed relationship between rat and human blood:air partition coefficients is assumed to be predictive, then the extent of uptake of the two isomers into human tissues would be roughly half that of the corresponding rat tissues shown below (Table 3-1).

Table 3-1. Tissue:air partition coefficients of the 1,2-dichloroethylene isomers in the rat

Tissue	Partition coefficient	
	cis-1,2-Dichloroethylene	trans-1,2-Dichloroethylene
Blood	21.6	9.58
Liver	15.3	8.96
Muscle	6.09	3.52
Fat	227	148

Source: Gargas et al. (1988).

3.3. METABOLISM

Henschler and Bonse (1977) proposed a metabolic scheme for cis- and trans-1,2-DCE, shown in Figure 3-1. Metabolism of 1,2-DCE is initially catalyzed by hepatic CYP450, and limited experimental evidence suggests that CYP2E1 may be the primary pathway for metabolism of cis- or trans-1,2-DCE in the rat (Costa and Ivanetich, 1984, 1982). Studies suggest that the metabolism of 1,2-DCE involves epoxidation of the ethylene double-bond-forming dichlorinated epoxides, which can undergo a nonenzymatic rearrangement (Costa and Ivanetich, 1984, 1982) and produce several metabolites. Following rearrangement of the intermediate epoxide, reduction of the resulting dichloroacetaldehyde to dichloroethanol may be catalyzed by alcohol dehydrogenase (ADH). Studies by Costa and Ivanetich (1984, 1982) provide evidence that dichloroacetaldehyde is the predominant metabolite of CYP450, which is extensively converted to dichloroethanol and dichloroacetate by dehydrogenases present in hepatocytes. Oxidative dechlorination of the minor metabolite, dichloroacetic acid (DCA) to glyoxylate is catalyzed by glutathione S-transferase zeta (GSTZ) (Costa and Ivanetich, 1982). The enzymes involved in further biotransformation of 1,2-DCE metabolites have not been characterized.

Sources: Adapted from U.S. EPA, 2003; Henschler and Bonse, 1977.

Figure 3-1. Proposed metabolic scheme for cis- and trans-1,2-dichloroethylene.

3.3.1. Metabolism in Animals

Bonse et al. (1975) studied the metabolism of several chlorinated C_2-compounds, among them cis- and trans-1,2-DCE, in isolated perfused livers from female Wistar rats (170–230 g). The perfusate was supplemented with various concentrations of the compounds in the gas phase.

Concentrations of 1,2-DCE and metabolites in liver tissue and perfusate were monitored with gas chromatography (GC). Bonse et al. (1975) expected to find epoxide isomers (oxiranes) as the primary metabolites of 1,2-DCE. Both cis- and trans-epoxides are unstable, however, and were shown to rearrange spontaneously to form dichloroacetaldehyde, which was then readily converted to DCA and 2,2-dichloroethanol. Levels of enzymes from liver cells in the perfusate (i.e., lactate dehydrogenase [LDH], aspartate aminotransferase [AST] (glutamate oxaloacetate transaminase [SGOT], or alanine aminotransferase [ALT] (glutamate pyruvate transaminase [SGPT]) increased with time. The authors interpreted these findings to be indicative of liver damage. Higher activity levels of these enzymes were detected in the cis-1,2-DCE perfusate compared with corresponding activities in the trans-1,2-DCE perfusate.

For cis-1,2-DCE, Bonse et al. (1975) identified 2,2-dichloroethanol as the major metabolite and DCA as a minor metabolite; for trans-1,2-DCE, only small amounts of these two metabolites could be identified. Uptake of cis-1,2-DCE in liver tissue was demonstrated to be at least 2 times faster than the uptake of trans-1,2-DCE, which may partially account for the lower concentrations of metabolites of trans-1,2-DCE in liver tissue. The authors also observed that the amount of metabolites in liver tissue did not correlate with tissue uptake for these two substances, thus confirming differing rates of metabolism as well. The authors further observed in this study that metabolic transformation increased with the number of chlorine substituents.

Leibman and Ortiz (1977) studied the metabolism of the 1,2-DCE isomers in rat liver homogenate supernatants (9000×g supernatant fraction [S9]) and suggested metabolic schemes for the chlorinated ethylenes. For 1,2-DCE (isomer not specified), they proposed the same sequence of events that Bonse et al. (1975) and Henschler and Bonse (1977) had proposed, although Leibman and Ortiz (1977) were not able to experimentally identify DCA as a metabolite of 1,2-DCE. They were, however, able to mechanistically describe the chemical rearrangement via an epoxide intermediate that explains the formation of asymmetrically substituted DCA from both symmetrically substituted 1,2-DCE isomers.

Costa and Ivanetich (1982) investigated the metabolism of the chlorinated ethylenes ex vivo, using the S9 fraction from the livers of male Long-Evans rats. Some of the rats were pretreated with enzyme inducers, such as β-naphthoflavone or phenobarbital, prior to sacrifice and microsome preparation. The DCEs were added as ethanolic solutions to the microsomal preparations. Metabolite identification was performed by gas/liquid chromatography. Following treatment with both cis- and trans-1,2-DCE, measurable amounts of 2,2-dichloroethanol and dichloroacetaldehyde were detected, with trans-1,2-DCE yielding about 25% the amount of 2,2-dichloroethanol that cis-1,2-DCE yielded. DCA was also formed from both substances, although the amount was about 6 times less from trans-1,2-DCE than from cis-1,2-DCE. The authors could not identify any of the dechlorination metabolites of cis- or trans-1,2-DCE shown in the metabolic scheme in Figure 3-1, possibly because the S9 mix used did not contain considerable glutathione S-transferase (GST) activity. Overall, the authors estimated the in vitro

CYP450-mediated metabolism of cis-1,2-DCE to be 4 times that of trans-1,2-DCE. Suicide inhibition of the CYP450 activity via covalent binding of a reactive intermediate to the heme moiety was also observed; this propensity to bind heme was independent of the enzymatic degradation of any DCE substrate. However, it was noted that substances whose epoxides rearranged to an aldehyde (e.g., cis- or trans-1,2-DCE) bound to heme, while those that formed acylchlorides (e.g., 1,1-DCE) did not bind to heme.

Costa and Ivanetich (1982) also found that metabolite binding to hepatic microsomes induced a spectral shift indicative of binding to the active center of CYP450. Hanes plots of substance concentration vs. spectral shift revealed two binding constants, suggesting that more than one CYP450 isoform in the microsomes was involved. Pretreatment of the animals with phenobarbital increased the affinity of the substrate for the low affinity binding site but did not affect that of the high affinity binding site. Biphasic Hanes plots were observed with cis-1,2-DCE when either phenobarbital-noninduced or -induced liver microsomes were used; with trans-1,2-DCE, the plots were monophasic unless phenobarbital-induced microsomes were used. Treatment with the nonspecific inhibitors carbon monoxide and SKF-525A suppressed the formation of dichloroacetaldehyde or 2,2-dichloroethanol from both cis- or trans-1,2-DCE. However, while metyrapone, a specific CYP3A4 inhibitor, was minimally effective in inhibiting metabolism of cis-1,2-DCE, it was most effective in suppressing trans-1,2-DCE metabolism. Accordingly, pretreatment with phenobarbital, which induces CYP3A4, among others, increased the metabolism of trans-1,2-DCE more than cis-1,2-DCE. Therefore, CYP3A4 may play a role in the metabolism of 1,2-DCE, but the exact nature and extent of this role need to be further characterized. These researchers also conducted experiments that suggested that the formation of 2,2-dichloroethanol from dichloroacetaldehyde was catalyzed by an NADPH-dependent ADH that contaminated their microsomal preparations. Filser and Bolt (1980) also reported that disulfiram, an ADH inhibitor, caused changes in the response of rats to inhaled trans-1,2-DCE that were suggestive of ADH involvement in its metabolism.

In a subsequent publication, Costa and Ivanetich (1984) used hepatocytes from male Long-Evans rat livers to study the metabolism of cis- and trans-1,2-DCE. After incubation, cells were destroyed with sulfuric acid and sodium tungstate and the supernatants extracted for gas/liquid chromatography. Isolated rat hepatocytes metabolized cis-1,2-DCE primarily to 2,2-dichloroethanol (2.4 nmol/10^6 cells/10 minutes) with the formation of smaller amounts of DCA (0.3 nmol/10^6 cells/10 minutes) and dichloroacetaldehyde (0.04 nmol/10^6 cells/ 10 minutes). No other chlorinated metabolites were produced from cis-1,2-DCE in measurable amounts. The metabolism of trans-1,2-DCE in isolated rat hepatocytes gave rise to DCA (0.05 nmol/10^6 cells/10 minutes), traces of dichloroacetaldehyde (0.008 nmol/10^6 cells/ 10 minutes), and 2,2-dichloroethanol (0.01 nmol/10^6 cells/10 minutes). This study by Costa and Ivanetich (1984) showed that the total amount of trans-1,2-DCE metabolized was 8–25 times less

than that of cis-1,2-DCE, yielding only small amounts of DCA and trace amounts of 2,2-dichloroethanol and dichloroacetaldehyde.

Costa and Ivanetich (1984) estimated Michaelis constant (KM) values of 0.67 mM for the formation of DCA from cis-1,2-DCE (the metabolic yield with trans-1,2-DCE was too small for rate estimation), 2.15 mM for the formation of dichloroacetaldehyde, and 2.55 mM for the formation of 2,2-dichloroethanol when phenobarbital-induced hepatocytes were used. These researchers also incubated the known metabolites of cis- and trans-1,2-DCE with cultured hepatocytes. They observed that DCA and dichloroacetaldehyde were largely (~90%) metabolized within 60 minutes in phenobarbital-induced hepatocyte culture. Degradation of dichloroacetaldehyde yielded primarily DCA, with the formation of a small amount of 2,2-dichloroethanol.

The question of further metabolism of 2,2-dichloroethanol or DCA has not been investigated in the context of cis- and/or trans-1,2-DCE metabolism. Barton et al. (1995), in an attempt to model the toxicokinetics of chloroethylene mixtures, found that exposing male Sprague-Dawley rats to 40 ppm trans-1,2-DCE for 4.5 hours did not affect nonprotein sulfhydryl content (essentially reduced glutathione [GSH]) in the livers. This could be seen as an indication that metabolites of trans-1,2-DCE do not undergo GSH conjugation to any major extent. Similarly, Dowsley et al. (1999) were not able to detect any GSH conjugates of 1,1-DCE in experiments with microsomal preparations from female CD-1 mice, although 1,1-DCE forms the same metabolite, dichloroacetaldehyde, as the 1,2-isomers. According to a metabolic scheme provided in that paper, formation of acetyl chloride or its derivative would be a prerequisite for GSH conjugation. McMillan (1986) found slight yet statistically significant reductions in hepatic GSH concentrations following high single doses of cis- or trans-1,2-DCE (10% reduction following 4.4 g/kg trans-1,2-DCE orally, 22% reduction following 1.9 g/kg trans-1,2-DCE intraperitoneally [i.p.], and 17% reduction following 2 g/kg cis-1,2-DCE i.p.).

The IRIS *Toxicological Review for Dichloroacetic Acid* (U.S. EPA, 2003) indicates that DCA is metabolized via oxidative dechlorination, a cytosolic process that does not involve CYP450 but instead GSH, NADPH, and GSTZ. The resulting metabolite is glyoxylate, which can undergo further oxidation to oxalate, reduction to glycolic acid, and transamination to glycine with subsequent formyl group transfer to form serine. This pathway is also presented in Figure 3-1. DCA stimulates peripheral glucose utilization and therefore has been proposed as an agent for treatment of several metabolic disorders, including diabetes and myocardial ischemia (Stacpoole, 1989). Oxalate can form insoluble crystals of calcium oxalate that can cause kidney damage. The ultimate products of glyoxylate biotransformation, glycine and serine, are utilized in protein synthesis.

Nakajima (1997) presented some evidence that both cis- and trans-1,2-DCE are metabolized by CYP2E1. By using microsomal preparations from untreated, fasted, or ethanol-pretreated rats, they found a twofold increase in the rate of metabolism (RAM) of cis-1,2-DCE in

microsomes from fasting rats and a threefold increase in its metabolism in microsomes from ethanol-treated rats. Fasting and dietary ethanol are widely known to induce the activity of CYP2E1 (Cederbaum, 2006; Wan et al., 2006). A comparatively low RAM of trans-1,2-DCE by microsomes from ethanol-treated rats was reported, which was not measurable using microsomes from untreated or fasted rats. The results obtained with ethanol-induced liver microsomes provide inferential evidence that CYP2E1 is involved in the metabolism of 1,2-DCE.

3.3.2. Metabolism in Human Preparations In Vitro

Doherty et al. (1996) investigated the potential clastogenic activity of several chlorinated hydrocarbons, among them 1,2-DCE (likely a mixture of both isomers), using several human cell lines with variable CYP450 enzyme expression profiles. Their findings suggest that a direct-acting genotoxic effect without the need for metabolic activation is possible and the production of a metabolite that was less genotoxic than the parental compounds is also possible.

3.4. ELIMINATION

No reports of studies in animals or humans that assessed the elimination of cis- or trans-1,2-DCE or its metabolites, via any route, were identified in the available literature. However, Pleil and Lindstrom (1997) have estimated elimination rate constants for the disappearance from human blood of certain halogenated volatile organic compounds (VOCs), including cis-1,2-DCE. Estimates were based on decay of exhaled breath concentrations following a 10-minute shower exposure to contaminated water and published blood/air partition coefficients for the VOC in question. Two volunteers (recruited with informed consent in compliance with local institutional standards) were exposed in separate showering episodes, in which estimated total absorbed doses of cis-1,2-DCE were 1.19 and 2.34 µg, respectively. The kinetics of elimination of parent compound from breath suggested the existence of two biological distribution compartments, which were presumed to represent the blood and "highly perfused tissues" (e.g., liver). In the first fast-elimination compartment (presumed to represent disappearance of cis-1,2-DCE from the blood), elimination half-lives of 0.82 and 2.37 minutes were estimated in the two subjects; corresponding half-lives in the slower, highly perfused tissue compartment were 8.96 and 29.33 minutes, respectively. These limited data suggest the potential for variability in the elimination of cis-1,2-DCE in humans.

Considering the metabolic fates of the various possible metabolites of 1,2-DCE, it may be assumed that whatever portion of dichloroethanol is not transformed to DCA will be ultimately exhaled. For dichloroacetaldehyde and DCA, the IRIS *Toxicological Review for Dichloroacetic Acid* (U.S. EPA, 2003) provides some useful information. Accordingly, glyoxylate formed via GSTZ is ultimately broken down to carbon dioxide or oxidized to oxalate, which is excreted in the urine. Dechlorination products, such as monochloroacetic acid, also are said to exist, but, for the case of cis- or trans-1,2-DCE, this is at odds with the findings of Costa and Ivanetich (1984,

1982) who could not detect dechlorination products of 1,2-DCE in vitro with rat microsomes or hepatocytes. A possible explanation is that, given the comparatively poor uptake and slow metabolism of cis- and trans-1,2-DCE, tissue levels of DCA never become high enough to allow for any measurable dechlorination reaction to occur.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

A toxicokinetic description of distribution and elimination of inhaled cis- and trans-1,2-DCE in rats was reported by Filser and Bolt (1979), who analyzed experimental data by using a simplified compartmental model. However, their interpretation of the metabolic clearance of 1,2-DCE failed to address the inactivation of CYP2E1 that had been observed both in vivo and in vitro by Freundt and Macholz (1978) and later quantified in hepatic microsomal preparation in vitro by Costa and Ivanetich (1982). This metabolic inactivation phenomenon also complicated the fitting of experimental data, which had been obtained with different concentrations of cis- and trans-1,2-DCE vapors in a closed gas chamber, to a typical PBTK model for VOCs, using only the metabolic constants, V_{max} and KM.

Gargas et al. (1990) updated the PBTK model for rats with an algorithm that described CYP2E1 suicide inhibition-resynthesis. In this algorithm (Clewell and Andersen, 1987), the rate of enzyme inactivation was proportional to a second order rate constant (k_d), multiplied by the square of the initial RAM, thereby representing the reaction of free metabolite(s) with the enzyme-substrate complex. The algorithm also included a term for the zero-order rate of enzyme resynthesis (k_s) during exposure. Subsequently, the PBTK model for cis- and trans-1,2-DCE in rats was extended by Lilly et al. (1998) to describe quantitatively the mechanisms of both suicidal inhibition of CYP2E1 by metabolic intermediate(s) and CYP2E1 resynthesis. This algorithm ("Model 1" in Lilly et al., 1998) required four parameters, or kinetic constants: V_{maxC} (maximum rate of metabolism), K_M (pseudo-Michaelis constant), k_d (inhibition constant), and K_{de} (enzyme degradation constant). The model-estimated kinetic constants V_{maxC} and K_M were 4.53 mg/hour/kg and 0.19 mg/L for cis-1,2-DCE and 4.27 mg/hour/kg and 0.08 mg/L for trans-1,2-DCE, respectively, with cis-1,2-DCE metabolite(s) being less potent inhibitor(s) of CYP2E1 (k_d = 2.07 [mg/hour] × [hour]$^{-1}$) than the metabolite(s) of trans-1,2-DCE (k_d = 496 [mg/hour] × [hour]$^{-1}$), under a similar enzyme degradation constant (K_{de} about 0.025 [hour]$^{-1}$) (Lilly et al., 1998).

The PBTK model structure (Figure 3-2) consists of five dynamic tissue compartments representing the lungs, fat, rapidly perfused tissues, slowly perfused tissues, and liver. All perfusion-limited tissue compartments are linked through blood flow, following an anatomically accurate, typical, physiologically based description (Lilly et al., 1998).

Source: Lilly et al. (1998) (reproduced with permission of Springer Verlag, Heidelberg/New York).

Figure 3-2. PBTK model for cis- and trans-1,2-dichloroethylene in rats.

Briefly, because cis- and trans-1,2-DCE are retained by the tissue(s) in each compartment according to their tissue/blood partition coefficients (measured in vitro by Gargas et al., 1988), the concentrations of both chemicals in venous blood (leaving the tissue) are lower than those in arterial blood during the equilibration phase. Therefore, the rate of change in the amount of either chemical in each tissue compartment ($_i$) is given by the difference between concentration in blood entering (C_a) and exiting (C_{vi}) the tissue, multiplied by the blood flow (Q_i). The differential equations for each tissue compartment (except lungs) are integrated over time, giving the amounts of cis- or trans-1,2-DCE present in the tissue. Because the partition coefficient (P_i) and the actual volume of each tissue are known from the literature (Ramsey and Andersen, 1984), concentrations of cis- or trans-1,2-DCE in each tissue can be calculated over time.

For the lung compartment with two mass inputs (mixed venous blood and inhaled air) and two outputs (arterial blood and exhaled air), at steady state the amount of either chemical in alveolar air is in equilibrium with the amount in lung blood, and, thus, concentrations of cis- or trans-1,2-DCE in arterial blood can be calculated from the simple mass balance equations, taking into account the alveolar ventilation rate and the rate of blood flow through the lung (equal to

cardiac output), both known from the literature (Ramsey and Andersen, 1984). For the liver compartment, with mass input from blood and two outputs (venous blood and metabolism; biliary excretion was not considered), the chemical mass transfer is given by the difference between concentrations in portal (C_a) and venous (C_{vl}) blood multiplied by hepatic blood flow (Q_l) and corrected for metabolic clearance of cis- or trans-1,2-DCE.

The rates of amount metabolized (RAMs) (see Figure 3-2) of cis- and trans-1,2-DCE are calculated from the Michaelis-Menten equation (using "metabolic capacity remaining" instead of initial velocity V_{max0}) and subtracted from the rate of change in chemical mass in the liver. Rates of change of inhibitable metabolism (RVMT) (see Figure 3-2), under the assumption that a reactive metabolite reacts with enzyme-substrate complex ("Model 1" in Lilly et al., 1998), can be calculated also from the Michaelis-Menten equation with a negative inhibition term (rate constant $-k_d$ multiplied by RAM), whereas rates of change of metabolism due to enzyme resynthesis can be calculated by a zero order term, multiplying V_{max} by K_{de} (Bae et al., 2005; Lilly et al., 1998).

A simplified scheme of the mass flow in the PBTK model for cis- and trans-1,2-DCE is shown in Figure 3-2, according to Lilly et al. (1998). This model was calibrated with data obtained in closed-chamber gas uptake studies with rats, as reported by Gargas et al. (1990). From four different algorithms tested by Lilly et al. (1998), "Model 1," which assumes that reactive metabolite(s) of cis- and trans-1,2-DCE inactivate the CYP2E1 enzyme-substrate complex, gave the best approximation of experimentally obtained data (Bae et al., 2005). One could extrapolate the model to humans by allometrically scaling V_{max} in the absence of exposure and the resynthesis rate for CYP2E1, while assuming that the molecular rate of suicide inhibition is the same for human and rat CYP2E1. However, in the absence of human data with which to validate or calibrate this model, such an extrapolation would involve considerable uncertainty, much greater than cases without suicide inhibition. (The data on human exhalation subsequent to exposure in a shower is likely most sensitive to the parameters describing respiration, cardiac output, and the blood:air partition coefficient, and these data are expected to provide little information on metabolic rates.) Therefore, such extrapolation of the model is not attempted in this assessment.

Since this PBTK model was not calibrated with human data, it cannot be scaled allometrically to humans, whose liver CYP2E1 activity, resynthesis rate, and sensitivity to inhibition differ from those in rats. Given the current state of knowledge, this PBTK model is not useful for estimating the human equivalent dose from the available animal data for cis- or trans-1,2-DCE. No other valid PBTK models of cis- or trans-1,2-DCE were identified. A data gap exists with respect to PBTK data relevant to cis- and trans-1,2-DCE in humans.

4. HAZARD IDENTIFICATION

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

There are limited data available from studies of effects of 1,2-DCE in humans. In an early study (Lehmann and Schmidt-Kehl, 1936), the threshold for odor detection of trans-1,2-DCE by two human subjects was reported to be 280 ppm (1,100 mg/m^3). Slight eye irritation occurred after 30 minutes inhalation exposure to 830 ppm (3,300 mg/m^3), while at exposure concentrations of 1,200 ppm (4,800 mg/m^3) to 2,200 ppm (8,800 mg/m^3) for 5–10 minutes, both subjects reported symptoms of nausea, drowsiness, fatigue, vertigo, and a feeling of intracranial pressure. Hamilton (1934, as cited in Dow Chemical Co. [Dow, 1962]) reported that a worker who entered a vat containing rubber dissolved in 1,2-DCE of unknown isomeric composition was found dead the following morning. The exposure concentration and duration of exposure were unknown. A human threshold limit value (TLV) of 200 ppm for cis- or trans-1,2-DCE and mixtures of the two isomers has been established by ACGIH (2001).

4.2. SHORT-TERM, SUBCHRONIC, AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

A number of studies in animals have investigated the short-term and subchronic toxicity of cis- or trans-1,2-DCE by either the oral or inhalation route. Presented below are summaries of these investigations. No chronic studies for cis- or trans-1,2-DCE or their mixtures were identified. No cancer studies for cis- or trans-1,2-DCE or their mixtures were identified.

4.2.1. Oral Exposure
4.2.1.1. *Short-term Studies*
4.2.1.1.1. *cis-1,2-DCE.* McCauley et al. (1990, unpublished) conducted a 14-day gavage study of cis-1,2-DCE in male and female Sprague-Dawley rats. The study was subsequently published (McCauley et al., 1995). Upon review and comparison of the unpublished McCauley report (McCauley et al., 1990) and the published study (McCauley et al., 1995), errors in the documentation of doses and other minor inconsistencies were noted. These errors were not considered to compromise the reliability of the findings. Cis-1,2-DCE was administered by gavage in corn oil vehicle to approximately 10-week-old Sprague-Dawley rats (10/sex/dose) at doses of 0, 1, 3, 10, and 20 mmol/kg-day (equivalent to 0, 97, 291, 970, and 1,940 mg/kg-day, respectively).[1] At the end of the exposure period, animals were sacrificed and the brain, gonads, heart, kidneys, adrenals, liver, spleen, and thymus were weighed and examined for gross

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[1]Doses in the 1995 study were incorrectly converted from mmol/kg-day to mg/kg-day. The doses presented here are the correctly converted doses.

pathology. Blood samples were collected for hematological and clinical chemistry examination. Tissues from controls and the high-dose group animals were examined for histopathologic changes.[2]

During the study, male and female rats in the 1,940 mg/kg-day groups released excessive clear secretions around the nose and/or mouth and appeared agitated, followed by lethargy and ataxia. These symptoms were most common immediately after dosing. Gavage-related deaths were reported in the 1,940 mg/kg-day group (2/10 males and 3/10 females) and 970-mg/kg-day group (1/10 males and 1/10 females). Increases in water consumption were also seen in both male and female rats in the 1,940 mg/kg-day groups.

With the exception of a 10% decrease in male rats in the 1,940 mg/kg-day dose group, there were no significant changes in the final mean body weights. Significant dose-related increases in relative liver weight were reported in both males (16–38%) and females (15–39%) at all dose levels. Statistically significant increases were observed for relative kidney weights in females in the 970 and 1,940 mg/kg-day groups (14 and 12%, respectively) and for relative testes weights in males in the 1,940 mg/kg-day group (23%). Serum phosphorus levels were significantly elevated in females in all experimental groups, and serum cholesterol was increased in the 1,940 mg/kg-day group. Serum calcium was statistically significantly increased in male groups dosed with 970 and 1,940 mg/kg-day. Decreases in blood urea nitrogen (BUN) occurred in females at doses of 291 (14%), 970 (28%), and 1,940 mg/kg-day (17%). (Increases in BUN are generally indicative of an effect on kidney function.) Hematocrit values for females were decreased by 8 to 11% at the 291, 970, and 1,940 mg/kg-day dose groups; similar effects did not occur in males. The authors considered most of the clinical chemistry and hematology effects to be marginal and not biologically meaningful or dose related. No compound-related histopathological changes were found at sacrifice. The authors noted that cis-1,2-DCE affected organ-to-body-weight ratios at relatively low exposure levels, but in light of negative histopathology, these data were difficult to interpret.

4.2.1.1.2. *trans-1,2-DCE.* Barnes et al. (1985) conducted a 14-day gavage study in male and female CD-1 mice. Concentrations of trans-1,2-DCE were prepared so that each mouse received approximately 1/100 and 1/10 of the lethal dose (LD_{50}) (21 and 210 mg/kg) daily. No significant differences in weight gain were observed among the treated groups. Weights of the brain, liver, spleen, lungs, thymus, kidney, and testes were not altered by DCE exposure. All organ weights were within the limits of historical controls, and there was no treatment effect when the weights were expressed as absolute weight, percent of body weight, or organ-to-brain ratios. There were no changes seen in hematocrit or hemoglobin values. Fibrinogen levels were decreased by 12% in the 210 mg/kg treatment group, and prothrombin activity increased slightly

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[2]According to the unpublished report (McCauley et al., 1990), only half the controls, rather than all controls as reported in McCauley et al. (1995), were examined for histopathologic changes.

as manifested by a 7% decrease in prothrombin time. There were no significant differences in SGPT (ALT) activity or BUN levels; however, a statistically significant decrease (29%) in the LDH levels of the 210 mg/kg trans-1,2-DCE group was observed. In a study by the same laboratory, discussed below in Section 4.4.3.2, values for leukocyte counts, hematocrit, hemoglobin, fibrinogen, and prothrombin time did not differ significantly from control values when identical experiments were conducted in male CD-1 mice (Munson et al., 1982).

4.2.1.1.3. *Mixtures of cis- and trans-1,2-DCE.* In a dissertation, McMillan (1986) reported a statistically significant increase in kidney weight in male Sprague-Dawley-derived rats (6/group) administered a dose of 5 mmol (485 mg/kg-day) of a 50:50 mixture of cis-1,2-DCE and trans-1,2-DCE (in a sesame seed oil vehicle, 1 mL/kg) in a 14-day oral gavage study. Slight reductions (statistically significant) in plasma creatinine and BUN levels, and an increase in plasma calcium levels were also recorded at termination.

4.2.1.2. *Subchronic Studies*
4.2.1.2.1. *cis-1,2-DCE.* In a 90-day study, 10 Sprague-Dawley rats/sex/group, approximately 70 days old at study initiation, were administered 97% pure cis-1,2-DCE in corn oil by gavage (3 mL/kg) at doses of 0, 32, 97, 291, and 872 mg/kg-day (McCauley et al., 1995, 1990). Comparison of the unpublished McCauley report (McCauley et al., 1990) and the published study (McCauley et al., 1995) revealed errors in the documentation of administered doses and other minor inconsistencies.[3] These errors and inconsistencies were not considered to compromise the reliability of the 90-day study findings. At the end of the 90-day exposure period, animals were sacrificed and the brain, gonads, heart, kidneys, adrenals, liver, spleen, and thymus were weighed and examined for gross pathology. Blood samples were collected for hematological and clinical chemistry examinations. Tissues from controls and the high-dose group animals were examined for histopathologic changes.[4]

Clinical observations during the study were reported by the authors as minimal and not compound-related. Gavage deaths were present in both the treated and control groups (1/10 female rats at 32 mg/kg-day; 1/10 female rats at 97 mg/kg-day; 1/10 male controls; 3/10 male rats at 291 mg/kg-day; 4/10 male rats at 872 mg/kg-day).

Terminal body weights in male rats at the two highest dose groups were lower than controls by 10–11%, but were not considered by the author as statistically significant; no

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[3]The administered doses in McCauley et al. (1995) were reported as 0, 0.33, 1, 3, and 9 mmol/kg-day, which when converted to mg/kg-day, are 0, 32, 97, 291, and 872 mg/kg-day. McCauley et al. (1995), however, reported the converted doses incorrectly as 0, 10, 32, 98, and 206 mg/kg/day. The doses presented here are the correctly calculated doses of doses of 0, 32, 97, 291, and 872 mg/kg-day, as reported in McCauley et al. (1990). In addition, the summary of clinical chemisty findings in McCauley et al. (1995) did not adjust for early gavage-related deaths in the number of animals studied.
[4]According to the unpublished report (McCauley et al., 1990), only half the controls, rather than all controls as reported in McCauley et al. (1995), were examined for histopathologic changes.

treatment-related effects on body weight were reported in female rats. Relative liver weights were statistically significantly increased in a dose-related manner in males and females of the 97, 291, and 872 mg/kg-day dose groups (Table 4-1). The increases were 15, 17, and 32% for males and 14, 19, and 30% for females at 97, 291, and 872 mg/kg-day, respectively. Histopathological evaluation revealed no specific hepatic injury. The authors concluded that there was a consistent, dose-related increase in relative liver weight in both sexes and that this effect, in light of the negative histopathology findings, may reflect hypertrophy and hyperplasia.

Table 4-1. Relative organ weights of rats exposed to cis-1,2-DCE by gavage for 90 days

		Dose (mg/kg-day)			
	Control	**32**	**97**	**291**	**872**
Males[a]					
Kidney	0.70 ± 0.06	0.80 ± 0.06^b (14%)[c]	0.83 ± 0.06^b (19%)[c]	0.83 ± 0.10^b (19%)[c]	0.89 ± 0.06^b (27%)[c]
Liver	2.85 ± 0.26	3.15 ± 0.27 (10%)[c]	3.28 ± 0.18^b (15%)[c]	3.34 ± 0.44^b (17%)[c]	3.75 ± 0.20^b (32%)[c]
Females[a]					
Kidney	0.69 ± 0.06	0.71 ± 0.05 (3%)[c]	0.82 ± 0.23 (19%)[c]	0.85 ± 0.21 (23%)[c]	0.85 ± 0.06 (23%)[c]
Liver	2.82 ± 0.19	2.91 ± 0.18 (3%)[c]	3.21 ± 0.22^b (14%)[c]	3.36 ± 0.18^b (19%)[c]	3.67 ± 0.27^b (30%)[c]
Thymus	0.99 ± 0.18	1.40 ± 0.27	1.00 ± 0.29	1.11 ± 0.33	1.16 ± 0.31^b

[a]Values are mean ± SD.
[b]Statistically significantly different from control group; $p \leq 0.05$ by Tukey's multiple comparison test.
[c]Values are percent increases from control group.

Source: McCauley et al. (1995).

Statistically significant increases in relative kidney weights were recorded in male rats in all dose groups (14, 19, 19, and 27% at 32, 97, 291, and 872 mg/kg-day, respectively) (Table 4-1). Female rats exhibited increased (although not statistically significant) relative kidney weights in the three highest doses (19, 23, and 23% at 97, 291, and 872 mg/kg-day, respectively). Relatively large variances in the female dose groups may explain why relative kidney weight increases in females were not statistically significant. Histopathological findings for kidney effects were negative, leading the authors to hypothesize that the increases in relative kidney weight may be due at least in part to decreased body weight gain.

Sporadic changes (although noted as statistically significant) in some clinical chemistry parameters were observed. BUN levels were significantly decreased (40%) at the highest dose in males but not in females. Serum calcium levels were significantly elevated by 8 and 10% in males at the 32 and 97 mg/kg-day doses, respectively, and serum phosphorus was significantly

decreased by 14% in males exposed to 32 mg/kg-day. In females, serum phosphorus was significantly increased by 34 and 25% in the groups dosed with 97 and 291 mg/kg-day, respectively. No significant changes were reported in AST activity. Hemoglobin and hematocrit levels, and red blood cell (RBC) counts were significantly decreased in female rats dosed at 291 mg/kg-day, while only hematocrit was significantly decreased in females dosed with 872 mg/kg-day. In males, similar decreases (ranging from 6 to 10% compared with the control) occurred in hemoglobin in the 291 and 872 mg/kg-day groups and in hematocrit in the 97, 291, and 872 mg/kg-day groups. Overall the changes in clinical chemistry and hematology parameters were considered by the authors to be marginal and of questionable biological significance. No noteworthy compound-related histopathological changes were observed in any dose group.

4.2.1.2.2. *trans-1,2-DCE.* There are three subchronic studies that evaluated oral exposure to trans-1,2-DCE (NTP, 2002; Hayes et al., 1987; Barnes et al., 1985). In a 90-day study by Hayes et al (1987), groups of 20 male and 20 female rats, approximately 29–37 days of age, were administered 98% pure trans-1,2-DCE in drinking water containing 1% emulphor to promote solubility. The experimental groups consisted of an untreated control group, a 1% emulphor control group, and three test groups receiving drinking water containing trans-1,2-DCE sufficient to provide approximate daily doses of 500, 1,500, and 3,000 mg/kg. Based on fluid consumption measured twice weekly, actual mean doses were 0, 402, 1,314, and 3,114 mg/kg-day for males and 0, 353, 1,257, and 2,809 mg/kg-day for females. Effects on body weight, organ weights, hematology, urine, and blood chemistries were examined. Gross pathological examinations were performed following removal and weighing of selected organs.

A dose-related increase in fluid consumption was observed among the groups receiving drinking water with emulphor, but the differences did not attain statistical significance. No trans-1,2-DCE-related changes in behavior or interim deaths were observed. The mean body weight of male rats increased from 100 to approximately 500 g for all dose groups during the study. Female rats increased in body weight from 100 g at the beginning of the study to 250 g at the end of the study. Although the male rats gained considerably more weight than the females during the course of the study, at termination no statistically significant compound-related differences in body weights or body weight gains were found in either the males or females among the five groups. The authors reported that there were no consistent, remarkable compound-related, dose-dependent effects on any of the hematological, serological, or urinary parameters evaluated. No significant changes in organ weights or relative organ weights were seen in males, and only absolute kidney weights (Table 4-2) and kidney weights relative to brain weights were statistically significantly elevated in the mid- and high-dose groups of female rats. These increases were 8 and 9% for absolute kidney weight and 11 and 11% for kidney weights relative to brain weights for the mid- and high-dose female rats, respectively. Dose-related

increases (although not statistically significant) in liver weights occurred in both sexes. A limited number of organs (livers, kidneys, testes, and ovaries from 10 rats/sex/dose) were examined microscopically at termination, and no compound-related histopathological changes were reported.

Table 4-2. Absolute kidney weights in female rats treated with trans-1,2-DCE via drinking water for 90 days

Dose (mg/kg-day)	Vehicle	353	1,257	2,809
Number of animals	19	20	19	17
Kidney weight (g)[a]	2.20 ± 0.04	2.26 ± 0.04	2.37 ± 0.04[b]	2.40 ± 0.03[b]

[a]Mean ± Standard error.
[b]Statistically significant, $p \leq 0.05$.

Source: Hayes et al. (1987).

NTP (2002) conducted a 14-week study with trans-1,2-DCE in rats and mice. F344/N rats, 10/sex/dose, were fed diets containing microcapsules with a chemical load of 45% trans-1,2-DCE at dietary concentrations of 0, 3,125, 6,250, 12,500, 25,000, and 50,000 ppm, resulting in average daily trans-1,2-DCE doses of 0, 190, 380, 770, 1,540, and 3,210 mg/kg-day for males and 0, 190, 395, 780, 1,580, and 3,245 mg/kg-day for females, respectively. B6C3F$_1$ mice (10/sex/group) received 0, 480, 920, 1,900, 3,850, and 8,065 mg/kg-day for males and 0, 450, 915, 1,830, 3,760, and 7,925 mg/kg-day for females (NTP, 2002). Additional groups (10 males and 10 females) of rats and mice served as untreated and vehicle controls (animals that received feed with unloaded microcapsules). Animals were evaluated for survival, body weight (weekly), and feed consumption (weekly). Necropsies were performed on all animals. Organ weights were measured for the heart, right kidney, liver, lung, right testis, and thymus. Clinical findings, including hematology (rats only), clinical chemistry, and histopathology, were performed. Hematology parameters included hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials. Complete histopathology was performed on all rats and mice in the untreated control, vehicle control, and 50,000 ppm groups (3,210 and 3,245 mg/kg-day in male and female rats; 8,065 and 7,925 mg/kg-day in male and female mice, respectively). In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach

(forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, uterus, and Zymbal's gland.

In the rat study, there were no exposure-related deaths. The final mean body weight and body weight gain of male rats exposed to trans-1,2-DCE in the 3,210 mg/kg-day group were reduced by about 6% (statistically significant) below controls (Table 4-3). Feed consumption in the exposed groups was similar to that in the vehicle controls. On day 21 and at week 14, there were mild decreases (generally less than 5% below the values in controls) in hematocrit values, hemoglobin concentrations, and erythrocyte counts in 1,540 and 3,210 mg/kg-day males and 1,580 and 3,245 mg/kg-day female rats. At week 14, these effects were also seen in male rats exposed to 380 and 770 mg/kg-day trans-1,2-DCE. However, it was noted that females exposed to ≥780 mg/kg-day had statistically significantly decreased serum alkaline phosphatase (ALP) activities compared with the vehicle controls on day 21. These decreases were noted by the authors to be minimal in severity, no greater than about 13%, and transient, with activities in the affected groups returning to vehicle control levels by week 14. On day 21, it was also noted that there was a minimal suppression of serum 5'-nucleotidase activities in the 3,210 mg/kg-day male and the 3,245 mg/kg-day female rats. According to the authors, these sporadic differences in clinical chemistry parameters at various time points generally did not demonstrate exposure concentration relationships or were inconsistent between males and females. These differences were not considered by the authors to be toxicologically relevant.

Table 4-3. Reduced body weights in male rats exposed to trans-1,2-DCE in the feed for 14 weeks

	Dose (mg/kg-day)					
	Vehicle	**190**	**380**	**770**	**1,540**	**3,210**
Body weight - g (mean ± SE)	360 ± 6	365 ± 5	361 ± 3	357 ± 5	350 ± 6	339 ± 4[a]

[a]Statistically significant difference from controls, $p < 0.05$.

Source: NTP (2002).

NTP (2002) reported mild decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts at week 14 in male and female rats in all but the lowest dose groups. Of these parameters, only RBC counts showed a dose-response and robust statistical significance at the $p \leq 0.01$ level. This effect was well demonstrated in male rats, with RBC count statistically significantly decreased at all but the lowest dose. However, the maximum decrease in RBC was only 7% in males and 5% in females at the highest dose (3,210 mg/kg-day in male rats and 3,245 mg/kg-day in female rats). The experimental data are displayed in Table 4-4.

Table 4-4. Red blood cell counts in male and female rats exposed to trans-1,2-DCE in the feed for 14 weeks

	Males[a]					
	Dose (mg/kg-day)					
	Vehicle	**190**	**380**	**770**	**1,540**	**3,210**
RBC ($10^6/\mu L$) (mean ± SE)	8.14 ± 0.08	8.17 ± 0.05	7.93 ± 0.10[b]	7.84 ± 0.09[b]	7.79 ± 0.08[c]	7.56 ± 0.15[c]
	Females[d]					
	Dose (mg/kg-day)					
	Vehicle	**190**	**395**	**780**	**1,580**	**3,245**
RBC ($10^6/\mu L$) (mean ± SE)	7.59 ± 0.06	7.58 ± 0.10	7.50 ± 0.08	7.49 ± 0.04	7.34 ± 0.05[c]	7.20 ± 0.08[c]

[a]Ten animals in each group except for the 380 mg/kg-day group with only nine animals.
[b]Statistically significant difference, $p \leq 0.05$.
[c]Statistically significant difference, $p \leq 0.01$.
[d]Ten animals per group.

Source: NTP (2002).

In female rats exposed to ≥395 mg/kg-day the absolute and relative liver weights were approximately 8–17% and 6–10% higher (statistically significant), respectively, than those of the vehicle controls (see Table 4-5). The greatest increases were observed at the 395 mg/kg-day dose. Absolute kidney weights of male rats exposed to 1,540 or 3,210 mg/kg-day trans-1,2-DCE were decreased by about 7%. No gross or histological lesions were observed in rats that were attributed to exposure to trans-1,2-DCE.

In the mouse study, no exposure-related deaths occurred. Mean body weights of 8,065 mg/kg-day males and 7,925 mg/kg-day females were significantly less (both by about 7%) than those of the vehicle controls. Mean body weight gains of female mice in the 1,830 and 3,760 mg/kg-day groups were also significantly less (6 and 4%, respectively) than in vehicle controls. Feed consumption in the exposed groups was similar to that in the vehicle controls. No exposure-related alterations in clinical chemistry parameters were observed.

As shown in Table 4-5, the relative liver weights of male mice exposed to ≥1,900 mg/kg-day and female mice exposed to 3,760 or 7,925 mg/kg-day were significantly greater than those of the vehicle controls. Relative liver weight increases in the male mice were 10% or less except for the high dose (8,065 mg/kg-day), which showed a 14% increase compared with the vehicle control. The relative liver weights in the two highest female dose groups (3,760 mg/kg-day and 7,925 mg/kg-day) were increased by about 12% over vehicle controls. Other than a statistically significant increase of 16% in the 915 mg/kg-day females, there was no significant dose-related change in absolute liver weight. No gross or microscopic lesions were observed in mice that could be attributed to trans-1,2-DCE exposure.

Table 4-5. Relative liver weights in male and female mice and rats exposed to trans-1,2-DCE in the feed for 14 weeks

	Mice - males					
	Dose (mg/kg-day)					
	0	**480**	**920**	**1,900**	**3,850**	**8,065**
Relative liver weights (mean ± SE)[a]	4.347 ± 0.056	4.552 ± 0.113	4.597 ± 0.115	4.745 ± 0.084[b]	4.736 ± 0.079[b]	4.979 ± 0.111[b]
	Mice - females					
	Dose (mg/kg-day)					
	0	**450**	**915**	**1,830**	**3,760**	**7,925**
Relative liver weights (mean ± SE)[a]	4.621 ± 0.07	4.738 ± 0.068	4.970 ± 0.127	4.813 ± 0.05	5.115 ± 0.139[b]	5.117 ± 0.08[b]
	Rats - males					
	Dose (mg/kg-day)					
	0	**190**	**380**	**770**	**1,540**	**3,210**
Relative liver weights (mean ± SE)[a]	3.465 ± 0.058	3.538 ± 0.032	3.658 ± 0.099	3.524 ± 0.050	3.492 ± 0.048	3.634 ± 0.056
	Rats - females					
	Dose (mg/kg-day)					
	0	**190**	**395**	**780**	**1,580**	**3,245**
Relative liver weights (mean ± SE)[a]	2.937 ± 0.038	3.040 ± 0.052	3.220 ± 0.066[b]	3.100 ± 0.051[b]	3.132 ± 0.052[b]	3.216 ± 0.051[b]

[a]Ten animals per group
[b]Statistically significant, $p \leq 0.01$.

Source: NTP (2002).

It was concluded by NTP (2002) that little toxicity was associated with ingestion of microencapsulated trans-1,2-DCE, and that the histopathology and clinical chemistry data combined with organ and body weight data revealed that the maximum tolerated dose (MTD) had not been reached in this study.

In a 90-day drinking water study conducted by Barnes et al. (1985), groups of male and female CD-1 mice (24 mice/sex in the control group and 16 mice/sex in the treatment groups) were exposed to trans-1,2-DCE, purity 98%, dissolved in deionized water at 0, 0.1, 1.0, or 2.0 mg/mL. Target daily doses were 1/100, 1/10, and 1/5 the acute oral LD_{50}; actual time-weighted average daily doses calculated on the basis of water consumption were 0, 17, 175, and 387 mg/kg-day for males and 0, 23, 224, and 452 mg/kg-day for females. Body and organ weights, hematology, serum chemistries, and hepatic microsomal activities were measured. Fluid consumption by both male and female mice progressively decreased throughout the duration of the experiment, with comparable changes occurring in the control group. Few trans-1,2-DCE-induced changes in terminal body weight gain or gross pathology were observed at the time of necropsy in either sex. As shown in Table 4-6, male mice receiving 175 mg/kg-

day trans-1,2-DCE demonstrated a statistically significant increase in mean relative liver weights; however, relative liver weights were less than those of the controls in the low- and high-dose groups. Females receiving 452 mg/kg-day demonstrated an 11% decrease (statistically significant) in relative lung weights. Additionally, absolute thymus weight was reduced at the high dose in females and relative thymus weights in the mid- and high-dose females.

Table 4-6. Results of 90 day study in male and female CD-1 mice exposed to trans-1,2-DCE in the drinking water

Parameter	Males[a]			
	Dose (mg/kg-day)			
	Vehicle	17	175	387
Liver weight (mg) (mean ± SE)[d]	2,029 ± 43	2,007 ± 62	2,288 ± 60[b] (8%)	2,022 ± 85
Lung weight (mg) (mean ± SE)[d]	232 ± 4	228 ± 5	236 ± 6	223 ± 5
Thymus weight (mg) (mean ± SE)[d]	48 ± 3	47 ± 3	54 ± 2	48 ± 2
Prothrombin time (sec) (mean ± SE)	10.0 ± 0.2	8.5 ± 0.2[b] (15%)	8.8 ± 0.3[b] (12%)	9.8 ± 0.2
Leukocytes (10^3/mm^3)	5.30 ± 0.32	4.95 ± 0.42	4.83 ± 0.24	5.16 ± 0.40
Glucose (mg %)	153 ± 7	195 ± 8[b] (27%)	184 ± 5[b] (20%)	190 ± 7[b] (24%)
LDH (IU/L)	677 ± 33	605 ± 47	449 ± 22[b] (34%)	587 ± 56
SGPT (IU/L) (or ALT)	44.3 ± 3.3	55.1 ± 7.1	45.0 ± 6.8	41.2 ± 4.7
SGOT (IU/L) (or AST)	74.0 ± 6.5	110.0 ± 7.8[b] (48%)	65.3 ± 5.0	69.9 ± 5.8
SAP (IU/L) (or ALP)	34.3 ± 1.8	37.6 ± 5.1	55.5 ± 5.4[b] (62%)	45.6 ± 2.4[b] (33%)
Parameter	Females[c]			
	Dose (mg/kg-day)			
	Vehicle	23	224	452
Liver weight (mg) (mean ± SE)	1,712 ± 57	1,839 ± 51	1,864 ± 38	1,741 ± 57
Lung weight (mg) (mean ± SE)	254 ± 11	255 ± 7	244 ± 7	222 ± 8[b] (11%)
Thymus weight (mg) (mean ± SE)	71 ± 3	67 ± 4	61 ± 4	54 ± 4[b] (24%)
Prothrombin time (sec) (mean ± SE)	9.7 ± 0.2	9.8 ± 0.3	9.1 ± 0.2	9.0 ± 0.6
Leukocytes (10^3/mm^3)	7.27 ± 0.32	6.98 ± 0.50	8.95 ± 0.61[b] (23%)	7.79 ± 0.60
Glucose (mg %)	122 ± 3	156 ± 6[b] (28%)	147 ± 5[b] (20%)	156 ± 6[b] (28%)
LDH (IU/L)	511 ± 22	377 ± 20[b] (26%)	452 ± 23	559 ± 42
SGPT (IU/L) (or ALT)	49.9 ± 6.4	38.3 ± 3.0	33.5 ± 3.6[b] (33%)	30.4 ± 1.6[b] (39%)
SGOT (IU/L) (or AST)	91.7 ± 6.6	77.8 ± 6.0	66.9 ± 4.5[b] (27%)	58.9 ± 8.8[b] (36%)
SAP (IU/L) (or ALP)	44.0 ± 2.3	47.6 ± 4.2	51.0 ± 3.0	45.4 ± 2.9

[a]Twenty-three animals/sex in the control group and 15–16 animals/sex in the treatment groups.
[b]Differ statistically significantly from controls, $p \le 0.05$.
[c]Twenty-four animals/sex in the control group and 16 animals/sex in the treatment groups.
[d]All organ weights are absolute values

Source: Barnes et al. (1985).

Few changes in hematological parameters were seen; prothrombin time was significantly decreased by 15 and 12% in male mice exposed to 17 and 175 mg/kg-day trans-1,2-DCE, respectively, and only decreased by 2% at the high dose (387 mg/kg-day) in the male mice. In female mice exposed to 224 mg/kg-day, an increase (23%) in blood leukocytes and decrease (42%) in polymorphonuclear leukocytes occurred. Slight changes in several clinical chemistry parameters were observed. Although some values were statistically significantly different from those of the controls, there were no consistent trends or any large deviations from historical

control values. The most noteworthy change was a statistically significant increase in the serum glucose levels at all dose levels in both males and females. However, the toxicological significance of these increases is unknown because the values were well within the wide range of measured values for control mice and a dose response was not demonstrated, even though the range of doses was 20-fold.

In male mice significant changes in liver function enzymes LDH, AST (SGOT), and ALP activities were reported. Significant increases of 62 and 33% were observed in serum ALP levels at the 175 and 387 mg/kg-day levels, respectively, in male mice. Such increases, however, showed no dose-response relationship and were not found in the female mice. In female mice, ALT and AST were depressed at all levels of exposure to trans-1,2-DCE; the decreases were statistically significant at the two higher dose levels. In both sexes, sporadic elevations in serum potassium and depressions in serum sodium and calcium were not considered biologically significant. In males, serum glutathione (GSH) levels were depressed 21% in the highest dose group.

In this same study (Barnes et al., 1985), possible effects of trans-1,2-DCE exposure on hepatic microsomal drug metabolism potential were assessed by measuring hexobarbital sleeping time and by evaluating microsomal protein/g liver, CYP450 and cytochrome b5 concentrations, and microsomal activities for aminopyrine N-demethylase and aniline hydroxylase. Hexobarbital sleeping times were not affected in the various dose groups exposed to trans-1,2-DCE in either sex. In male mice, exposure to 175 mg/kg-day trans-1,2-DCE significantly decreased the microsomal metabolizing activities of both aminopyrine N-demethylase (17%) and aniline hydroxylase (27%). In contrast, no significant changes in these enzyme activities were observed in the 387 mg/kg-day exposure group in males. In female mice, aniline N-hydroxylase activity was statistically significantly depressed in all exposure groups, although the decreases (21, 33, and 28%, respectively) were not dose-dependent.

4.2.1.2.3. *Mixtures of cis- and trans-1,2-DCE.* McMillan (1986) conducted a 30-day subchronic study with a 50% mixture of the cis- and trans-isomers orally administered in sesame seed oil (1 mL/kg) to male Sprague-Dawley-derived rats (six/group) at a daily dose of 5 mmol (485 mg/kg-day); control rats received the vehicle alone. At termination, the mean relative weight of the liver in the treated group was 19% greater (statistically significant) than that of control rats, while the mean relative weight of the lungs was significantly reduced by 14%. Mean values for plasma AST activity and creatinine levels in the treated group were significantly reduced by 25 and 17%, respectively, at sacrifice. The mean plasma calcium level in the treatment group was elevated by about 14%, as was plasma chloride by about 3%, while plasma K and CO_2 were slightly depressed. Reductions in erythrocyte count, hemoglobin, and hematocrit were also recorded as decreases of 6, 5, and 5%, respectively.

4.2.1.3. *Chronic Studies*

No chronic toxicity studies for the cis- and trans- isomers of 1,2-DCE administered by the oral route were found.

4.2.2. Inhalation Exposure

4.2.2.1. *Short-term Studies*

No short-term inhalation studies of cis-1,2-DCE, trans-1,2-DCE, or mixtures of cis- and trans-1,2-DCE were identified.

4.2.2.2. *Subchronic Studies*

4.2.2.2.1. ***cis-1,2-DCE.*** No subchronic inhalation studies of cis-1,2-DCE were identified.

4.2.2.2.2. ***trans-1,2-DCE.*** Freundt et al. (1977) exposed six mature female SPR Wistar rats/group for 8 hours/day, 5 days/week to air containing 200 ppm (792 mg/m^3) trans-1,2-DCE for 1, 2, 8, and 16 weeks. Concentrations were monitored by GC. The results of this study, shown in Table 4-7, indicate changes in alveolar septal distension of the lungs and slight to severe fatty degeneration of the liver lobules and Kupffer cells. Pathological changes in the lung were noted and consisted of pulmonary capillary hyperemia and alveolar septal distention in all six rats in all four exposure groups. These changes in the lung were considered by the authors to be slight. These changes were also seen in one of the control animals exposed for 1 week and in two of the control animals exposed for 2 weeks, but not in any of the control animals exposed at either 8 or 16 weeks. This is the only reported study of lung pathology in animals exposed to trans-1,2-DCE. This evaluation of respiratory effects has a few weaknesses: several of the control rats also developed pulmonary capillary hyperemia and alveolar septal distention, a small number of animals were examined, and the upper respiratory tract was not examined for pathology. In addition, a statistical evaluation of the histological data on the respiratory system was not presented in this study.

Table 4-7. Histopathological changes in subchronic inhalation study of trans-1,2-DCE

Exposure	Rat	Liver effect: fat accumulation— liver lobule[a]	Liver effect: fat accumulation— Kupffer cells	Rat	Lung effect: capillary hyperemia, alveolar septum distention
Controls	1–6	0	0	1–5 6	0 +
200 ppm/8 hr for 1 week (5 days)	1–4 5,6	0 +	0 +	1–6	+
Controls	1–6	0	0	1–4 5,6	0 +
200 ppm/8 hr for 2 weeks (5 days/week)	1,2 3–6	0 +	0 +	1–6	+
Controls	1–5 6	0 0	0 ++	1–6	0
200 ppm/8 hr for 8 weeks (5 days/week)	1–3 4–6	0 +	0 ++	1–6	+
Controls	1–4 5,6	0 +	0 +	1–6	0
200 ppm/8 hr for 16 weeks (5 days/week)	1 2,3 4–6	0 + ++	0 + +	1–6	+

0 = no change; + = slight change; ++ = severe change.

Source: Freundt et al. (1977).

The other effects noted were histopathological changes observed in the liver and included fat accumulation of liver lobules and Kupffer cells. After exposure to 792 mg/m^3 for 1 week, slight fat accumulation in liver lobules and Kupffer cells occurred in two of the six rats but not in any of the controls. When rats were exposed for 2 weeks under the same conditions, slight fat accumulation in liver lobules and Kupffer cells occurred in four of the six rats but not in any of the controls. After exposure to 792 mg/m^3 for 8 weeks, three of the six rats showed evidence of slight changes in the liver lobules and severe changes in the Kupffer cells. At this exposure for 8 weeks, severe fat accumulation was also noted in the Kupffer cells in one of the six controls. When rats were exposed for 16 weeks under the same conditions, slight changes in the Kupffer cells and severe changes in the liver lobule were noted in three of the six exposed rats. Slight changes in both the Kupffer cells and in the liver lobules occurred in two other treated animals in this 16-week exposure group for a total of five of the six rats showing some liver effect in this exposure group. However, slight changes in both the Kupffer cells and in the liver lobule also occurred in two of the control animals in the 16-week exposure group. For each of the exposure durations (1, 2, 8, and 16 weeks) there was no statistically significant difference between the controls and the exposed groups with respect to the incidence of liver effects (fat accumulation

or Kupffer cells). However, in general, the incidence and severity of fat accumulation increased with increasing exposure duration.

As described in an abstract (Kelly et al., 1999), the subchronic toxicity of trans-1,2-DCE (>99.4% pure) was evaluated in Crl:CD®BR male and female rats exposed to analytically determined mean concentrations of 0, 200, 1,000, or 4,000 ppm (0, 792, 3,960, or 15,800 mg/m^3) for 6 hours/day, 5 days/week for 90 days. There were no effects on body weight or on food consumption. Clinical symptoms during exposures or during an observation period of 1 month post exposure were not remarkable. Liver cell proliferation was evaluated in five rats of both sexes after 7, 45, and 90 days, while clinical pathology was evaluated in an equal number of males and females (10/sex/group) at 45 and 90 days. Anatomical pathology was evaluated at 90 days (10/sex/group) and after a 1-month post exposure period (5/sex/group). No exposure-related effects were seen in clinical or anatomic pathology parameters or on liver cell proliferation.

4.2.2.2.3. *Mixtures of cis- and trans-1,2-DCE.* A subchronic inhalation study was conducted by Dow (1962) in which rats, rabbits, guinea pigs (strains not stated), and beagle dogs were exposed to 0, 500, or 1,000 ppm (0, 1,980, or 3,960 mg/m^3) 1,2-DCE mixture (58% cis-, 42% trans-isomer), 7 hours/day for 6 months. The 1,980 mg/m^3 exposure groups consisted of 24 male and 35 female rats, 7 male and 8 female guinea pigs, 3 male and 3 female rabbits, and 2 female dogs, while the 3,960 mg/m^3 exposure groups consisted of 12 male and 12 female rats and 2 male and 2 female rabbits. In addition to the animals receiving daily 7-hour exposures, separate groups of 10 male rats were exposed to 1,980 mg/m^3 1,2-DCE for 4, 2, or 1 hour(s)/day for a total duration of 5 months. In all studies each animal was weighed twice per week until growth was determined to be normal; afterwards, each animal was weighed once per week. Hematological analyses and clinical chemistry determinations were performed on all rabbits, on five male and five female rats exposed to 3,960 mg/m^3, and on all dogs exposed to 1,980 mg/m^3.

Rats and rabbits exposed to 3,960 mg/m^3 of 1,2-DCE, 7 hours/day (136 exposures in 195 days) did not exhibit increased mortality or clinical signs of toxicity. Growth of animals was normal, and final body weights and weights of lungs, heart, spleen, and testes were not significantly different from controls. Hematology and biochemical values were within normal limits. The average relative kidney weights in male and female rats were increased by 16 and 9%, respectively (statistically significant only in the males). Average relative liver weights of female rats were statistically significantly increased by 23%. Liver weights in both male and female rabbits were also increased, but statistical significance was not determined because of the small number of rabbits tested.

Rats exposed to 1,980 mg/m^3 of 1,2-DCE, 7 hours/day, for six months did not demonstrate excess mortality or adverse clinical effects. Hematology and clinical chemistry values were within normal limits. Terminal body weights and relative weights of lungs, heart,

spleen, and testes were not significantly different from controls, but relative kidney weights of male and female rats were increased by 9 and 18%, respectively (statistically significant only in the males). Liver weights of female rats were significantly increased by 19%. No noteworthy effects on mortality, behavior, or appearance were observed in the guinea pigs exposed to 1,980 mg/m^3 on 81 of 117 days. Final average body weights and organ weights were not significantly different from controls. Rabbits exposed to 1,980 mg/m^3 for 131 exposures in 181 days exhibited no effects, except that increases in liver weights of both male and female rabbits occurred at termination (statistical evaluations were not performed because of the small number of experimental animals). Female dogs exposed to 1,980 mg/m^3 tolerated 129 exposures in 183 days without biologically significant effects. Clinical chemistry and hematology data were essentially identical to values obtained prior to initiation of the 1,980 mg/m^3 exposure regimen.

In rats exposed to 1,980 mg/m^3 1,2-DCE for shorter periods of 4, 2, or 1 hour/day, 5 days per week, for 5 months (136 exposures in a period of 195 days), no clinical or behavioral abnormalities were seen, and final body weight and organ weight data were not significantly different from control values. The BUN and ALP values were within normal limits.

4.2.2.3. *Chronic Studies*

No chronic inhalation exposure studies were identified for either cis- or trans-1,2-DCE.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

The available studies of reproductive and developmental outcomes are limited for both the cis- and trans- isomers of 1,2-DCE. There is a negative inhalation teratogenicity study that investigated reproductive and developmental toxicity of trans-1,2-DCE via inhalation (DuPont, 1988a). In this study, few developmental parameters were affected by treatment and these were observed only in the high-exposure groups. In addition, range-finding studies on the developmental toxicity of a mixture of 1,2-DCE (composition of isomers unknown) via the oral route of exposure (NTP, 1991a, b, c) showed no signs of developmental or maternal toxicity at any of the initial doses tested (up to 2,918 mg/kg-day), but at higher doses (up to 6,906 mg/kg-day) showed maternal toxicity in the form of reduced maternal body weight and reduced maternal weight gain.

4.3.1. Oral Exposure
4.3.1.1. *cis-1,2-DCE*

No studies of reproductive or developmental toxicity of cis-1,2-DCE in animals following oral exposure were found.

4.3.1.2. *trans-1,2-DCE*

In a 14-week toxicity study described above in Section 4.2.1.2.2, NTP (2002) fed F344/N rats and B6C3F$_1$ mice diets containing microcapsules with a chemical load of 45% trans-1,2-DCE. The rats (10/sex/group) received average daily trans-1,2-DCE dietary doses of 0, 190, 380, 770, 1,540, and 3,210 mg/kg-day for males and 0, 190, 395, 780, 1,580, and 3,245 mg/kg-day for females. In the mouse study (10/sex/group), males received 0, 480, 920, 1,900, 3,850, and 8,065 mg/kg-day and females received 0, 450, 915, 1,830, 3,760, and 7,925 mg/kg-day (NTP, 2002). There were no organ weight changes or gross or microscopic lesions observed in the reproductive organs of rats or mice that would suggest that trans-1,2-DCE targets the reproductive system.

4.3.1.3. *Mixtures of cis- and trans-1,2-DCE*

NTP conducted a series of developmental toxicity range-finding studies in mice and rats with a mixture of 1,2-DCE isomers (composition unknown). The test compound was administered in the feed in the form of microcapsules. Macroscopic or microscopic testing for malformations was not conducted. In the mouse study (NTP, 1991a), 12 pregnant CD-1 mice/group were given feed containing 1,2-DCE mixture at concentrations of 0, 0.05, 0.25, 0.5, 1, and 1.5% on gestation days (GDs) 6–16. Doses were calculated based on feed intake and body weight as 0, 97, 505, 979, 2,087, and 2,918 mg/kg-day, respectively. Body weight, feed consumption, and any signs of toxicity were monitored. Dams were sacrificed on GD 17 and their uteri examined. Gravid uterus weights, fetal body weights, and numbers of fetuses (live/dead), implantation sites, and resorptions were recorded. None of the parameters showed any deviation from control values. The authors concluded that, based on this range-finding study, 1,2-DCE treatment did not cause maternal or developmental toxicity in mice at any of the tested dose levels.

Ten pregnant Sprague-Dawley rats/group were subjected to the same experimental protocol as above (NTP, 1991b) and received feed containing 0, 0.2, 1, 2, or 4% 1,2-DCE mixture on GDs 6–16, resulting in doses of 0, 135, 672, 1,228, 1,966, and 2,704 mg/kg-day, respectively. Dams were sacrificed on GD 20. The same parameters as in the mouse study were examined. There were no signs of developmental or maternal toxicity at any of the levels tested. Four animals of the highest dose group were found not to be pregnant; the study authors considered this to be an isolated event not related to chemical treatment. A repeat of this study was then undertaken with higher doses (NTP, 1991c). Pregnant Sprague-Dawley rats were exposed on GDs 6–16 to feed containing 0, 4, 7.5, or 10% 1,2-DCE mixture, corresponding to doses of 0, 3,134, 5,778, and 6,906 mg/kg-day. Dams were sacrificed on GD 20. The same parameters as above were monitored. There was no mortality. Feed intake was dose-dependently reduced. Maternal weight gain was dose-dependently reduced and statistically significantly different from controls in the mid- and high-dose groups. Maternal body weights

were statistically significantly reduced at the 3,134 (3 and 15% on GDs 14 and 16, respectively), 5,778 (6, 7, 7, 9, and 8% on GDs 9, 11, 14, 16, and 20, respectively), and 6,904 mg/kg-day doses (11, 12, 12, 14, and 10% on GDs 9, 11, 14, 16, and 20, respectively). Pregnancy outcome numbers or fetal body weights were not affected by the treatment. Based on the dose ranges used in this dose range-finding study, the authors concluded that DCE treatment caused maternal toxicity at all dose levels based on reduced body weight. However, no changes were noted in the limited number of fetal parameters evaluated in the study.

4.3.2. Inhalation Exposure
4.3.2.1. *cis-1,2-DCE*

No studies of reproductive or developmental toxicity of cis-1,2-DCE in animals following inhalation exposure were found.

4.3.2.2. *trans-1,2-DCE*

In a study conducted by DuPont (1988a), and published in Hurtt (1993), trans-1,2-DCE was administered to 24 pregnant female Crl:CD$^®$BR rats/group by inhalation, 6 hours daily, on GDs 7–16. Selection of the exposure levels was based on an MTD study conducted with pregnant female rats prior to the actual experiment. On the basis of the pilot study, exposure levels chosen for the actual study were 0, 2,000, 6,000, and 12,000 ppm (0, 7,920, 23,760, and 47,520 mg/m^3, respectively). The low-exposure group concentration level was chosen to be 10 times the ACGIH TLV. Maternal body weight and feed consumption data were observed and analyzed. Fetal weights were also noted. During the first two days of dosing, dams exposed to 23,760 mg/m^3 showed slight weight gain suppression, and the 47,520 mg/m^3 exposure group showed statistically significant weight loss. Additionally, a statistically significant suppression of body weight gain was noted in animals at the 23,760 mg/m^3 concentration on GDs 11–13. For the entire dosing period, a significantly reduced weight gain was observed only at the 47,520 mg/m^3 concentration. Feed consumption was significantly reduced in the 23,760 and 47,520 mg/m^3 groups throughout the exposure period. In the 7,920 mg/m^3 group, there was a significant decrease in feed consumption during GDs 13–15, although no significant effects on body weight were reported. No significant changes in body weight or food consumption were observed in any other group. As seen in maternal body weight change, no significant differences were noted in feed consumption in the pre- or post exposure periods. Eye irritation was observed in rats at all exposure levels. The only other compound-related clinical or postmortem findings were increases in alopecia, salivation, and lethargy in rats during the periods of exposure, especially in the high-exposure group.

Dams were sacrificed on GD 22 and their uteri examined. The mean number of resorptions per litter was statistically significantly increased in dams in the 23,760 mg/m^3 and 47,520 mg/m^3 exposure groups. The values for resorptions in mid- and high-exposure groups

were within the range of historical controls in recent studies (past 2 years) conducted by the laboratory and were not considered to be biologically significant but rather an artifact of the unusually low resorption rate in the concurrent control group (0.3 mean resorptions per litter). There were no differences in the pregnancy rate, fetuses per litter, number of stunted fetuses, or number of corpora lutea observed per female. Developmental variations per litter were not significantly increased in any of the exposure groups. No significant differences were detected in the mean percent of fetuses per litter with malformations at any exposure level.

In conclusion, treatment-related maternal and developmental toxicities were only observed in high-concentration groups. Maternal toxicity was evidenced by statistically significant decreases in body weight and feed consumption at 47,520 mg/m^3 (the highest exposure concentration tested), and by significant decreases in feed consumption at 23,760 and 7,920 mg/m^3. The decrease in feed consumption at 23,760 mg/m^3 was expressed as an observed effect on body weights only on GDs 11–13. At 7,920 mg/m^3, the effect on feed consumption, seen only on GDs 13–15, was minimal and not accompanied by a significant body weight change. Although the body weight change was lower for this group compared with controls, their feed consumption was consistently lower than controls throughout the study. According to the authors, this change was not accompanied by a statistically significant decrease in body weight, and, therefore, its biological significance is questionable. Additionally, a statistically significant trend was noted in the incidence of females with clinical findings on GDs 7–16, but this was the result of ocular irritation in most animals. Significant developmental toxicity (decreased mean fetal weight) was evident among fetuses exposed to 47,520 mg/m^3 trans-1,2-DCE.

4.3.2.3. *Mixtures of cis- and trans-1,2-DCE*

No studies of reproductive or developmental toxicity of mixtures of 1,2-DCE in animals following inhalation exposure were found.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES
4.4.1. Acute Studies
4.4.1.1. *Oral Exposure*
4.4.1.1.1. *cis-1,2-DCE.* In an acute hepatotoxicity study reported in a dissertation by McMillan (1986), cis-1,2-DCE was administrated orally by gavage at doses of 26 mmol/kg (2,521 mg/kg) and 51 mmol/kg (4,944 mg/kg) in sesame seed oil to six male Sprague-Dawley rats/dose. The GSH levels were statistically significantly elevated 19 and 28% for the 2,521 and the 4,944 mg/kg doses, respectively. The ALT activity was unchanged by either dose, but AST activity was statistically significantly elevated 56% for the 4,944 mg/kg dose of cis-1,2-DCE.

4.4.1.1.2. *trans-1,2-DCE.* Male and female Sprague-Dawley-derived CD rats, 22–30 days of age, were administered a range of single doses of trans-1,2-DCE via corn oil gavage in a study conducted by Hayes et al. (1987). The total volume of solution administered in this acute study was 10 mL/kg. There were five dosage groups (exact doses not given), consisting of 10 rats/sex/group. Symptoms of dose-dependent central nervous system (CNS) depression, ataxia, and depressed respiration were observed at all doses; all deaths occurred within 30 hours of dosing. Although the exact dosages were not reported in this study, the authors determined that the LD_{50} was 7,902 mg/kg (95% confidence interval [CI] 6,805–9,175 mg/kg) and 9,939 mg/kg (CI 6,494–15,213 mg/kg) for male and female rats, respectively. Gross necropsy findings of all rats that died were negative. No consistent compound-related gross pathological findings were observed at necropsy.

  An oral LD_{50} test was performed by Freundt et al. (1977) in which mature female SPF Wistar rats received doses of 2–8 mL/kg trans-1,-2-DCE dissolved in olive oil (totaling 10 mL/kg each dose) via gavage. The LD_{50} was reported to be 1.0 mL/kg (95% CI: 0.9–1.1 mL/kg) (1,280 mg/kg) for the rats treated via gavage. (The action of trans-1,2-DCE given orally is more pronounced than after i.p. doses; described in Section 4.4.4.1.). One rat exhibited gross pathology including pulmonary capillary hyperemia and alveolar septal distention and fibrous swelling and hyperemia with incipient disorganization of the cardiac muscle. In two rats, severe fatty infiltration of the liver lobules and Kupffer cells was found.

  In an acute hepatotoxicity study reported in a dissertation by McMillan (1986), trans-1,2-DCE was administered orally as a single dose of 51 mmol/kg (4,944 mg/kg). As with the study conducted on the cis- isomer, six male rats were in each dose group. No differences were seen between the controls and rats administered the single 4,922 mg/kg-day dose of trans-1,2-DCE.

  Barnes et al. (1985) also evaluated the acute oral toxicity of trans-1,2-DCE in 6-week-old male and female CD-1 mice. Trans-1,2-DCE was administered via gavage as a single dose after an 18-hour fast. Nine different doses, ranging from 800–3,500 mg/kg of trans-1,2-DCE in 0.01 mL 1:9 emulphor:water vehicle per gram body weight, were used to generate dose-response curves for DCE-induced mortality. The mice were observed continuously for 4 hours following gavage and then twice daily for 14 days. All decedents and mice surviving the 14-day period were subjected to gross necropsy. The LD_{50} was determined by log probit analysis to be 2,122 mg/kg (95% CI: 1,874–2,382) for male mice and 2,391 mg/kg (95% CI: 2,055–2,788) for female mice, respectively. Upon gross necropsy, target organs were the lungs and liver. The lethality of the test agent was attributed to depression of the CNS, as signs of decreased activity, ataxia, suppression of the righting reflex, ruffled fur, and hunched back were seen. Hyperemia of the mucosal surface of the stomach and small intestines was also observed at necropsy.

4.4.1.1.3. *Mixtures of cis- and trans-1,2-DCE.* Dow (1960) reported that a dose of 2,000 mg/kg 1,2-DCE (isomer composition not stated) as a 10% solution in corn oil administered by gavage was not lethal to the exposed rats in this range-finding study. The Dow (1960) study noted that "some kidney injury" was observed at necropsy, but that no other reactions were noteworthy.

4.4.1.2. *Inhalation Exposure*

4.4.1.2.1. *cis-1,2-DCE.* A 4-hour inhalation median lethal concentration (LC_{50}) study with groups of five male and five female Crl:CD$^®$BR rats was conducted with cis-1,2-DCE (DuPont, 1999). The exposure concentrations were 0, 12,100 (47,900 mg/m^3), 13,500 (53,400 mg/m^3), 15,700 (62,200 mg/m^3), and 23,200 ppm (91,900 mg/m^3). During a 14-day recovery period, rats were weighed and observed for signs of clinical toxicity. All rats underwent gross pathological examination immediately after death or at the end of the recovery period and the liver, kidney, heart, and lung were evaluated histologically. The LC_{50} was calculated to be 54,200 mg/m^3. It was noted that the rats were prostrate, with eyes open, but unresponsive to alerting stimuli during exposure. Other clinical signs included weakness and irregular respiration immediately after exposure. After the 47,900 mg/m^3 cis-1,2-DCE exposure, rats showed weakness, but there were no effects on body weight at this concentration. Rats that did not die (two of five males and two of five females) at the 53,400 mg/m^3 exposure showed weakness and irregular respiration immediately after the exposure and showed slight to severe weight loss for one day after exposure, followed by a normal weight gain rate. All five of the male rats exposed (all of which had died during the study) to 62,200 mg/m^3 of the cis-isomer had minimal hepatic centrilobular vacuolation. One male rat in each of the lower exposure levels (47,900 and 53,400 mg/m^3) also had minimal hepatic centrilobular vacuolation upon microscopic examination. There was one male rat in the highest exposure group with this lesion. Since all five male rats at the high concentration died during exposure, these animals may not have survived long enough to develop the lesion. There were no exposure-related effects observed in female rats exposed to cis-1,2-DCE. No effects were seen in the heart, kidney, or lungs in exposed rats.

4.4.1.2.2. *trans-1,2-DCE.* In studies similar to those conducted with the cis-isomer, DuPont (1999) conducted a 4-hour acute inhalation study with trans-1,2-DCE, using five male and five female Crl:CD$^®$BR rats per exposure level. The exposure concentrations used in this study were 0, 12,300 (48,700 mg/m^3), 22,500 (89,100 mg/m^3), 28,100 (111,300 mg/m^3), and 34,100 ppm (135,000 mg/m^3). At the 48,700 mg/m^3 exposure, rats recovered and resumed a normal appearance within about 30 minutes after the end of the exposure. There were no effects on body weight at this concentration. Rats that survived the 89,100 mg/m^3 exposure showed lethargy and irregular respiration immediately after exposure and showed slight weight loss for one day, followed by a normal weight gain rate. Rats exposed to 111,300 mg/m^3, showed

weakness immediately after exposure and slight to severe weight loss for one day. Unlike effects seen with cis-1,2-DCE, no compound-related effects were observed in livers of rats exposed to trans-1,2-DCE at concentrations up to 135,000 mg/m^3. No effects were seen in heart, kidneys, or lungs of exposed rats. The LC_{50} was determined at 95,400 mg/m^3; the authors concluded that trans-1,2-DCE was about half as acutely toxic as the cis-isomer by the inhalation route.

Freundt et al. (1977) exposed six mature female SPR Wistar rats/group once to trans-1,2-DCE concentrations of 0, 200, 1,000, and 3,000 ppm (0, 792, 3,960, and 11,880 mg/m^3, respectively) for 8 hours. Test agent concentrations were monitored by GC. Various parameters including symptoms of CNS depression, quantitative determination of serum components (cholesterol, calcium, inorganic phosphate, total bilirubin, albumin, total protein, uric acid, urea nitrogen, glucose, ALP), and hematological parameters (hemoglobin, cell volume, mean corpuscular volume, mean corpuscular hemoglobin) were evaluated.

No symptoms of CNS depression were observed at the concentrations used. Clinical chemistry values for serum albumin, BUN, and ALP were statistically significantly depressed (11, 20, and 16%, respectively) in the rats exposed to 3,960 mg/m^3; values for 11,880 mg/m^3 were not reported. Freundt et al. (1977) noted pathological changes in the hearts of rats exposed to trans-1,2-DCE after a single 8-hour exposure to 11,880 mg/m^3 but not after exposures to lower levels. These changes were described as severe fibrous swelling of the myocardium and hyperemia. No other reports of cardiac toxicity were identified elsewhere in the literature. Blood leukocyte counts were reduced in rats exposed to 792 and 3,960 mg/m^3, while erythrocyte counts were significantly reduced in rats exposed to 3,960 mg/m^3 trans-1,2-DCE. (The actual blood leukocyte counts and erythrocyte counts were not given.) Histopathological changes were seen, including fatty degeneration of liver lobules and Kupffer cells and capillary hyperemia with distension of the alveolar septa of lungs. At 792 mg/m^3 (the TLV), slight fatty degeneration of the liver occurred in one of six rats, and hyperemia of the lung with alveolar septum distention occurred in all members of the group. At 3,960 mg/m^3, liver degeneration was seen in two of six rats, and lung changes were seen in five of six rats. The same incidence (2/6 rats) of liver degeneration was noted at 11,880 mg/m^3, and five of six rats had lung effects. In addition to effects on liver and lungs, severe fibrous swelling and hyperemia with barely maintained striation of the myocardium were noted (% not indicated) at 11,880 mg/m^3.

Gradiski et al. (1978) evaluated the toxicity of trans-1,2-DCE by using groups of 20 female OF1 mice exposed for 6 hours to five airborne concentrations. The LC_{50} was determined graphically to be 21,723 ppm (86,000 mg/m^3). On the basis of the high LC_{50}, the inhalation toxicity of trans-1,2-DCE was judged by the author to be lower than that of nine other chlorinated aliphatic solvents that were tested concurrently.

4.4.1.2.3. *Mixtures of cis- and trans-1,2-DCE.* Lehmann (1911) reported inhalation experiments in which four cats were exposed to 50–72 mg/L (50,000–72,000 mg/m^3) 1,2-DCE;

the isomer composition was not stated. The cats demonstrated varying degrees of narcosis; with symptoms of salivation, sneezing, disturbance of balance, and prostration. Two of the cats died.

Acute inhalation studies with 1,2-DCE (isomer composition not stated) were conducted by Dow (1960). Exposure levels of 0, 7,297, 14,814, 16,810, 29,035, and 50,123 ppm (28,900, 58,740, 66,650, 115,120, and 198,700 mg/m^3) were utilized; nine male rats/group were exposed for periods up to 7 hours. Rats exposed to concentrations above 115,120 mg/m^3 rapidly became unconscious, with rapid breathing and tremors, and exposures lasting longer than 0.2 hours were fatal. Six of nine rats exposed to 66,650 mg/m^3 for 4 or 7 hours died. One hour after exposure at this concentration, the rats developed tremors and made running movements while lying on their sides. Slight liver and lung pathology were observed 1 day after exposures; 1 week after exposures, slight liver and lung injury plus moderate kidney injury were reported. No rats died after exposure to 28,900 mg/m^3 1,2-DCE for 7 hours or after exposure to 58,740 mg/m^3 for 1 hour. No LC$_{50}$ was calculated.

4.4.2. In Vivo Neurological Behavioral Studies

Inhibition of propagation and maintenance of an electrically evoked seizure discharge was used as a criterion for neurotropic effects in experimental animals by Frantik et al. (1994). The studies were designed to measure the concentrations of 1,2-DCE (isomer not stated) and 47 other volatile solvents required to inhibit electrically evoked acute neurotoxic symptoms, a measure of subclinical CNS depression. Effect-air concentration regressions for 1,2-DCE and 47 other volatile solvents were determined after 4-hour inhalation exposures in adult male Wistar-derived rats (0.5–1 year old) and H strain female mice (2–4 months old). Four exposed animals and four untreated controls were tested at four to five solvent concentrations, ranging from 90 to 21,000 ppm in rats and 300 to 24,000 ppm in mice. Each experiment was repeated.

As measured by the tonic extension of hind limbs in rats and the velocity of tonic hind-limb extension in mice, the mean latency of responses to short electrical stimuli (0.2 seconds, 50 Hz, 180 V in rats and 90 V in mice) was evaluated graphically. The critical level of effect (the effect in the lower third part of the dose-response function corresponding to the shortening of the tonic extension of hind-limbs by 3 seconds in rats and the lengthening of the latency of extension by 0.6 seconds in mice) and the threshold for slowing the propagation or shortening of the duration of seizures by 10% of the maximum effect possible (EC$_{10}$) were determined. Values for this critical level of effect were generally several times lower than airborne concentrations evoking behavioral inhibition in animals and 1–2 orders of magnitude lower than concentrations inducing narcosis. The mean concentration of 1,2-DCE evoking a 30% depression in response in rats was 1,810 ppm with a one-sided 90% CI of 245 ppm and a slope of the regression line of 0.022%/ppm. Equivalent data for mice were 3,400 ppm for a 30% depression in response with a one-sided 90% CI of 490 ppm and a slope of the regression line of 0.02%/ppm. Frantik et al. (1994) proposed that their EC$_{10}$ values could be used to evaluate the efficacy of short-term

exposure limits for protection of workers from acute nervous depression and other subnarcotic effects, such as headaches, impairment of vigilance, and lowered reliability of performance.

Concentration-dependent behavioral changes in male Swiss OF1 mice, following a 4-hour exposure to 1,2-DCE (isomer not stated) and to 12 other aliphatic and aromatic solvents, were evaluated by DeCeaurriz et al. (1983). Tests were conducted to determine whether the test agent reduced immobility developed in the "behavior despair" swimming test. Concentration-related reductions in immobility during a 3-minute test were seen for all solvents; the percent decreases in immobility vs. exposure concentration for each agent (4–5 concentrations) were graphically depicted. The concentration of 1,2-DCE required for a 50% decrease in immobility (ID_{50}) was 1,983 ppm (95% CI: 1,708–2,309 ppm). Most of the solvents tested were considerably more effective than 1,2-DCE as inhibitors of immobility; only methyl ethyl ketone and 1,1,1-trichloroethane were less effective than 1,2-DCE. A good correlation (r = 0.93) between ID_{50} values and 1981 ACGIH occupational exposure standards for the chemicals tested was demonstrated.

Kallman and Balster (1983) studied disruption of reinforced operant behavior in groups of nine mice. The animals were trained to depress a lever, and the correct behavior was reinforced with sweetened milk. Mice were gavaged with daily doses of 100 mg/kg or more 1,2-DCE (isomer not stated) 30 minutes after the daily operant session for a minimum period of 1 week. A dose of 300 mg/kg-day or more disrupted the reinforced operant behavior. Continued exposure at this level produced initial decreases with a gradual return to baseline performance within 2 days. This pattern was maintained at doses below the MTD (800 mg/kg-day). When 1,2-DCE exposure was terminated, the mice recovered their conditioned behavior within 15 days.

Taste aversion to saccharin induced by 1,2-DCE in male CD-1 mice was also reported by Kallman et al. (1983). In conditioning trials for a period of 7 days, groups of seven mice were accustomed to 30-minute sessions of drinking from two spouts that provided access to 0.3% sodium saccharin or deionized water. Five minutes after session completion, gavage doses of 30–2,000 mg/kg 1,2-DCE in 1:9 emulphor:water were administered. Twenty-four hours after the final conditioning treatment, the groups of mice were subjected to the 30-minute two-bottle choice test (saccharin vs. deionized water), with careful monitoring of fluid consumption of saccharin and water from the bottles. Doses of 300–2,000 mg/kg 1,2-DCE (but not lower concentrations of 30 and 100 mg/kg) significantly depressed consumption of sodium saccharin offered in the 30-minute preference test. The effective dose (ED_{50}), the dose of 1,2-DCE that reduced saccharin solution consumption by 50%, was graphically determined to be 144.5 mg/kg. Intake of deionized water was also reduced when offered after daily gavage doses of 1,2-DCE but was statistically significantly reduced only after a dose of 2,000 mg/kg. Other halogenated compounds, such as chloral, 1,1,2-trichloroethane, and 1,2-dichloroethane, were more potent

than 1,2-DCE in inducing conditioned taste aversion. The threshold for behavioral effects of 1,2-DCE in these studies was about 100 mg/kg.

4.4.3. Immunological Studies
4.4.3.1. *cis-1,2-DCE*
No immunotoxicity studies of cis-1,2-DCE were located.

4.4.3.2. *trans-1,2-DCE*
In a subchronic assessment of immunotoxicity, 4-week-old male CD-1 mice (10–12/group) with an average initial weight of approximately 30 g were gavaged with solutions of trans-1,2-DCE (0, 22, and 222 mg/kg, or 1/100 and 1/10 the LD_{50}) on 14 consecutive days (Munson et al., 1982). At necropsy, there were no significant effects on liver, spleen, lungs, thymus, kidney, or brain weights. Leukocyte counts for the experimental groups did not differ significantly from the untreated control group. Munson et al. (1982) evaluated humoral immune function as indicated by the ability of the spleen cells to produce IgM antibody-forming cells (AFCs) following challenge with sheep red blood cells (sRBCs). The authors reported the antibody response to sRBCs challenge on day 11 as the number of AFCs per spleen and per 10^6 spleen cells from animals killed 24 hours after the last treatment. A trend towards suppression of the number of AFCs expressed per spleen basis (significant at $p < 0.1$ level) was observed with trans-1,2-DCE; however, this response was not statistically significant at the $p < 0.05$ level or when expressed per 10^6 spleen cells. Munson et al. (1982) also assessed cell-mediated immune response as measured by the delayed-type hypersensitivity (DTH) response to sRBCs. The response was characterized as slight but significant ($p < 0.05$) and not dose-dependent in the abstract of the journal article. However, in the results section of the article the authors stated that trans-1,2-DCE showed no effect in the DTH response. This contradictory presentation of the data between the abstract and results sections renders these study findings unreliable. It is unknown whether the slight reduction in DTH was associated with trans-1,2-DCE or trichloroethylene, another chemical tested in the study. The authors concluded that mice exposed to trans-1,2-DCE for 14 days at doses up to 222 mg/kg-day showed no significant change in cell-mediated or humoral immunity (Munson et al., 1982).

The immunotoxicity of trans-1,2-DCE was also investigated in studies in which three concentrations, 0.1, 1.0, and 2.0 mg/mL, were provided to male and female CD-1 mice (10 mice/group) in drinking water containing 1% emulphor (Barnes et al., 1985; Shopp et al., 1985). These drinking water concentrations were equivalent to doses of 17, 175, and 387 mg/kg-day in male mice and 23, 224, 452 mg/kg-day in female mice. The study by Shopp et al. (1985) reported assays for effects of the test agent on the immune system, while Barnes et al. (1985) reported the study details and systemic toxicity findings.

In a preliminary 14-day study involving gavage exposure to the test agent at 0.1 or 1.0 mg/mL (21 or 210 mg/kg), Shopp et al. (1985) reported no statistically significant effects of trans-1,2-DCE on the humoral immune status of male mice as measured by the production of AFCs against sRBCs. Cell-mediated immune status, measured by the DTH response to sRBCs, was also unaffected in male mice dosed with trans-1,2-DCE for 14 days. Body weight was not affected in male or female mice at either dose of trans-1,2-DCE in the 14-day study.

In the same study by Shopp et al. (1985), three assays were utilized to evaluate humoral immune status in both male and female mice following 90 days of exposure to trans-1,2-DCE in drinking water at concentrations up to 2.0 mg/ml. These assays included quantification of spleen AFCs directed against sRBCs on days 4 and 5 after antigen presentation, hemagglutinin titers to sRBCs, and spleen cell response to the B cell mitogen lipopolysaccharide (LPS).

Body weight was not affected in male or female mice at any dose of trans-1,2-DCE following 90 days of exposure. The AFC results are shown in Table 4-8. The number of AFCs per 10^6 spleen cells was reduced by 26% in male mice exposed to trans-1,2-DCE at doses of 175 and 387 mg/kg-day (significantly different at $p < 0.05$ from control mice given deionized water). When expressed on a per spleen basis, the numbers of AFCs in male mice were significantly reduced at all exposure concentrations tested (equivalent to doses 17, 175, and 387 mg/kg-day). However, the expression of AFCs on a per spleen basis is affected by changes in the relative size of the spleen. Therefore, to avoid effects due to differences in relative spleen size, the number of AFCs per 10^6 spleen cells is considered the more appropriate measure. Spleen weights were not significantly affected by the treatments. Females responded normally except for mice in the 0.1 mg/mL group (23 mg/kg-day), which demonstrated a 32% decrease in AFC response on a total spleen basis.

Table 4-8. Humoral immune response to sRBCs in CD-1 mice exposed to trans-1,2-DCE in drinking water for 90 days (Day 4)

Exposure group	Spleen weight (mg)	Antibody-forming cells per spleen ($\times 10^{-5}$)	Antibody-forming cells per 10^6 cells
Males[a]			
Control	202 ± 30	4.48 ± 0.32	$2{,}200 \pm 125$
0.1 mg/mL	164 ± 13	3.28 ± 0.28[b]	$2{,}048 \pm 152$
1.0 mg/mL	178 ± 6	3.34 ± 0.39[b]	$1{,}625 \pm 136$[b]
2.0 mg/mL	173 ± 10	2.87 ± 0.37[b]	$1{,}618 \pm 226$[b]
Females[a]			
Control	228 ± 13	4.38 ± 0.37	$1{,}765 \pm 110$
0.1 mg/mL	176 ± 11[b]	2.97 ± 0.49[b]	$1{,}478 \pm 211$
1.0 mg/mL	230 ± 12	4.51 ± 0.24	$1{,}967 \pm 89$
2.0 mg/mL	191 ± 13[b]	3.47 ± 0.50	$1{,}518 \pm 184$

[a]Values are mean ± SE for 12 mice in the control group and 8 mice in treatment groups, measured on day 4 after antigen presentation.
[b]Values differ significantly from control group ($p < 0.05$).

Source: Shopp et al. (1985).

Hemagglutinin titers in CD-1 mice exposed to trans-1,2-DCE at all dose levels were not significantly changed from control values. Spleen lymphocyte responsiveness to LPS was not altered in the males, but the female mice at the highest dose level demonstrated a statistically significantly enhanced spleen cell response to LPS.

Three assays were also used to evaluate the status of cellular immunity: (1) DTH response to sRBCs challenge, (2) popliteal lymph node proliferation in response to sRBCs, and (3) spleen cell response to concanavalin A (Con A). Male mice exposed to trans-1,2-DCE did not show changes in either the DTH or popliteal lymph node proliferation response to sRBCs, but females exposed to 1.0 mg/mL had a slight increase in the DTH response. No alterations in spleen lymphocyte response to Con A were noted. In addition, the ability of bone marrow cells from mice exposed to trans-1,2-DCE for 90 days to incorporate ^{125}I-labeled deoxyuridine was essentially unaffected by the treatments (Shopp et al., 1985).

In summary, repeated exposure of mice to trans-1,2-DCE in drinking water for 90 days had no effect on the cell-mediated immune status of either sex or on the humoral immune status of females. Shopp et al. (1985) concluded that there was marked suppression in humoral immune status in male mice and that the decrease in AFCs was significantly decreased in these mice. However, the authors also suggested that the decrease in AFCs was not severe enough to depress the functional ability of the humoral immune system because there was no change in hemagglutination titers to sRBCs or lymphoproliferative response of spleen cells to the B-cell

mitogen LPS. Overall, the authors concluded that the immune system of CD-1 mice was not overly sensitive to the effects of trans-1,2-DCE and that the few effects that were seen were probably the result of general toxicity rather than specific target organ toxicity. Additional discussion of these study findings is given in Section 4.6.1.2.

Freundt et al. (1977) reported that inhalation exposure of female SPF Wistar rats to ≥200 ppm caused slight to severe fatty degeneration of Kupffer cells in the liver. In addition, decreased leukocyte counts were observed in rats exposed to 200 ppm and 1,000 ppm trans-1,2-DCE for 8 hours, and pneumonic infiltration was observed in the lungs after exposure to 200 ppm for 8 and 16 weeks, suggesting that inhalation of the test agent may have immunological effects.

4.4.3.3. *Mixtures of cis- and trans-1,2-DCE*

No immunotoxicity studies of mixtures of cis- and trans-1,2-DCE were located.

4.4.4. **Toxicity Studies by Other Routes**

4.4.4.1. *Intraperitoneal Injection*

4.4.4.1.1. *cis-1,2-DCE.* In an acute hepatotoxicity study reported in a dissertation by McMillan (1986), cis-1,2-DCE was administrated i.p. at doses of 21 mmol/kg (2,039 mg/kg) and 26 mmol/kg (2,521 mg/kg) in sesame seed oil to six male Sprague-Dawley rats/dose, so that each rat received 4 mL/kg of body weight. The GSH levels and plasma enzyme activities (ALT, AST, sorbitol dehydrogenase [SDH]) were measured. The GSH and ALT activities were not significantly altered by either dose, but both plasma AST and SDH activities were statistically significantly elevated. For AST, both doses had more than a twofold increase over that seen in controls, while for SDH there was at least a threefold increase when compared with the control. Significant elevations of the activities of the plasma enzymes AST and SDH are indicative of possible liver damage.

In a study (Plaa and Larson, 1965) to obtain data regarding the relative nephrotoxic properties of a series of chlorinated methane, ethane, and ethylene derivatives in mice, kidney function was assessed for cis- and trans-1,2-DCE as well as other chlorinated derivatives by measurement of the excretion of phenolsulfonephthalein (PSP) and by the use of an indicator strip to measure protein and glucose in the urine. In addition to these tests of kidney function, kidney sections were examined histologically. For evaluating cis-1,2-DCE's potential for nephrotoxicity, doses of 0.1 (10 mice), 1.0 (10 mice), and 2.0 mL/kg (6 mice) were dissolved in corn oil and administered i.p. The results show that cis-1,2-DCE failed to cause renal dysfunction. None of the mice examined histologically showed necrosis or swelling.

4.4.4.1.2. *trans-1,2-DCE.* An LD_{50} test was performed by Freundt et al. (1977) in which mature female SPF Wistar rats and mature female NMRI mice were exposed to trans-1,2-DCE

via the i.p. route. The LD$_{50}$ was reported to be 6.0 mL/kg (95% CI: 5.1–7.1 mL/kg)
(7,680 mg/kg) for the rats. The LD$_{50}$ for mice was 3.2 mL/kg (95% CI: 2.8–3.7 mL/kg)
(4,096 mg/kg). The mouse is more sensitive to the effects of trans-1,2-DCE than the rat after i.p.
dosage. In the rat the action of trans-1,2-DCE given orally is more pronounced than after i.p.
doses (see Section 4.4.1.1.2.). The postmortem gross pathology in the mice after administration
of trans-1,2-DCE showed hyperemia involving the liver, kidneys, urinary bladder, and intestines.
The number of dead mice ranged from 1 to 10/10 per dose group. Clinical signs of toxicity were
not reported for mice. In one rat gross pathology included pulmonary capillary hyperemia and
alveolar septal distention and fibrous swelling and hyperemia with incipient disorganization of
the cardiac muscle.

In an acute hepatotoxicity study reported in a dissertation by McMillan (1986),
trans-1,2-DCE was administered i.p. at doses of 20 mmol/kg (1,939 mg/kg-day) and 25 mmol/kg
(2,424 mg/kg) in sesame oil (4 mL/kg) to male Sprague-Dawley rats. As with the study
conducted on the cis- isomer, six male rats were in each dose group. The results of this set of
experiments show that trans-1,2-DCE administered i.p. depressed GSH content (statistically
significant) at the 2,424 mg/kg dose and increased plasma AST and SDH activities in a dose-
related (although not statistically significant) manner. Plasma ALT activity was unchanged.
Also in this study the effects of trans-1,2-DCE on GSH content and plasma ALT, AST, and SDH
activities with respect to time were examined. Two groups of 30 male rats each were used, one
group was the control and the other group was treated i.p. with trans-1,2-DCE. At time intervals
of 2, 4, 8, 12, 24, and 48 hours, five animals from each group were killed. Blood was collected
for plasma enzyme determination, and livers were removed for GSH analysis. SDH activity was
maximally elevated at 4 hours after administration of trans-1,2-DCE and remained elevated.
This SDH activity was 5 times over that of controls at the maximally elevated point at 4 hours.
The AST activity was elevated over the entire time course (5 times over that of controls at the
maximally elevated point at 4 hours), while ALT activity was elevated over control levels for the
first 8 hours of the study (as well as 5 times over the controls at the maximally elevated point at
4 hours). Histopathological results of slight to moderate necrosis show that the greatest potential
hepatotoxicity occurred at 4 and 8 hours. The effects of the trans-isomer were maximal at
4 hours after administration, using plasma enzyme elevations as the indicator of toxicity.
Glutathione depression occurred between 4 and 8 hours after administration. It is important to
note that all the parameters measured, with the exception of AST activity, returned to near
control levels by 12 hours (i.e., the effects were not sustained). The histopathological results
indicated the same time course.

The LD$_{50}$ for trans-1,2-DCE in female OF1 mice via i.p. injection was reported by
Gradiski et al. (1978) to be 2,940 mg/kg. Freundt et al. (1977) reported that the i.p. LD$_{50}$ for
trans-1,2-DCE was 3.2 mL/kg (4,100 mg/kg) for female NMRI mice and 6.0 mL/kg
(7,680 mg/kg) for mature female Wistar rats. Nakahama et al. (2000) treated 7-week-old Wistar

rats (sex not stated) with 0.5 g/kg cis- or trans-1,2-DCE with or without co-treatment with phenobarbital (80 mg/kg). Animals were sacrificed 24 hours after treatment, and body weights as well as relative liver and lung weights were measured. The cis-1,2-DCE caused a small but statistically significant decrease in body weight gain. Both isomers caused increases (although not statistically significant) in relative liver weights. Lung weights were not affected. Pretreatment with phenobarbital had no noteworthy effect on these observations.

4.4.4.2. *Dermal Application*

In dermal toxicity studies (Brock, 1990; DuPont, 1988b), a single dose of 5,000 mg/kg trans-1,2-DCE was applied onto the clipped, intact skin of two male and three female New Zealand White rabbits under an occlusive wrapping. At the end of a 24-hour exposure period, the test material was removed. Test rabbits were examined for clinical signs of toxicity and mortality for 14 days after treatment. No animals died, but signs of severe skin irritation remained throughout the observation period. Mild-to-severe erythema and no-to-severe edema, necrosis, and fissuring of the skin with raw areas and epidermal scaling were observed. Body weight losses of up to 3% of initial weight were observed in three rabbits 1 day following treatment. Under conditions of the assay, the dermal LD_{50} was greater than 5,000 mg/kg body weight.

4.4.4.3. *Eye Irritation*

Brock (1990) reported results of an irritation test with trans-1,2-DCE (99.64% pure) that was conducted at DuPont (1988c). The test agent (0.01 mL) was instilled into the lower conjunctival sac of two female New Zealand White rabbits. Twenty seconds later, the eyes of one rabbit were washed with lukewarm tap water, while the eye of the other rabbit remained unwashed. Eyes were scored for irritation at 1 and 4 hours and after 1, 2, and 3 days. Severe corneal opacity was observed in the washed eye, and moderate iritis and conjunctivitis were observed in both the washed and unwashed treated eyes. Copious blood-tinged discharge was seen in both treated eyes, with moderate and mild chemosis in the washed eye and unwashed eye, respectively. The maximum Draize score was 17/110 for the unwashed eye and 41/110 for the washed eye. Fluorescein stain examinations were positive for corneal opacity in the washed eye and negative in the unwashed eye. Three days after treatment the eyes of both rabbits had returned to normal. Under conditions of the study, trans-1,2-DCE was a severe eye irritant.

Moderate pain and conjunctivitis were reported after 1,2-DCE (isomer not stated) was administered to the eyes of rabbits (Dow, 1960). Some of the eyes were washed after administration. Reactions to the test agent had not completely subsided 1 week after dosing.

4.4.4.4. *Skin Irritation*

Brock (1990) reported results of a skin irritation test with trans-1,2-DCE (99.64% pure) conducted at DuPont (1988d). The test agent, 0.5 mL, was applied onto the clipped, intact skin of one female and five male New Zealand White rabbits under an occlusive wrapping. At the end of a 24-hour exposure period, the material was removed. The site of application was scored for irritation at 24, 48, and 72 hours post treatment. Mild or moderate erythema was observed at all observation times. Under conditions of the study, trans-1,2-DCE was a moderate skin irritant.

In skin irritation studies, 1,2-DCE mixture was applied undiluted 10 times to the intact skin of ears of white rabbits (Dow, 1960). Essentially no irritation was reported following the first eight applications, but slight hyperemia was observed thereafter. The ears of the rabbits appeared normal 21 days after cessation of treatments. Four applications of undiluted 1,2-DCE to the intact belly skin of rabbits caused slight to moderate hyperemia. Slight edema and moderate necrosis of the skin appeared after the third and fourth applications. Undiluted 1,2-DCE was also applied twice to the abraded belly of rabbits. Slight to moderate hyperemia and edema with slight necrosis occurred after the first application, and moderate edema and necrosis were seen after the second application. Slight exfoliation, scabs, and scars were seen 21 days after treatments.

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

4.5.1. Hepatotoxicity Studies

Trans-1,2-DCE (20 mmol [1,940 mg]/kg i.p.) depressed liver aniline hydroxylation (AH), ethylmorphine N-demethylation (EN-D), and CYP450 content, but no effect was seen on NADPH:cytochrome c reductase activity (McMillan, 1986). Administration of phenobarbital with trans-1,2-DCE caused a further depression of all parameters except CYP450. Treatment with β-naphthoflavone and trans-1,2-DCE elevated AH and CYP450 levels but slightly depressed NADPH:cytochrome c reductase activity. When given prior or subsequent to trans-1,2-DCE, SKF-525A had a cumulative depressive effect on all parameters except NADPH:cytochrome c reductase activity. According to McMillan (1986) these data indicate an enhanced depressive effect of trans-1,2-DCE plus phenobarbital with respect to aniline hydroxylase and ethylmorphine N-demethylase activity. The author also concluded that, with respect to CYP450 and cytochrome c reductase, exposure to phenobarbital plus trans-1,2-DCE partially alleviated the depression seen in these parameters with trans-1,2-DCE treatment only.

Freundt and Macholz (1978) provided single 8-hour inhalation exposures of cis- or trans-1,2-DCE to adult female Wistar rats (10/group). Exposure levels of 0, 200, 600, or 1,000 ppm (0, 792, 2,380, or 3,960 mg/m^3) for either isomer resulted in statistically significant, concentration-related increases in hexobarbital sleeping time and zoxazolamine paralysis time at all exposure levels, with the exception of the zoxazolamine paralysis time for rats exposed to the

trans-isomer at 200 ppm, the current TLV. The cis-isomer was the more potent of the two isomers at every exposure level.

Inhalation of cis- and trans-1,2-DCE for 8 hours also caused a statistically significant, exposure-dependent inhibition of renal excretion of 4-aminoantipyrine (AAP) after gavage administration of 20 mg aminopyrine immediately following the exposures. The cis-isomer was also the more potent isomer in this experiment. The 1,2-DCE-induced effect was reversible by 6 hours after termination of exposures (Freundt and Macholz, 1978). These experiments indicated that phase I oxidative metabolism of hexobarbital, zoxazolamine, and aminopyrine was inhibited by exposures to either isomer of 1,2-DCE; the lowest-observed-adverse-effect level (LOAEL) was 200 ppm (792 mg/m^3). The actual concentration to these rats was estimated to be 198 mg/kg, based on a conversion factor of 3.96 mg/m^3 per ppm, 0.30 m^3/day breathing rate for adult female rats weighing 0.2 kg, and 8 hours/day of exposure with an estimated net inhalation retention of 50%. N-acetylation of AAP and O-glucuronidation of 4-hydroxyantipyrine were not affected by an 8-hour exposure of rats to 1,000 ppm trans-1,2-DCE, indicating that phase II enzymes are considerably less sensitive to induction or inhibition by 1,2-DCE. In in vitro studies, N-demethylation of aminopyrine and O-demethylation of p-nitroanisole were competitively inhibited by addition of trans-1,2-DCE to a reaction mixture containing liver microsomes from untreated rats (Freundt and Macholz, 1978). On the basis of these findings, the authors concluded that 1,2-DCE competed for the type I binding site of CYP450.

Jenkins et al. (1972) evaluated the effects of cis- and trans-1,2-DCE on enzyme activities in liver and plasma of adult male Holtzman rats. Twenty hours after administration of 400 or 1,500 mg/kg (4.1 or 15.5 mmol/kg) cis- or trans-1,2-DCE in corn oil (2 mL/kg) to 3–4 rats/group, liver glucose-6-phosphatase (G-6-Pase), ALP, and tyrosine transaminase activities were statistically significantly increased by the cis-isomer; with the exception of G-6-Pase activity in animals receiving 400 mg/kg, these enzyme activities were not significantly elevated after treatment with trans-1,2-DCE. In most instances, plasma ALP and ALT activities did not differ significantly from controls after treatment with either isomer. The authors concluded that the cis-isomer caused a slightly greater biochemical response than the trans-isomer.

Moore (1978), in an abstract, reported that administration of 0.1–1 mL/kg 1,2-DCE (isomer not stated) to rats (strain not given) inhibited the hepatic microsomal calcium pump 24 hours after treatment in a dose-dependent manner by up to 70% at the highest dose. This finding is of some interest in light of several other studies, where increases in blood calcium levels were observed following 1,2-DCE treatment (McCauley et al., 1995; McMillan, 1986; Barnes et al., 1985) because it points to a possible calcium-disturbing action of 1,2-DCE.

Sipes and Gandolfi (1980) used uninduced and phenobarbital-, 3-methylcholanthrene-, or Aroclor 1254-induced rat liver microsomes to demonstrate that several halogenated hydrocarbons, including 1,2-DCE (isomer not stated), bind covalently to protein and lipid, and that binding was increased up to eightfold when induced microsomes were used. However,

1,2-DCE displayed low protein and lipid binding compared with halogenated methanes and ethanes. Furthermore, these authors could not demonstrate any DNA binding with 1,2-DCE, even when phenobarbital-induced microsomal preparations were used, while all other compounds tested positive for this characteristic. The lowest DNA-binding activities were observed with dichloromethane, iodomethane, 1,2-dichloroethane, and 1,1,1-trichloroethane, while the highest activities came from 1,2-dibromoethane, bromotrichloromethane, chloroform, and carbon tetrachloride (Sipes and Gandolfi, 1980).

4.5.2. Nephrotoxicity Studies

The degree of nephrotoxicity elicited by cis- or trans-1,2-DCE administered i.p. to male Swiss mice was evaluated by monitoring urinary excretion of PSP and by detection of urinary protein and glucose excretion with indicator strips (Plaa and Larson, 1965). The chemicals were dissolved in corn oil and administered to 10 mice/dose at doses of 0.1, 1.0, or 2.0 mL/kg (128, 1,280, and 2,560 mg/kg) and 1.0, 2.0, or 4.0 mL/kg (1,280, 2,560, and 5,120 mg/kg) for the cis- and trans-isomer, respectively. Surviving mice in the treatment groups where lethality occurred exhibited a noteworthy delay in excretion (<40% excreted within 2 hours) of the administered dose of 1 mg/kg PSP, while normal untreated mice excreted 67% of the administered dose within 2 hours. Mice in dose groups where no animals died exhibited normal urinary excretion of PSP. Urine was collected from mice surviving 24 hours for evaluation of excretion of protein and glucose. An increased occurrence of urinary excretion of protein (≥100 mg/100 mL) in high-dose animals (Table 4-9) was observed with both isomers. There was no detectable amount of glucose in 60 control mice, and no detectable protein in 32 of the controls; 23 controls exhibited trace amounts of protein and 5 contained 30 mg. Histologic examination of kidneys of mice treated with 1.0 mL/kg cis-isomer or 2.0 mL/kg trans-isomer failed to reveal proximal convoluted tubule necrosis or swelling.

Table 4-9. Effect of 1,2-DCE isomers on urinary protein and glucose 24 hours after intraperitoneal treatment of male Swiss mice

Agent	Dose (mL/kg)	Number of mice tested[a]	Number of mice with urinary protein[b]
cis-1,2-DCE	0.1	10	2
	1.0	10	2
	2.0	6	3
trans-1,2-DCE	1.0	10	0
	2.0	10	1
	4.0	5	3

[a]Each group originally contained 10 mice; at the high dose, only survivors were tested.
[b]Significant urinary protein excretion if ≥100 mg/100 mL.

Source: Plaa and Larson (1965).

4.5.3. Studies with Cell Cultures

Mochida et al. (1995) used cultured human oral carcinoma cells (KB cells) to compare the toxicity of cis- and trans-1,2-DCE. Cells were exposed to various concentrations of the test agent for 72 hours. On the basis of cell counts, the ID_{50} was determined to be 3,900 µg/mL culture medium for the trans-isomer but 5,800 µg/mL culture medium for the cis-isomer. No possible explanation for the relative toxicity of the two isomers in KB cells was provided. Isomers of 1,2-DCE were considerably less toxic to KB cells than other chlorinated organic compounds that are common contaminants in groundwater.

The cytotoxicity of cis-1,2-DCE to isolated rat hepatocytes was reported by Suzuki et al. (1994). Hepatocyte cultures were incubated for 2 hours in Eagle's medium, containing 10% calf serum and 10 mM cis-1,2-DCE. No effect was seen on release of LDH or formation of thiobarbituric acid-reactive substances (TBARS). Extracted cellular lipids from hepatocytes exposed to cis-1,2-DCE did not show significant increases in phospholipid hydroperoxides, in contrast to cells exposed to carbon tetrachloride, 1,1,1-trichloroethane, and 1,3-dichloropropene. Thus, no indication of cis-1,2-DCE-induced lipid peroxidation in hepatocyte membranes was seen at the dose level tested.

No effect of trans-1,2-DCE on lipid peroxidation, measured as TBARS, was observed in cultured bovine pulmonary arterial endothelial cells or rabbit aortic smooth muscle cells (Tse et al., 1988). However, lipid peroxidation was seen in the presence of 2% (volume/volume) trans-1,2-DCE in Medium 199 supplemented with 10–20% fetal calf serum in the presence of extracellular Fe(III)ADP (6.2 µM), suggesting that a synergistic interaction between iron and the test agent may occur (Tse et al., 1990).

4.5.4. Genotoxicity

A number of studies have evaluated the genotoxicity of 1,2-DCE, its isomers (cis- and trans-), and a mixture of both isomers. In vitro studies include tests in prokaryotic organisms such as *Salmonella typhimurium* (Ames assay) and *Escherichia coli*, and eukaryotic organisms including *Saccharomyces cerevisiae* and *Aspergillus nidulans*. Genotoxic effects of 1,2-DCE have also been studied in Chinese hamster cells and human lymphocytes in vitro. Further, in vivo studies have been conducted in the host-mediated assay, micronucleus test in mice, and mitotic recombination in *Drosophila melanogaster*.

4.5.4.1. *In Vitro Studies*

Gene mutation studies in *S. typhimurium* and *E. coli* using cis- or trans-1,2-DCE or a mixture of both isomers were mainly nonpositive (Mersch-Sundermann, 1989; Mersch-Sundermann et al., 1989; Zeiger et al., 1988; Calandra et al., 1987; Strobel and Grummt, 1987; Mortelmans et al., 1986; Nohmi et al., 1985; Cerna and Kypenova, 1977; Greim et al., 1977, 1975; Simmon et al., 1977). However, Mersch-Sundermann (1989) reported positive results in a Salmonella strain, TA98, for the trans-isomer with or without metabolic activation by S9. Nonpositive results were reported in the same study for the cis-isomer with or without metabolic activation.

Strobel and Grummt (1987) tested trans-1,2-DCE at concentrations of 0.01–1 mg/plate in Salmonella strains TA97, TA98, TA100, and TA104. Although there were increases in the number of revertants in some strains (TA97, TA100), they were not dose-dependent. Up to a 5.5-fold increase in revertant numbers was observed in strain TA97. 1,2-DCE did not have any effect on strains TA97 and 98 in the absence of S9. However, in the presence of S9, TA97 showed a maximum (5.5-fold) response both at doses 0.025 and at 0.25 mg/plate. Furthermore, even at the lowest concentration (0.01 mg/plate), a fivefold increase in revertants was observed. In the case of strain TA98, an approximate twofold increase in revertants at 1 mg/plate was observed compared with the control. TA100 displayed up to a 2.5-fold increase in revertants at different doses both in the absence and presence of S9. Exposure of 1,2-DCE to the TA104 strain resulted in an increase in the number of revertants both in the absence and presence of S9. However the response was not dose-dependent. Since many strains had responses, even at the lowest concentrations, that were close to the maximum (TA97 and TA98, +S9; TA104, + and – S9), the results are difficult to interpret. The authors offered no discussion or rationale for the high revertant rates that occurred at the low concentrations. Cerna and Kypenova (1977) similarly reported decreasing number of revertants with increasing concentrations of DCE. However, these concentrations were lower than in assays that measured similar gene reversions and, therefore, it is unlikely that these concentrations were causing cell toxicity.

Studies in yeast, using the diploid *S. cerevisiae* strain D7 for gene conversion, reverse mutation, or mitotic recombination, were mostly nonpositive for cis- and trans-1,2-DCE (Koch et

al., 1988; Galli et al., 1982; Bronzetti et al., 1981; Simmon et al., 1977). However, Bronzetti et al. (1984) reported positive results in *S. cerevisiae* D7 for both isomers with metabolic activation and for the cis-isomer only without metabolic activation. In addition, a positive result was reported by Koch et al. (1988) for aneuploidy in *S. cerevisiae* D61.M with the trans-isomer with or without metabolic activation. However, Koch et al. (1988) cautioned that the effect noted in the D61.M strain could have been intensified due to the long incubation period required with this strain, and storage of the test tubes in an ice bath during part of the incubation period. Positive results were also seen for aneuploidy and mitotic segregation in *A. nidulans* diploid strain P1, for a mixture of both isomers (Crebelli et al., 1992; Crebelli and Carere, 1987).

No chromosome aberrations or sister chromosome exchanges were reported in Chinese hamster cells for either cis- or trans-1,2-DCE (Sawada et al., 1987; Sofuni et al., 1985). However, Doherty et al. (1996) investigated the activation and deactivation of chlorinated hydrocarbons including 1,2-DCE in metabolically competent human cells. The authors used human lymphoblastoid cell line AHH-1 (containing native CYP1A1 activity), MCL-5 (stably expressing human CYP1A2, 2A6, 3A4, 2E1 and microsomal epoxide hydrolase) and h2E1 (containing cDNA for CYP2E1) cell lines. 1,2-DCE produced an increase in micronuclei at concentrations between 0 and 10mM in the AHH-1 and h2E1 cell lines. The micronuclei contained approximately equal frequencies of both kinetochore-positive and kinetochore-negative signals. At concentrations up to and including 10 mM, no increase in micronuclei was observed in the MCL-5 cell line.

Tafazoli and Kirsch-Volders (1996) compared the cytototoxic, genotoxic, and mutagenic activity of a number of chlorinated aliphatic hydrocarbons including 1,2-DCE. The mutagenicity and cytotoxicity of 1,2-DCE was evaluated in an in vitro micronucleus assay using human lymphocytes in the presence or absence of S9. A low but positive response ($p < 0.05$) was obtained at 20 mM concentration both with and without S9. The authors also stated that this increase was not accompanied by a substantial decrease in cell proliferation. In addition to the micronucleus assay, a comet assay was employed to examine the capacity of 1,2-DCE to induce DNA damage in in vitro isolated human lymphocytes. Positive responses for tail length were found at 6 and 8 mM ($p < 0.01$) and for tail movement at 2 mM ($p < 0.01$) with S9. A summary of the in vitro genetic toxicology studies is presented in Table 4-10.

Table 4-10. In vitro genotoxicity studies using cis- and trans-1,2-dichloroethylene

Test system	Strain/ cell line	Result −S9	Result +S9	Dose/plate	Compound	Effect	Reference
Bacterial systems							
S. typhimurium	TA98 TA100 TA1535 TA1538 TA1950 TA1951 TA1952	−	NT	0.5–50 μL	cis, trans	Reverse mutation	Cerna and Kypenova, 1977
	TA1535 TA1538	−	−	NA	cis, trans	Reverse mutation	Greim et al., 1977
	TA98 TA100 TA1535 TA1537 TA1538	−	NT	Up to 5 mg	cis, trans	Reverse mutation	Simmon et al., 1977
	TA98 TA100 TA1535 TA1537	− −	− −	33–5,555 μg 10^1–10^4 μg	mixture trans	Reverse mutation	Mortelmans et al., 1986
	TA97 TA98 TA1535 TA1537	−	−	33–10,000 μg	cis	Reverse mutation	Zeiger et al., 1988
	TA97[a] TA98 TA100 TA102	NT	−	NA	trans	Reverse mutation	Calandra et al., 1987
	TA97 TA98 TA100	−	−	NA	cis	Reverse mutation	Mersch-Sundermann, 1989
	TA97 TA98 TA100	− + +	− + +	NA	trans	Reverse mutation	Mersch-Sundermann, 1989
	TA97 TA98 TA100 TA104	− − + +?	+?[a] +? + +?	0.01–1.0 mg	trans	Reverse mutation	Strobel and Grummt, 1987
	TA87 TA98 TA100 TA102	−	−	1.0–50 mg	cis	Reverse mutation	Nohmi et al., 1985
E. coli	K12	−	−	2.9 mM 2.3 mM	cis trans	DNA damage	Greim et al., 1977, 1975
	PQ37	−	−	NA	cis, trans		Mersch-Sundermann et al., 1989

Table 4-10. In vitro genotoxicity studies using cis- and trans-1,2-dichloroethylene

Test system	Strain/ cell line	Result −S9	+S9	Dose/plate	Compound	Effect	Reference
S. cerevisiae	D7	−	NA	100 mM	cis, trans	Gene conversion, reverse mutation, or mitotic recombination	Bronzetti et al., 1981
	D7	+	+	100 mM 40 mM	cis		Bronzetti et al., 1984
		−	+	100 mM 80 mM	trans		
	D7	−	−	100 mM	cis, trans		Galli et al., 1982
	D3	−	NT	Up to 0.2 mL	cis, trans		Simmon et al., 1977
	D7	−	−	77.3 mM	trans		Koch et al., 1988
	D61.M	+	+	77.3 mM	trans	Aneuploidy	Koch et al., 1988
A. nidulans	Diploid P1	−	NT	1–2.5 mL in 20 L (24-hour vapor)	mixture[b]	Mitotic recombination, mutation	Crebelli and Carere, 1987
		+				Aneuploidy	
	Diploid P1	+	NT	0.05–0.175% (v/v)	mixture	Mitotic segregation	Crebelli et al., 1992
Mammalian cells							
Chinese hamster	CHL	−	−	7.5 mg/mL	cis	Chromosome aberrations	Sofuni et al., 1985
	V79 lung	+	NT	6.5 x 10^{-3} M	trans	c–Mitosis, aneuploidy	Önfelt, 1987
	CHL	−	−	2.0 mg/mL	cis, trans	Chromosome aberrations, sister chromatid exchange	Sawada et al., 1987
	CHO	ND	?	160–5,000 µg/mL	cis	Sister chromatid exchange	Galloway et al., 1987
		−	?		trans		
	CHO	+	+	126–12,630 µg/mL	mixture		Galloway et al., 1987
	CHO	−	−	500–5,000 1,600–5,000 455–12,630 µg/mL	cis trans mixture	Chromosome aberrations	Galloway et al., 1987

Table 4-10. In vitro genotoxicity studies using cis- and trans-1,2-dichloroethylene

Test system	Strain/ cell line	Result –S9	Result +S9	Dose/plate	Compound	Effect	Reference
Human lymphoblastoid	AHH-1 h2E1 MCL-5	+ + −	NT	2.5 mM 2.5 mM 10 mM	mixture	Micronucleus assay	Doherty et al., 1996
Human lymphocytes		+	+	20 mM	mixture	Micronucleus assay	Tafazoli and Kirsch-Volders, 1996
		+	+	6 mM 4 mM	mixture	Comet assay, DNA breakage	Tafazoli and Kirsch-Volders, 1996

[a]Increase in revertants in mid-dose range, decrease at high doses; poor dose response (see text).
[b]Authors state CASRN for mixture, but chemical name is given as 1,2-dichloroethane.

+ = positive; – = nonpositive; ?= inconclusive; NT = not tested; NA = not available

4.5.4.2. *In Vivo Studies*

In the host-mediated assay in mice, Cerna and Kypenova (1977) reported an increase in mutation and chromosomal aberrations for the cis- isomer, with no increase noted for the trans- isomer. Also, in a similar host-mediated assay, Bronzetti et al. (1984) reported positive results for the cis-isomer and nonpositive results for the trans-isomer. No increase in micronucleus induction was reported in the bone marrow of CD-1 mice exposed by i.p. injection to a mixture of the cis- and trans-isomers (Crebelli et al., 1999). Since none of the 10 halogenated aliphatic hydrocarbons studied (including 1,2-DCE) showed any evidence of micronucleus induction, the authors concluded that the in vivo mouse bone marrow test may not be sensitive enough to detect the genotoxic effects of this group of compounds. However, an increase in mitotic recombination was observed in Drosophila larvae exposed to the vapors of a mixture of both isomers at 2,000 ppm (Vogel and Nivard, 1993). See Table 4-11 for a summary of the in vivo genetic toxicology studies using cis- and trans-1,2-DCE.

Table 4-11. In vivo genotoxicity studies using cis- and trans-1,2-dichloroethylene

Test system	Strain/cells	Result[a]	Dose (LED/HID)[b]	Compound	Effect	Reference
Host: mouse; *S. cerevisiae*	CD D7	−	3,000 mg/kg	cis, trans	Host-mediated assay	Bronzetti et al., 1981
		+ −	1,300 mg/kg	cis trans		Bronzetti et al., 1984
Host: mouse; *S. typhimurium*	ICR TA1950 TA1951 TA1952	+ −	½, 1 LD$_{50}$ (i.p.)	cis trans		Cerna and Kypenova, 1977
Mouse, female	Bone marrow	+ −	5 × 1/6 LD$_{50}$ (i.p.)	cis trans	Chromosomal aberrations	Cerna and Kypenova, 1977
Mouse, male		−	500–2,000 mg/kg	cis, trans		Tice et al., 1987
Mouse, male and female	Peripheral erythrocytes	−	280–490 mg/kg (i.p.)	mixture	Micronucleus test	Crebelli et al., 1999
Mouse, male and female		−	3,125–50,000 ppm in feed for 14 weeks	trans		MacGregor et al., 1990
Mouse, male	Bone marrow	−	500–2,000 mg/kg	cis, trans	Sister chromatid exchange	Tice et al., 1987
D. melanogaster larvae	Cross of y × w	+	2,000 ppm (vapor)	mixture	Eye mosaic assay	Vogel and Nivard, 1993

[a]+ = Positive; − = nonpositive.
[b]LED = lowest effective dose, HID = highest ineffective dose.

In conclusion, both cis-1,2-DCE and trans-1,2-DCE have been evaluated for genotoxicity and mutagenicity using various in vitro and in vivo assays in both non-mammalian and mammalian systems. Most gene mutation assays both in *S. typhimurium* strains and *E. coli* were nonpositive as a result of exposure to 1,2-DCE. Studies in yeast, using the diploid *S. cerevisiae* strain for gene conversion, reverse mutation, or mitotic recombination, were also mostly nonpositive for cis- and trans-1,2-DCE. No chromosome aberrations or sister chromosome exchanges were reported in Chinese hamster cells when exposed to either isomer of 1,2-DCE; however, micronucleus formation was observed in human lymphocytes. Overall, data for 1,2-DCE are generally nonpositive for genotoxicity and mutagenicity. The positive studies are inconsistent and need further confirmation.

4.5.5. Quantitative Structure-Activity Relationship Studies

Greim et al. (1975) used a number of chlorinated ethylenes in an *E. coli* mutation assay with metabolic activation by S9. They observed that ethylenes with an asymmetric arrangement of the chlorines across the double bond were mutagenic (vinyl chloride > trichloroethylene >

1,1-DCE), while those with a symmetric arrangement (tetrachloroethylene, cis- and trans-1,2-DCE) were not. No mutagenic activity was observed in any test in the absence of S9. Greim et al. (1975) explained this finding on the basis of likely differences in the chemical stabilities of the respective oxiranes that were formed by S9. Jones and Mackrodt (1982) developed a theoretical model for oxirane reactivity, specifically targeting the energy required to split the weaker of the two C–O bonds in the oxirane ring. They compared these energies to the mutational potencies observed by Greim et al. (1975) and confirmed the C–O bond split energy to be a good predictor of mutational potency. In a correlation of mutagenic potency vs. C–O bond split energy, there was a region of no mutational potency with decreasing bond strength, and both cis- and trans-1,2-DCE fell within that portion of the curve. There followed a region where mutational potency increased strongly with decreasing bond strength (trichloroethylene; maximal potency with chloroethylene) but then turned and decreased with decreasing bond strength; this part of the curve was represented by 1,1-DCE. In a subsequent paper, Jones and Mackrodt (1983) included carcinogenicity data for several halogenated ethylenes in their calculations and found that carcinogenicity and mutagenicity data formed almost overlapping bell-shaped curves when correlated with C–O bond split energy.

Loew et al. (1983) used a molecular orbital method to evaluate the carcinogenic potencies of chloroethylenes, including cis- and trans-1,2-DCE. They proposed that CYP450 metabolism of the parent compound results in an initial radical intermediate from which either epoxides or reactive carbonyl compounds could be formed, suggesting three possible alternate pathways of toxic activation. The authors considered the carbonyl compound (i.e., the acyl chloride or aldehyde) rather than the epoxide to be the ultimate carcinogen in a genotoxic (DNA adduct formation) or epigenetic (macromolecule alkylation and necrosis) process. Their findings indicated that the amount of reactive carbonyl compound formed by metabolism, rather than its electrophilic reactivity, was a determinant of carcinogenic potency. For the purpose of their evaluations, they assumed that carbonyl compounds were formed by both the radical and the epoxidation pathway and that the protonated forms of the epoxides could also act as the ultimate carcinogens.

Loew et al. (1983) used three parameters as molecular indicators of carcinogenic potency: the activation energy required to create a reactive intermediate in all three alternate pathways, the electrophilic potency of the putative active carcinogen to form a covalent bond, and its long-range electrostatic interactions. They used the "Modified Neglect of Diatomic Overlap" method in their calculations. Activation energy turned out to be a useful predictor only for compounds with few chlorine substituents, and predictions became increasingly inaccurate with increasing degree of chlorination. Electrophilicity turned out to be unsuitable as a predictor of carcinogenicity. Assuming the carbonyl compounds to be the ultimate carcinogen, the authors proposed a carcinogenicity ranking of vinyl chloride = 1,1-DCE > 1,2-DCE > tetrachloroethylene > trichloroethylene. Assuming the epoxide carbocation as the ultimate

carcinogen, the authors proposed a similar ranking in which 1,2-DCE was placed tentatively in the same position. Vinyl chloride is a confirmed human and animal carcinogen; the other compounds, with the exception of 1,2-DCE, are all animal carcinogens without evidence of carcinogenicity in humans. The approach and ranking, as proposed by Loew et al. (1983), are therefore of limited use. In the evaluation of Jones and Mackrodt (1983), vinyl chloride was listed as about 100 times as potent a carcinogen as 1,1-DCE, with no oncogenicity attributed to 1,2-DCE.

Crebelli et al. (1995) evaluated a set of 55 halogenated hydrocarbons for their ability to induce mitotic chromosome malsegregation, mitotic arrest, and lethality in *A. nidulans*. The 1,2-DCE isomer mixture was about one-half as potent in inducing malsegregation as 1,1-DCE but only slightly less potent in inducing growth arrest. The most potent malsegregation inducers in this test were 1,1,1-trichloropropene and 1,1,2,2-tetrabromoethane, with more than 20 times the potency of 1,2-DCE. Tetrabromomethane was about 2,500 times more effective in arresting *A. nidulans* growth than 1,2-DCE. The quantitative structure-activity relationship (QSAR) evaluations did not include 1,2-DCE; although the authors reported high correlation coefficients between measured and calculated values for 20 compounds that were subjected to QSAR, a visual inspection of the values for individual compounds was not convincing.

Liu et al. (1997) used the computer software MultiCASE (designed to identify as yet unknown portions in a chemical structure that confer specific reactivity) to evaluate 93 chemicals, including trans-1,2-DCE and several mono-, tri-, or tetrachlorinated alkanes and alkenes that had been tested experimentally for their ability to induce chromosome malsegregation in *S. cerevisiae*. A subset of the NTP salmonella mutagenicity database was used for comparison. They identified the trans-1,2-DCE structure and the vinylidene chloride portion of the tetrachloroethylene structure as having activity in the malsegregation assay.

Cronin (1996) used the original values of Frantik et al. (1994) (see Section 4.4.2) for a QSAR analysis of the neurotoxicity of 44 compounds that comprised benzene and many of its alkylated congeners, halogenated alkanes and alkenes (including the 1,2-DCE mixture), alcohols, ketones, esters, and a few unsubstituted alkanes (n-pentane, n-hexane, n-heptane, and cyclohexane). In a first attempt, using rat data, stepwise regression through all 44 data sets (that included boiling and melting points, a hydrophobicity factor, and several other specific molecular parameters for each compound) afforded an equation to describe the neurotoxicities of these compounds that included the respective boiling points and specific molecular parameters but no term for hydrophobicity. After removing four evident outliers from the data set— interestingly, those were the four unsubstituted alkanes mentioned above—another equation was obtained that was based only on a specific hydrophobicity factor. By using mouse data, an equation was obtained that again did not contain the hydrophobicity factor. The same four unsubstituted alkanes were outliers, and their removal resulted in an equation that was based on boiling point, hydrophobicity, and an additional molecular factor. Regression curves described

by the respective equations for rats and mice had similar slopes and intercepts. The author was able to obtain a description that separated highly neurotoxic compounds from less neurotoxic ones. The author pointed out that solubility of the compounds played a minor role in their neurotoxicity, while hydrophobicity was more useful in predicting this effect.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

A general overview of toxicity studies conducted with cis- or trans-1,2-DCE indicates that both compounds display low toxicity. Although most of the 1,2-DCE literature suggests that the liver is the organ most affected by exposure to 1,2-DCE at high doses, evidence of any specific pathological event is limited based on the available information (NTP, 2002; McCauley et al., 1995; Hayes et al., 1987; Freundt et al., 1977). Table 4-12 presents a summary of the major subchronic studies and the observed effects for both oral and inhalation exposure to cis- and trans-1,2-DCE.

Table 4-12. Summary of major noncancer subchronic studies for oral and inhalation exposure to 1,2-DCE

Reference	Isomer	Dosing route	Treatment period	Species, number of animals	Oral dose (mg/kg-day) or Inhalation concentration (mg/m³)	Observed effects (doses of statistical significance)	NOAEL[a] (mg/kg-d)	LOAEL (mg/kg-d)
NTP (2002)	trans	Oral (feed)	14 weeks	Rat, 9–10/sex/dose	M: 0, 190, 380, 770, 1,540, or 3,210 F: 0, 190, 395, 780, 1,580, or 3,245	M: ↓ Bw (3,210) ↓ RBC count (≥380) F: ↑ Abs. liver wt (≥395) ↑ Rel. liver wt (≥395)	3,245 (F)	NA
NTP (2002)	trans	Oral (feed)	14 weeks	Mouse, 10/sex/dose	M: 0, 480, 920, 1,900, 3,850, or 8,065 F: 0, 450, 915, 1,830, 3,760, or 7,925	M: ↑ Rel. liver wt (≥1,900) F: ↑ Rel. liver wt (≥3,760)	7,925 (F)	NA
Hayes et al. (1987)	trans	Oral (dw)	90 days	Rat, 20/sex/dose	M: 0, 402, 1,314, or 3,114 F: 0, 353, 1,257, or 2,809	F: ↑ Abs. kidney wt (≥1,257)	2,809 (F)	NA
Barnes et al. (1985)	trans	Oral (dw)	90 days	Mouse, 15–23/sex/dose	M: 0, 17, 175, or 387 F: 0, 23, 224, or 452	M: ↑ Rel. liver wt (175) ↑ ALP (≥175) F: ↓ Abs. thymus wt (452) ↓ Rel. thymus wt (≥224) ↓ Abs. lung wt (452) ↓ Rel. lung wt (452)	387 (M)	NA
Shopp et al. (1985)	trans	Oral (dw)	90 days	Mouse, 8–12/sex/dose	M: 0, 17, 175, or 387 F: 0, 23, 224, or 452	M: ↓ sRBC-responsive cells (≥175) F: ↓ sRBC-responsive cells (23)	17 (M)	175 (M)
McCauley et al. (1995)	cis	Gavage	90 days	Rat, 10/sex/dose	Reported: 0, 10, 32, 98, 206 mg/kg-day; EPA calculated: 0, 32, 97, 291, 872	M: ↑ Rel. liver wt (≥97) ↑ Rel. kidney wt (≥32) ↓ Hemoglobin (≥291) ↓ Hematocrit (≥97) ↓ BUN (872) ↓ Serum phosphorus (32) ↑ Serum calcium (32, 97) F: ↑ Rel. liver wt (≥97) ↓ Hemoglobin (291) ↓ Hematocrit (≥291) ↓ RBC count (291) ↑ Serum phosphorus (97, 291)	unknown	unknown

Table 4-12. Summary of major noncancer subchronic studies for oral and inhalation exposure to 1,2-DCE

Reference	Isomer	Dosing route	Treatment period	Species, number of animals	Oral dose (mg/kg-day) or Inhalation concentration (mg/m^3)	Observed effects (doses of statistical significance)	NOAEL[a] (mg/kg-d)	LOAEL (mg/kg-d)
Dow (1962) report	mix	Inhalation	7hr/day for 6 months	Rat, 12–35/sex/conc.	0, 1,980, or 3,960	M: ↑ Rel. kidney wt (≥1980) F: ↑ Rel. liver wt (≥1,980)	3,960 (M, F)	NA
Freundt (1977)	trans	Inhalation	8 hr/day, 5 days/week for 1,2,8, and 16 weeks	Female rat, 6/sex/conc.	792 (only concentration)	Fat accumulation in the liver and Kupffer cells	NA	792

[a]NOAEL = No-observed-adverse-effect level

M = males; F = females; ↑ = increase; ↓ = decrease; bw = body weight; Abs. = absolute; Rel. = relative; wt = weight; dw = drinking water; conc. = concentration

4.6.1. Oral

4.6.1.1. *cis-1,2-DCE*

No studies of the effects of oral exposure to cis-1,2-DCE in humans were identified, and the experimental toxicity database for this isomer is limited. The only investigation of repeat-dose toxicity of cis-1,2-DCE by the oral route is McCauley et al. (1995, 1990). As noted in Sections 4.2.1.1 and 4.2.1.2, comparison of the unpublished report (McCauley et al., 1990) and the published study (McCauley et al., 1995) revealed certain errors and inconsistencies in the documentation of administered doses, in the study protocol, and in the number of animals available for examination (e.g., McCauley et al. [1995] did not correct for animals that died in the first week of dosing as a result of gavage error). In addition, some errors in the values for a few of the relative organ weights were identified, which may have been due to either transcription errors or calculation errors. These errors did not compromise the integrity of the data since the inconsistencies were more likely an issue of quality of the report writing than an issue with the findings themselves.

McCauley et al. (1995, 1990) reported statistically significant increases in relative liver weights in rats following both 14- and 90-day exposures (up to 38 and 32% in males and 38 and 30% in females, respectively). These investigators reported that there were no histopathological changes in the liver. Clinical chemistry indicators of liver function were limited to serum AST. There were no statistically significant changes in AST levels at any dose in either sex. The absence of compound-related histopathological changes in the liver, the absence of AST changes, and the lack of measurements of other clinical chemistry indicators of liver function in the McCauley et al. (1995, 1990) study create difficulties in interpreting the relative liver weight findings.

The observed increase in liver weight could represent an early indicator or precursor of liver toxicity; however, toxicity information is limited. Additionally, it is not possible to predict whether liver damage would or would not occur at higher concentrations or in studies of longer exposure duration (i.e., chronic studies). To better understand whether increased liver weight observed by McCauley et al. (1995, 1990) could be a precursor of liver toxicity for cis-1,2-DCE, acute and short-term toxicity studies for cis-1,2-DCE and the literature for 1,2-DCE mixtures were also considered.

Limited data from studies of shorter (acute and short-term) duration suggest that the liver is a target organ for cis-1,2-DCE, although responses, at least under the conditions studied, were minimal. Liver enzymes were measured in an acute toxicity study of cis-1,2-DCE (McMillan, 1986). Following a single oral gavage dose of 4,944 mg/kg-day cis-1,2-DCE, GSH levels were elevated by 28% and AST activity by 56%; ALT activity was unchanged. Minimal hepatic centrilobular vacuolation was reported in rats exposed to lethal or near lethal concentrations (≥47,900 mg/m^3) of cis-1,2-DCE in an acute inhalation study (DuPont, 1999), and concentration-

related increases in relative liver weights were reported in male and female rats in the 14-day oral toxicity study by McCauley et al. (1995, 1990).

In studies of 1,2-DCE mixtures, slight liver pathology was observed in rats 1 week after an acute 7-hour inhalation exposure to 66,740 mg/m^3 1,2-DCE (isomer composition not stated) —a concentration lethal to 6/9 exposed rats (Dow, 1960)—and relative liver weights were increased following exposure to 1,2-DCE (58% cis-, 42% trans-isomer) by inhalation intermittently for up to 195 days at concentrations of 1,980 mg/m^3 (rabbits) or 3,960 mg/m^3 (rats) (Dow, 1962). In the Dow (1962) study, clinical chemistry values were reported to fall within normal values. Overall, these studies show elevated liver weight to be the most consistent finding in studies of cis-1,2-DCE or mixed isomers of 1,2-DCE. Reported increases in liver enzymes have been small, and slight liver pathology has been documented only following acute inhalation exposure at or near lethal exposure concentrations.

The increased liver weight observed in the McCauley et al. (1995, 1990) study was related to administration of cis-1,2-DCE; however, in the absence of elevated liver enzymes or histopathology, the change in liver weight is difficult to interpret. Overall, the available data support the conclusion that the liver is a target organ for oral exposure to cis-1,2-DCE.

Increased relative kidney weight was less consistently observed by McCauley et al. (1995, 1990). In male rats, statistically significant increases in relative kidney weight were observed at all doses in the 90-day study but not in the 14-day study. In female rats, relative kidney weights were not statistically elevated following 90 days of exposure, but were elevated in the highest two concentration groups following 14 days of exposure. The absence of compound-related histopathological changes in the kidney in the McCauley et al. (1995, 1990) study raises questions about the biological significance of the relative kidney weight findings. BUN and creatinine, two clinical chemistry parameters that are indicators of kidney function (generally renal dysfunction), did not provide supporting evidence for functional damage to the kidney (McCauley et al., 1995, 1990). In the 90-day study, BUN and creatinine were only marginally decreased (although statistically significant) in high-dose (872 mg/kg-day) male rats; values in treated females were similar to controls.

McCauley et al. (1995, 1990) also reported decreases in hematological parameters (hemoglobin and hematocrit) in male and female rats at doses ≥97 mg/kg-day that were not dose-related and were considered by the study investigators to be marginal. Comparison of hemoglobin and hematocrit findings with normal values for Sprague-Dawley rats suggests that these hematological parameters were not affected by cis-1,2-DCE treatment. Based on blood samples collected from 25 male and 25 female Sprague-Dawley rats, Leonard and Rubin (1986) reported the following means (and ranges): hemoglobin—16.1 g/dL (13.3–17.3 g/dL) in males and 16.2 g/dL (14.6–17.2 g/dL) in females and hematocrit—42.4% (36.7–46.4%) in males and 41.6% (37.6–44.3%) in females. With the exception of the 291 mg/kg-day-dosed female rats, values for hemoglobin and hematocrit in cis-1,2-DCE-treated rats were within the normal range

reported by Leonard and Rubin (1986). Matsuzawa et al. (1993) examined hematological data from >2,700 male and >2,600 female Sprague-Dawley rats. Values within two standard deviations of the mean were considered by the study authors to be within the normal range. For hemoglobin and hematocrit in male and female rats, two standard deviations from the mean as reported by Matsuzawa et al. (1993) is equivalent to approximately 12–13% of the mean. This compares to decreases in hematological parameters in the McCauley et al. (1995, 1990) study of only 6–10% of the control mean. Thus, based on normal ranges for hematologic parameters, the hematology findings in McCauley et al. (1995, 1990) are not considered biologically significant.

There is limited evidence that oral exposures to cis-1,2-DCE affect the CNS. In a 14-day oral gavage study, McCauley et al. (1995, 1990) reported signs of CNS depression in male and female rats. Immediately following gavage dosing, animals appeared agitated followed by lethargy and ataxia. In the 90-day study (McCauley et al., 1995, 1990), however, the investigators reported no compound-related clinical effects.

There are no oral studies of chronic, reproductive, or developmental toxicity of cis-1,2-DCE. The findings from developmental toxicity range-finding studies for a mixture of 1,2-DCE isomers (NTP, 1991a, b, c) are summarized in Section 4.6.1.3. below.

4.6.1.2. *trans-1,2-DCE*

No human studies involving oral exposure to trans-1,2-DCE were identified. Information on the potential health effects of oral exposure to trans-1,2-DCE comes from acute (Hayes et al., 1987; McMillan, 1986; Barnes et al., 1985; Freundt et al., 1977) and subchronic toxicity studies (NTP, 2002; Hayes et al., 1987; Barnes et al., 1985), developmental range-finding studies on a mixture of 1,2-DCE isomers (NTP, 1991a, b, c), and subchronic studies of immunotoxicity (Barnes et al., 1985; Shopp et al., 1985; Munson et al., 1982). No chronic bioassays of trans-1,2-DCE toxicity have been performed.

Acute oral toxicity in animals including CNS depression was noted by Hayes et al. (1987) and Barnes et al. (1985). Freundt et al. (1977) reported hyperemia involving the liver, kidney, urinary bladder, and intestines from postmortem gross pathology in mice after i.p. administration; pulmonary capillary hyperemia and alveolar septal distention and fibrous swelling and hyperemia with incipient disorganization of the cardiac muscle in one rat via the gavage route of administration and in one rat via the i.p. route of exposure; and severe fatty infiltration of the liver lobules and Kupffer cells in rats exposed via gavage. Depressed GSH content and increased plasma AST and SDH activities were observed in rats administered trans-1,2-DCE i.p. (McMillan, 1986).

The oral toxicity of trans-1,2-DCE was evaluated in four subchronic toxicity studies— NTP (2002) (rats and mice), Barnes et al. (1985) (mice), Hayes et al. (1987) (rats) and Shopp et al. (1985) (mice). The drinking water study by Barnes et al. (1985) exposed mice at doses up to approximately 400 mg/kg-day, whereas the drinking water study by Hayes et al. (1987) and

dietary study by NTP (2002) exposed mice and rats to doses almost an order of magnitude higher. These three studies identified a range of effects associated with trans-1,2-DCE exposure, including decreased body weight gain, effects on organ weights (liver, kidney, thymus, and lung), minimal changes in liver function enzymes, decreased mean body weight, and minimal decreases in hematological parameters. A fourth subchronic study in mice (Shopp et al., 1985) evaluated the immunotoxic potential of trans-1,2-DCE.

Statistically significant effects on the liver were observed by Barnes et al. (1985) and NTP (2002), but not Hayes et al. (1987). In the 90-day Barnes et al. (1985) study, male and female mice were exposed to trans-1,2-DCE in drinking water at doses up to 387 mg/kg-day for males and up to 452 mg/kg-day for females. A significant increase in mean liver weights was noted at the mid-dose (175 mg/kg-day), but not at the highest dose, in male mice. No DCE-induced changes in terminal body weight were observed. Significant increases in serum ALP levels of 62 and 33% were reported at the 175 and 387 mg/kg-day doses, respectively, in male mice. These increases showed no dose-response relationship, were within the normal range for this mouse strain, and were not observed in female mice. In female mice, ALT and AST levels were depressed at all doses, with statistical significance at the two highest dose levels. Increases in ALT and AST levels are indications of liver damage; the implication of decreases in these enzymes is unknown. The findings of Barnes et al. (1985) suggest that trans-1,2-DCE, via drinking water, does not induce hepatotoxicity at doses up to 387 mg/kg-day in male mice and up to 452 mg/kg-day in female mice.

NTP (2002) conducted a 14-week dietary study of trans-1,2-DCE in rats and mice at doses ranging from approximately 190 to 3,200 mg/kg-day in rats and approximately 450 to 8,000 mg/kg-day in mice. Table 4-5 shows the relative liver weight changes in mice and rats. Absolute and relative liver weights of female rats exposed to ≥395 mg/kg-day were statistically significantly higher by 8–17% and 6–10%, respectively, than those of the vehicle controls; liver weights of male rats were not affected by trans-1,2-DCE exposure. In mice, relative liver weights were statistically significantly increased over controls in males (by 9–15%) exposed to doses of ≥1,900 mg/kg-day and in females (by 11%) exposed to doses of ≥3,760 mg/kg-day. Clinical chemistry data did not suggest hepatotoxicity in either species. Statistically significant decreases in serum ALP activities were reported in female rats exposed to the three highest doses compared with the vehicle controls; these decreases were minimal in severity (<13%) and transient (i.e., present at day 21 but not week 14). No exposure-related changes in ALP activities were observed in male rats or mice of either sex. No changes were observed in other clinical chemistry parameters, including cholesterol, ALT, and SDH levels, in rats or mice of either sex.

As with cis-1,2-DCE, consideration was given to the possibility that the observed liver effects for trans-1,2-DCE are early indicators or precursors of liver toxicity. It is not possible to predict whether liver damage would or would not occur at higher concentrations or in studies of longer exposure duration (i.e., chronic).

In interpreting the liver weight findings from subchronic oral exposure to trans-1,2-DCE, consideration was given to the entire database for trans-1,2-DCE, including acute oral gavage studies, an inhalation exposure study (Freundt et al., 1977), and 1,2-DCE mixture information. McMillan et al. (1986) reported no effects on ALT in rats following a single gavage dose of 4,944 mg/kg trans-1,2-DCE; acute i.p. injection caused transient increases in liver enzymes and histopathology (maximally elevated at 4 hours and near control levels at 12 hours post exposure). In an oral LD_{50} test in female Wistar rats (Freundt et al., 1977), severe fatty infiltration of the liver lobules and Kupffer cells was observed in some animals receiving a single oral gavage dose of trans-1,2-DCE. Barnes et al. (1985) indicated that based on gross necropsy findings, the liver was a target following single acute gavage administration of trans-1,2-DCE. By inhalation, Freundt et al. (1977) found fatty effects on the liver following single and repeated (up to 16 weeks) exposures to 792 mg/m^3; in contrast, DuPont (1999) found no compound-related effects on the liver following acute inhalation exposures at lethal concentrations (48,700–135,000 mg/m^3). In studies of 1,2-DCE mixtures, slight liver pathology was observed in rats one week after an acute 7-hour inhalation exposure to 66,740 mg/m^3 1,2-DCE (isomer composition not stated) —a concentration lethal to 6/9 exposed rats (Dow, 1960)— and relative liver weights were increased following exposure to 1,2-DCE (58% cis-, 42% trans-isomer) by inhalation intermittently for up to 195 days at concentrations of 1,980 mg/m^3 (rabbits) or 3,960 mg/m^3 (rats) (Dow, 1962). In the Dow (1962) study, clinical chemistry values were reported to be within normal values.

The increased liver weight observed in NTP (2002) and Barnes et al. (1985) was related to administration of trans-1,2-DCE; however, in the absence of elevated liver enzymes or histopathology, the change in liver weight is difficult to interpret.

In the 90-day drinking water study by Hayes et al. (1987), kidney weights (relative and absolute) were statistically significantly increased in female rats (by 11 to 13%) at doses of 1,257 and 2,809 mg/kg-day trans-1,2-DCE, but not in male rats in any dose groups. The kidney weight changes in female rats were not accompanied by histopathologic changes. In the dietary study by NTP (2002), absolute kidney weights were decreased (up to 9%) in female rats (1,580 and 3,245 mg/kg-day) and female mice (7,925 mg/kg-day), but relative kidney weights were similar to controls in all dosed groups. No gross or histopathological lesions in the kidney were observed in rats or mice that were attributed to exposure to trans-1,2-DCE (NTP, 2002). Similarly, clinical chemistry findings, BUN, creatinine, total protein, and albumin levels, did not provide evidence of any functional changes in the kidney. NTP (2002) observed that sporadic differences in clinical chemistry parameters at various time points generally did not demonstrate an exposure response relationship or were inconsistent between males and females.

Overall, the findings from Hayes et al. (1987) and NTP (2002) provide limited evidence that trans-1,2-DCE affects the kidney. The findings from these two studies are inconsistent, with Hayes et al. (1987) reporting an increase in relative kidney weight and NTP (2002) reporting a

decrease. Neither NTP (2002) nor Hayes et al. (1987) found any treatment-related histopathological changes of the kidney in rats and mice. Additionally, NTP (2002) did not find any clinical chemistry changes indicative of nephrotoxicity. Therefore, the kidney may not be a target organ of trans-1,2-DCE toxicity.

There is limited evidence in the trans-1,2-DCE database for effects on other organs. In a 90-day drinking water study, Barnes et al. (1985) reported increased relative lung weight in high-dose (452 mg/kg-day) female mice and decreased relative and absolute thymus weight in mid- (224 mg/kg-day) and high-dose (452 mg/kg-day) female mice, but not in any of the treated male mice. In other subchronic studies, neither Hayes et al. (1987) nor NTP (2002) found changes in the weights of these organs or histopathologic changes at doses almost 10-fold higher than the doses used in Barnes et al. (1985). Therefore, based on the available evidence, the lung and thymus are not targets of trans-1,2-DCE toxicity.

Some positive hematological findings have been associated with trans-1,2-DCE exposure. In a 90-day drinking water study, Barnes et al. (1985) reported sporadic changes in hematology parameters (prothrombin time, leukocytes, and polymorphonuclear leukocytes) in mice; changes in these parameters were not dose-related or consistent across sexes. In a second subchronic drinking water study of trans-1,2-DCE, Hayes et al. (1987) reported no treatment-related effects on hematologic parameters in rats at doses up to approximately 3,000 mg/kg-day.

NTP (2002) reported mild decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts at week 14 in male and female rats in all (380–3,245 mg/kg-day) but the lowest dose groups (190 mg/kg-day). Only decreased RBC counts showed a dose-response and statistical significance. This effect was demonstrated in male rats with significant decreases at doses $\geq$380 mg/kg-day ($p \leq 0.05$), although the maximum decrease in RBC was only 7% in males and 5% in females at the highest dose (3,210 and 3,245 mg/kg-day for males and females, respectively). NTP (2002) further observed the following: (1) McCauley et al. (1995) reported a decrease in the circulating erythroid mass in Sprague-Dawley rats exposed to 872 mg/kg cis-1,2-DCE by gavage for 90 days, but this response was not dose-related and not considered by the study investigators to be biologically relevant; and (2) no hematologic response was observed by Barnes et al. (1985) in male or female CD-1 mice exposed to 387 mg/kg-day trans-1,2-DCE for 90 days. NTP (2002) concluded that the trans- (and cis-) isomer may have an effect on hematologic endpoints but more consistency between studies is necessary before the biological significance (if any) is known.

The immunotoxicity associated with oral exposure to trans-1,2-DCE was investigated in mice treated for 14 or 90 days (Shopp et al., 1985; Munson et al., 1982). In male CD-1 mice administered trans-1,2-DCE for 14 consecutive days at doses up to 222 mg/kg-day by gavage, Munson et al. (1982) evaluated humoral immune function as indicated by the ability of spleen cells to produce IgM AFCs following challenge with sRBCs. Munson et al. (1982) also assessed cell-mediated immune function as indicated by the DTH response to sRBCs. The authors

described the antibody response to sRBCs as the number of AFCs per spleen and per 10^6 spleen cells. Munson et al. (1982) reported a trend toward suppression of the number of AFCs expressed on a per spleen basis (significant at $p < 0.1$), but this response was not statistically significant at the $p < 0.05$ level or when expressed per 10^6 spleen cells. The DTH response was characterized as slight but significant ($p < 0.05$) and not-dose dependent in the abstract of the journal article. However, in the results section of the article the authors state that trans-1,2-DCE showed no effect in the DTH response. This contradictory reporting of the DTH response data renders these findings questionable. It is unknown whether the slight reduction in DTH was associated with trans-1,2-DCE or some other test chemical evaluated in the study. The authors concluded that mice exposed to trans-1,2-DCE for 14 consecutive days at doses up to 222 mg/kg-day showed no significant change in cell-mediated or humoral immunity (Munson et al., 1982). Data from a longer duration (90-day) oral exposure study demonstrate that suppression of the antibody response to sRBCs is associated with exposure trans-1,2-DCE, but do not support an effect of trans-1,2-DCE on the DTH response.

In a 90-day drinking water study in mice (Shopp et al., 1985), a dose-related suppression of the humoral immune status, as measured by spleen cell antibody production directed against sRBCs, was observed in male mice treated with trans-1,2-DCE. When expressed as AFCs per 10^6 spleen cells, the number of AFCs was reduced by 26% in male mice at doses of 175 and 387 mg/kg-day (significantly different at $p < 0.05$ from control mice). Shopp et al. (1985) reported that there was marked suppression in humoral immune status in male mice as indicated by the significantly decreased number of AFCs in these mice. However, the authors also state that the decrease in AFCs was not severe enough to depress the functional ability of the humoral immune system because there was no change in hemagglutination titers to sRBCs or lymphoproliferative response of spleen cells to the B-cell mitogen LPS. The authors conclude that the immune system of CD-1 mice does not appear to be overly sensitive to trans-1,2-DCE and that the observed effects were probably the result of general toxicity as opposed to specific target organ toxicity.

EPA evaluated the findings from Shopp et al. (1985) and determined, in contrast to the study authors, that the suppression in the number of AFCs in male CD-1 mice represents functional suppression of the humoral immune system. Suppression of T-cell-dependent antibody response is a well validated endpoint that is highly predictive for immunotoxicity (Herzyk and Holsapple, 2007; Luster et al., 1992). The suggestion that the immune effects observed in Shopp et al. (1985) were the result of general toxicity is not supported. There were no indicators, such as reduced body weight, of general toxicity. There was no effect of trans-1,2-DCE on body weight in male or female mice at any dose tested.

EPA's testing guidelines for immunotoxicity require testing the antibody response to a T-cell-dependent antigen (and suggest the use of sRBCs) as the primary assay to determine functional responsiveness of major components of the immune system following chemical

exposure in mice and rats (U.S. EPA, 1998b). To evaluate the antibody response, EPA's testing guidelines require the measurement of splenic anti-sRBC AFCs or serum levels of anti-sRBC IgM. Reduction in either of these measures of the antibody response represents evidence of chemical immunosuppression. The AFC assay is a well validated endpoint in immunotoxicology and has been characterized across multiple labs (Ladics, 2007; Loveless, 2007). While serum measurements of anti-sRBC IgM levels such as the hemagglutination test are more convenient than AFC assays because of the ability to obtain values from frozen serum rather than viable animal cells, the two assays provide an evaluation of different aspects of the antibody response. The AFC assay provides a measure of the antibody producing cells of the spleen, and this measure is highly predictive of the overall immunotoxicity of a chemical (Luster et al., 1993; Luster et al., 1992). Serum anti-sRBC IgM values are a general measure of the antibody response because these values reflect antibodies produced from multiple sources, including spleen, lymph nodes, and bone marrow. Therefore, the AFC assay is not expected to provide evidence of chemical immunosuppression at the level of splenic antibody production that might not be identified by measurements of serum levels of anti-sRBC IgM. Data on the antibody response for two well known immunotoxicants (cyclophosphamide and dexamethasone) demonstrate that the AFC assay can be a more sensitive assay for the determination of suppression of the antibody response than measurement of serum levels of anti-sRBC IgM in an enzyme-linked immunosorbent assay (Loveless et al., 2007). Loveless et al. (2007) reported that the AFC assay was consistently better at identifying suppression of the T-dependent antibody response across laboratories and that the AFC detected suppression at lower concentrations for dexamethasone than were observed by measurement of serum levels of anti-sRBC IgM.

In addition to the negative data from the hemagglutination assay, Shopp et al. (1985) uses a lack of an observed effect of trans-1,2-DCE on the proliferative response of splenocytes to LPS to suggest that the functional ability of the humoral immune system in male mice was not suppressed. The proliferative response to LPS is not a reliable indicator of humoral immune suppression, and is listed as one of the poorest predictors for potential immunotoxicity in the review of sensitivity and predictability of immune tests by Luster et al. (1992). In contrast, suppression of T-cell-dependent antibody response as determined by the AFC assay is one of the most predictive assays for chemical immunotoxicity (Herzyk and Holsapple, 2007; Luster et al., 1992).

EPA determined that the 26% suppression in the number of sRBC-specific AFCs per 10^6 spleen cells of male mice in Shopp et al. (1985) is a biologically significant measure indicating suppressed immune function associated with oral exposure to trans-1,2-DCE that is not contradicted by a lack of observed change in the hemagglutination assay to sRBCs or proliferative response to LPS.

4.6.1.3. *Mixtures of cis- and trans-1,2-DCE*

There is inadequate information available on the mixtures of cis- and trans-1,2-DCE to support a separate health assessment. Dow (1960) reported that a dose of 2,000 mg/kg 1,2-DCE (isomer composition not stated) as a 10% solution in corn oil administered to rats by gavage was not lethal, but some kidney injury was observed at necropsy. In a 14-day oral gavage study in which a 50% mixture of both 1,2-DCE isomers was administered in a sesame seed oil vehicle (1 mL/kg) at a dose of 5 mmol (485 mg/kg-day) to male Sprague-Dawley-derived rats (six/group), McMillan (1986) reported a statistically significant increase in kidney weights, slight but significant reductions in plasma creatinine and BUN, and an increase in plasma calcium. In a 30-day study by McMillan (1986), the mean relative weight of the liver in the treated group at termination was significantly greater by 19% than that of control rats. Additionally, the treated rats exhibited significant reductions in mean relative weight of the lungs (14%), mean values for plasma AST (25%), and creatinine levels (17%); erythrocyte count, hemoglobin, and hematocrit levels were also reduced by 6, 5, and 5%, respectively.

4.6.2. Inhalation
4.6.2.1. *cis-1,2-DCE*

No studies of the effects of cis-1,2-DCE by inhalation exposure in humans were identified. There are no inhalation studies of subchronic, chronic, reproductive, or developmental toxicity of cis-1,2-DCE. Investigation of the inhalation toxicity of cis-1,2-DCE is limited to an acute 4-hour inhalation LC_{50} study in rats (DuPont, 1999). The LC_{50} was calculated to be 54,200 mg/m^3. Severe weight loss was noted at 53,400 mg/m^3, followed by normal weight gain during a 14-day observation period. Clinical signs noted in this study suggest effects on the CNS, including unresponsiveness, weakness, and irregular respiration immediately after exposure. Weakness was apparent after the 47,900 mg/m^3 cis-1,2-DCE exposure. At the 53,400 mg/m^3 exposure, weakness and irregular respiration immediately after the exposure was noted.

All five male rats exposed to 62,200 mg/m^3 cis-1,2-DCE and one male rat in the highest exposure group (91,900 mg/m^3) had minimal hepatic centrilobular vacuolation upon microscopic observation. One male rat in each of the lower exposure levels (47,900 and 53,400 mg/m^3) also had minimal hepatic centrilobular vacuolation upon microscopic examination. The authors noted that this change can often be seen in control rats and therefore did not consider the effects at these lower levels to be related to exposure to cis-1,2-DCE. Because all five male rats at the high concentration died during exposure, these animals may not have survived long enough to develop hepatic lesions. No exposure-related effects on the liver were observed in female rats, and no effects were seen in the heart, kidney, or lungs in exposed male or female rats following the acute (4-hour) inhalation exposure.

4.6.2.2. *trans-1,2-DCE*

The human database for trans-1,2-DCE is limited to one study from the 1930s involving only two subjects (Lehmann and Schmidt-Kehl, 1936). This study provides limited evidence that trans-1,2-DCE can cause eye irritation and CNS depression (nausea, drowsiness, fatigue, vertigo) following acute inhalation exposures. Information on the potential health effects of inhaled trans-1,2-DCE comes from studies in animals, including two acute inhalation studies (DuPont, 1999; Freundt et al., 1977) and two subchronic inhalation studies (Kelly et al., 1999; Freundt et al., 1977), of which only one (Freundt et al., 1977) is a published peer-reviewed study. In addition, one study evaluated the effects of inhalation exposure on developmental outcomes (DuPont, 1988a; published in Hurtt et al., 1993).

Inhalation studies provide evidence that the liver is likely the primary target of trans-1,2-DCE. In the only published peer-reviewed subchronic inhalation study, Freundt et al. (1977) reported slight to severe fatty accumulation in the liver lobules and Kupffer cells in rats exposed for 8 hours/day, 5 days/week to air containing 792 mg/m^3 trans-1,2-DCE for 1, 2, 8, or 16 weeks. These effects occurred in two of the six rats exposed for 1 week, in four of the six rats exposed for 2 weeks, in three of the six rats exposed for 8 weeks, and in five of the six rats exposed for 16 weeks. These effects were also seen in one of the six controls at 8 weeks and in two of the six controls at 16 weeks. In general, the incidence and severity of fat accumulation increased with increasing exposure duration. Similar effects were reported in an acute inhalation study by the same investigators (Freundt et al., 1977).

In a second subchronic study of rats exposed to analytically determined mean concentrations of 0, 792, 3,960, or 15,800 mg/m^3 trans-1,2-DCE for 6 hours/day, 5 days/week for 90 days (Kelly et al., 1999), investigators reported no effects on body weight or on food consumption, clinical symptoms were not remarkable, and no exposure-related effects were seen in clinical or anatomic pathology parameters or on liver cell proliferation. These study findings were reported as an abstract only, and the study methods and results could not be evaluated.

In a single-exposure concentration inhalation study by Freundt et al. (1977), histopathological changes of the lung (hyperemia and alveolar septal distension) were reported in rats exposed to 11,880 mg/m^3 for 8 hours. Similar effects were reported by these investigators in animals exposed to 792 mg/m^3 trans-1,2-DCE for 8 hours/day, 5 days/week for up to 16 weeks. The pathological changes in the lung were considered by the authors to be slight in severity and were present in all six rats in all four exposure groups, in one of the six control animals exposed for 1 week, and in two of the six control animals exposed for 2 weeks, but not in any of the control animals exposed at either 8 weeks or 16 weeks. The finding of lung effects in the Freundt et al. (1977) study is difficult to interpret as this study is the only report of lung pathology in animals exposed to trans-1,2-DCE, a small number of animals were examined, several of the controls also developed this effect, and the upper respiratory tract was not examined for pathology.

Only one study investigated the developmental toxicity of trans-1,2-DCE in pregnant rats that were exposed via inhalation to 7,930, 23,790, or 47,580 mg/m^3 trans-1,2-DCE for 6 hours/day (DuPont, 1988a; published in Hurtt et al., 1993). The two high concentrations were overtly maternally toxic, while the 7,930 mg/m^3 concentration (chosen at 10 times the TLV) was slightly maternally toxic (DuPont, 1988a; published in Hurtt et al., 1993). There were no changes in numbers of fetuses or implantations, but a statistically significant decrease in fetal weight was reported at the highest concentration. No malformations were observed. Oral administration of a 1,2-DCE mixture to pregnant mice and rats similarly provided no evidence for developmental toxicity and showed maternal toxicity only at high doses.

Evidence for CNS toxicity following trans-1,2-DCE exposure by the inhalation pathway comes from studies of acute inhalation exposure only. Freundt et al. (1977) reported no symptoms of CNS depression in rats that received an 8-hour inhalation exposure to concentrations up to 11,880 mg/m^3. Lethargy and irregular respiration were reported immediately after exposure to a concentration of 89,100 mg/m^3 (DuPont, 1999). In summary, the liver is considered to be the potential target organ from exposure via inhalation to trans-1,2-DCE.

4.6.2.3. *Mixtures of cis- and trans-1,2-DCE*

There is inadequate information available on the mixtures of cis- and trans-1,2-DCE to support a separate human health assessment. At concentrations of about 115,270 mg/m^3, rats exposed to a mixture of 1,2-DCE isomers (unspecified composition) rapidly became unconscious, and exposures lasting longer than 0.2 hours were fatal (Dow, 1960). In another Dow study (1962), rats, rabbits, guinea pigs, and beagle dogs were exposed to 0, 1,980, or 3,960 mg/m^3 of a 1,2-DCE mixture (58% cis-, 42% trans isomer) 7 hours/day for six months. The only notable effects in rats and rabbits exposed to the highest concentration (3,960 mg/m^3) were an increase in the average relative kidney weights in male and female rats by 16 and 9%, respectively (only statistically significant in males), and an increase of 23% in the average relative liver weights of female rats (statistically significant). Liver weights in both male and female rabbits were also increased, but statistical significance was not determined because of the small number of rabbits tested. At the mid-concentration of 1,980 mg/m^3, the relative kidney weights of male and female rats were statistically significantly increased by 9 and 18%, respectively; liver weights of female rats were also significantly increased by 19%. In addition, increases in liver weights of both male and female rabbits occurred at termination (statistical evaluations were not performed because of the small number of experimental animals). Thus, data from studies on the inhalation of mixtures of 1,2-DCE support the conclusion that the liver may be a target organ for 1,2-DCE. This is consistent with the findings from the oral subchronic studies of cis-1,2-DCE (McCauley, 1995, 1990), the oral subchronic studies of trans-1,2-DCE (NTP, 2002), and the inhalation subchronic studies of trans-1,2-DCE (Freundt et al., 1977).

4.6.3. Mode-of-Action Information

The available information on the toxic responses to either cis- or trans-1,2-DCE is limited and precludes the determination of a mode of toxic action. The acute toxicity (and possibly behavioral toxicity) of both isomers are likely the result of CNS toxicity related to the anesthetic and narcotic properties of both compounds. Although the liver is considered to be a potential target organ for cis- and trans-1,2-DCE-induced toxicity (NTP, 2002; McCauley et al., 1995; Hayes et al., 1987; Freundt et al., 1977), the nonspecific effects do not point to any particular mode of action (e.g., covalent binding of a metabolite). However, elucidating the reaction of metabolites of cis- and trans-1,2-DCE with cell components and their possible binding to a cell component may inform a possible mode of action.

In vitro studies indicate that the biotransformation of cis- and trans-1,2-DCE involves the hepatic CYP450 system. Furthermore, it has been proposed that multiple forms of hepatic CYP450 bind and metabolize cis- and trans-1,2 DCEs (Costa and Ivanetich, 1982). The study by Costa and Ivanetich (1982) suggests that the hepatic CYP450 system is closely associated with the metabolism and toxicity of 1,2-DCEs. In addition, a number of studies indicate that both the cis- and the trans-isomers are able to induce (at the protein synthesis levels) and/or inhibit (via suicide inhibition of the enzyme or suppression of protein synthesis) CYP450s. Based on the measurement of enzyme activities associated with specific CYP450s, Paolini et al. (1992) concluded that trans-1,2-DCE induced CYP2E1 (120%) and CYP2B1 (260%) but not CYP3A1/2 or CYP1A1/2; microsomal CYP450 content was elevated 50%. In a subsequent study, Paolini et al. (1995) evaluated the CYP2E1-inducing potency of trans-1,2-DCE in several species and organs. They found that trans-1,2-DCE induced CYP2E1 activity in the liver of hamster > rat > mouse, in the kidney of rat > mouse with no effect in the hamster, and in the lung of rat > mouse with no effect in the hamster. Nakahama et al. (2000) reported that cis- and trans-1,2-DCE suppressed CYP1A1/2 activity in rat liver microsomes by approximately 70 and 55% and 3A1/2 activity in liver by approximately 40 and 20%, respectively. CYP2B1/2 activity in lung microsomes was reduced by 73 and 96%, respectively. CYP2E1 activity, the enzyme that metabolizes both isomers in the liver, was not affected.

Testai et al. (1982), using a mixture of cis- and trans-1,2-DCE, found evidence that this compound inactivated CYP450 following metabolic activation by destroying the heme moiety of CYP450. Mathews et al. (1997) showed that CYP2E1 activity was affected by trans-1,2-DCE and that the effects of a single i.p. dose to male rats lasted less than 24 hours, at which time normal CYP2E1 levels had been restored. This effect of trans-1,2-DCE is so predictable that this isomer is routinely being used as a CYP2E1 inhibitor in toxicity studies (cf. Jackson et al., 2000). The reduction of CYP450 activity was also measured by an increase in hexobarbital sleeping time or zoxazolamine paralysis time (Freundt and Macholz, 1978). These authors reported that cis-1,2-DCE was more potent than trans-1,2-DCE and attributed this finding to a higher uptake

of cis-1,2-DCE in the liver, as compared with trans-1,2-DCE. In contrast, when rat liver microsomes were exposed in vitro to cis- or trans-1,2-DCE vapor, trans-1,2-DCE was found to be approximately 25% more potent than cis-1,2-DCE in inhibiting CYP2E1 activity; both isomers had no effect on CYP2B1/2 activity (Lilly et al., 1998; Thornton-Manning et al., 1994). The nature of the agent that binds to the heme portion of CYP450 has not been elucidated but is likely to be the epoxide (oxirane) (see Figure 3-1). One explanation for the relatively lower toxicity of trans-1,2-DCE in animal studies is the fact that it is metabolized poorly in vivo.

Hanioka et al. (1998) reported some compound- and gender-specific effects of cis- and trans-1,2-DCE, respectively. Rats were given i.p. injections of 7.5 mmol/kg (727 mg/kg) cis- or trans-1,2-DCE in corn oil on 4 consecutive days and sacrificed 24 hours after the last treatment. Liver microsomes were prepared and tested for CYP450 isozyme-specific protein contents. In males, cis-1,2-DCE reduced CYP2E1 and CYP4A1 levels to a minor extent, while those of 1A1/2, 2B1/2, 2C11/6, and 3A2/1 were reduced. In females, cis-1,2-DCE reduced only the level of CYP2B1/2 (the levels of 2C11/6 and 2A2/1 were below detection limits in females). In males, trans-1,2-DCE also had only a minor effect on CYP2E1, 3A2/1, and 4A1, reduced the level of 2C11/6 to a similar extent as cis-1,2-DCE, but increased the levels of CYP1A1/2 and 2B1/2. In female rats, trans-1,2-DCE caused an increase in CYP1A1/2 levels but no changes in the other CYP450 levels.

Filser et al. (1982, 1978) and Filser and Bolt (1980) observed the production of acetone following exposure to cis- and trans-1,2-DCE (and other halogenated ethanes). When male Wistar rats were exposed to cis- or trans-1,2-DCE at various concentrations in a closed-system chamber, the authors found acetone in the exhaled air (Filser et al., 1978). Chloroacetate, a known metabolite of haloethanes, also caused acetone exhalation; thus, the study authors proposed that the effect was caused by inhibition of the citric acid cycle. Similar to the results of Freundt and Macholz (1978), they found that cis-1,2-DCE was more potent than the trans-isomer in eliciting acetone production. Subsequently, Filser and Bolt (1980) reported that the amount of acetone exhaled far exceeded the amount of cis- or trans-1,2-DCE metabolized in the animals, suggesting that the exhaled acetone was not a metabolite of the test agent. The authors found that acetone formation did not increase further when cis- or trans-1,2-DCE exposure surpassed concentrations that saturated the metabolic capacity of the test animals; however, induction and inhibition of CYP450 increased and decreased acetone formation, respectively. They concluded that metabolic transformation of cis- or trans-1,2-DCE was a prerequisite for acetone formation. Based on a further study using only trans-1,2-DCE, Filser et al. (1982) suggested that the exhaled acetone was likely a by-product of increased lipid metabolism (a ketone body).

Keys et al. (2004) provided a summary of events by which DCA, a downstream metabolite of cis- and trans-1,2-DCE, brings about suicide inactivation of GSTZ, the enzyme that, by oxidative dechlorination, transforms DCA into glyoxylic acid (see Figure 3-1). DCA

can bind covalently to GSH, with the resulting compound either being hydrolyzed into glyoxylic acid and GSH or bound covalently to GSTZ, thus inactivating the enzyme. However, while it was shown that extended exposure to DCA slows down its own metabolism, the exact amount of DCA being formed during cis- or trans-1,2-DCE metabolism is not known. Therefore, the potential impact of this suicide inhibition on the overall metabolism of cis- or trans-1,2-DCE is also not known.

In summary, both cis- and trans-1,2-DCE induced synthesis of specific CYP450 isozymes to some extent (e.g., CYP1A1/2 2B1 and CYP2E1 in mice). The trans-1,2-DCE is an effective but transient inhibitor of CYP2E1 that by itself is metabolized poorly. The downstream metabolite DCA may affect the citric acid cycle and, secondarily, lipid metabolism, causing ketogenesis, but, because of the likely low in vivo concentrations of DCA resulting from 1,2-DCE exposure, it is probably biologically ineffective.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *inadequate information to assess the carcinogenic potential* of cis- and trans-1,2-DCE. This cancer descriptor is based on the absence of epidemiological studies in humans and animal studies in which tumors were detected or which exceeded an exposure period of 6 months.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

No epidemiologic studies evaluating possible long-term health effects of cis-1,2-DCE, trans-1,2-DCE, or their mixture in humans were identified. The longest duration animal study, a 6-month inhalation study in four species (Dow, 1962), did not evaluate any histology or cancer endpoints. The 90-day feeding study by NTP (2002) evaluated cancer endpoints but no positive findings were reported. This lack of primary information is supplemented by inconclusive evidence from genotoxicity and mutagenicity studies. For example, cis-1,2-DCE, trans-1,2-DCE, and their mixture have been mostly nonpositive in bacterial genotoxicity assays for gene reversion or DNA damage but gave positive results in some bacterial assays for mitotic recombination or aneuploidy, frequently in the absence of metabolic activation by S9. Results for chromosomal aberrations or sister chromatid exchanges in mammalian cells in culture were mixed, providing positive findings in the presence or absence of metabolic activation. Some in vivo assays gave positive results (host-mediated assay, chromosomal aberrations) for cis-1,2-DCE only, possibly reflecting the fact that hepatic uptake of cis-1,2-DCE is higher than that of trans-1,2-DCE. An additional factor contributing to the uncertainty is that DCA, a possible metabolite of both cis- and trans-1,2-DCE, is considered likely to be a human carcinogen (U.S. EPA, 2003).

4.7.3. Mode-of-Action Information

There is inadequate information to assess the carcinogenic potential of 1,2-DCE. However, it is worthwhile examining what is known about the biochemistry of the isomers of 1,2-DCE. Both cis- and trans-1,2-DCE are converted into reactive epoxides (oxiranes) by CYP450 enzymes. It is likely that epoxides are responsible for the inactivation of CYP2E1 by binding to its heme moiety, and protein adduct formation via sulfhydryl groups of amino acids has been shown to occur with 1,2-DCE (Maiorino et al., 1982; Sipes and Gandolfi, 1980). However, DNA adduct formation has not been demonstrated. DNA binding of 1,2-DCE was negative in an in vitro assay where other chlorinated hydrocarbons gave positive results (Sipes and Gandolfi, 1980).

Positive results have been obtained with cis-1,2-DCE in several genotoxicity assays in the absence of metabolic activation, suggesting that the C=C double bond positioned next to two chlorine substituents might be reactive on its own. However, Henschler (1977), in an evaluation of the mutagenicity of halogenated olefins, pointed out that asymmetric distribution of chlorine substituents across the C–C bond, such as exists in 1,1-DCE, was far more likely to give rise to mutagenic events because the resulting epoxides are unstable, as compared with a symmetric distribution of the chlorines as exists in both cis- and trans-1,2-DCE. Evidence for other effects that could potentially lead to tumor formation, such as redox cycling, GSH depletion, or lipid peroxidation, has not been shown for cis- or trans-1,2-DCE.

The fact that both cis- and trans-1,2-DCE form epoxides and/or radicals as active metabolites raises the question of whether these intermediates represent structural alerts. Laurence et al. (1984) performed a computational study of the reactivities of vinyl chloride and trans-1,2-DCE by evaluating the bond energies of protonated chlorine or oxygen in the corresponding chlorooxiranes. Their assessment indicated that the oxirane from trans-1,2-DCE should form a guanine N_7 adduct analogous to the one found after vinyl chloride exposure that is thought to be the cause of vinyl chloride-related cancer. However, this evaluation also predicted that the trans-1,2-DCE oxirane would be far more reactive than the one formed by vinyl chloride, rapidly reacting with other cellular nucleophiles before sufficient quantities could reach critical targets in the DNA, and thus predicting a lack of carcinogenicity associated with trans-1,2-DCE.

Carcinogenic activity of a metabolite of cis- and trans-1,2-DCE, DCA, has been established in several animal bioassays but not in humans (U.S. EPA, 2003). A mode of action has not been proposed. Existing evidence suggests that DCA exhibited some mutagenicity at high doses. U.S. EPA (2003) also discusses the possibility that DCA acts via cytotoxicity and compensatory hyperplasia for which some experimental evidence exists. Further evidence suggests that DCA might act via tumor promotion and hypomethylation (U.S. EPA, 2003). Therefore, there are several possible modes of action (i.e., epoxide formation, mutagenesis, redox cycling, GSH depletion or lipid peroxidation) that could be related to 1,2-DCE or one of its metabolites, but none of these have yet been established in animal bioassays or humans.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

No information is available concerning maternal exposure or health effects in developing humans exposed to cis- and/or trans-1,2-DCE. One animal study (DuPont, 1988a; published in Hurtt et al., 1993) investigated the potency of trans-1,2-DCE to induce fetotoxicity or developmental toxicity in pregnant rats exposed to this agent via inhalation at concentrations of 7,930 to 47,580 mg/m^3 for 6 hours daily on GDs 7–16. The results were negative; no malformations were identified, and fetal weight loss was associated only with concentrations that were overtly maternally toxic. On the basis of this study, trans-1,2-DCE is not expected to cause fetotoxicity or developmental effects in humans; however, the limited information does not support an assessment of potential developmental toxicity. No studies were conducted that would address childhood susceptibility to either cis- or trans-1,2-DCE.

4.8.2. Possible Gender Differences

Acute toxicity studies in animals provide suggestive evidence that males may be more sensitive than females to either cis- or trans-1,2-DCE (McCauley et al., 1995; Hayes et al., 1987). Hanioka et al. (1998) demonstrated that both cis- and trans-1,2-DCE were far more effective in affecting the activity of CYP450s in male rat hepatic microsomes as compared with female preparations. However, conclusions about gender differences in response to 1,2-DCE exposure cannot be drawn based on this limited information.

4.8.3. Other—Genetic Polymorphisms

Four specific enzymes have been associated with the metabolism of cis- or trans-1,2-DCE: CYP2E1, CYP3A4, ADH, and GSTZ. Of the CYP450s, CYP3A4 is active toward these compounds in rats (Costa and Ivanetich, 1982) but most likely not in humans (Guengerich et al., 1991) (for detail, see Sections 3.3.1 and 3.3.2). Alcohol dehydrogenases represent a whole family of enzymes, several members of which display gene polymorphism. Because the specific type of ADH, that according to Costa and Ivanetich (1982) and Filser and Bolt (1980) may be involved in 1,2-DCE metabolism, has not been characterized, possible variation in susceptibility associated with ADHs is not further considered here. CYP2E1 and GSTZ, as enzymes whose polymorphisms might affect the susceptibility of humans towards cis- or trans-1,2-DCE, are discussed below.

4.8.3.1. *Cytochrome P450 2E1*

CYP2E1 is constitutively expressed in human liver but is inducible by a variety of factors, prominently by ethanol consumption, diabetes, or hunger, with in vivo activity levels varying up to 20-fold (Rannug et al., 1995). Therefore, it is possible that a portion of the

population may experience susceptibility towards the toxic effects of cis- or trans-1,2-DCE. In addition, at least six allelic variants of CYP2E1 are known to exist in humans (Bartsch et al., 2000). In Caucasians, so far no variation in catalytic activity has been associated with genotype; >90% of Caucasians carry the homozygous wild-type c1/c1 allele. Asians, however, also carry the variant c2 allele, and the homozygous form of that allele, c2/c2, has been shown to have lower catalytic activity than the wild-type or the c1/c2 heterozygote (Bartsch et al., 2000). The frequency of the c2 allele has been reported to be 19–24% in Asians, and the frequencies of other variants are also much higher in Asians than in Caucasians (Rannug et al., 1995). There is evidence that the homozygous allele c2/c2 and, to a lesser extent, the heterozygote c1/c2 are associated with an increased risk for several cancer types (Bartsch et al., 2000). Thus, the possibility exists that polymorphism of the CYP2E1 gene may affect the susceptibility of humans to the toxic effects of cis- and/or trans-1,2-DCE.

4.8.3.2. *Glutathione S-Transferase*

Although DCA is likely a minor metabolite of cis- and trans-1,2-DCE, it is considered a likely human carcinogen, and therefore genetic polymorphism of the enzyme that metabolizes DCA, GSTZ, may play a role in human susceptibility. As outlined in the IRIS *Toxicological Review for Dichloroacetic Acid* (U.S. EPA, 2003), GSTZ is polymorphic in humans; at this time, five variants have been described that carry combinations of two possible A/G and/or two possible T/C transitions. The known variants are designated GSTZ1a-1a, GSTZ1b-1b, GSTZ1c-1c, GSTZ1d-1d, and GSTZ1e-1e (Blackburn et al., 2001, 2000; Tzeng et al., 2000). Blackburn et al. (2000) analyzed blood samples of Caucasians (68 female and 73 male Australians of European descent, ages 16–69) and demonstrated that allele frequencies for variants 1a, 1b, and 1c were 0.09, 0.28, and 0.63, respectively. In the following year, Blackburn et al. (2001) refined their analysis to comprise all five variants, using 128 Australian subjects of European descent, and found variant distributions of 0.086, 0.285, 0.473, 0.156, and 0 for GSTZ1a-1a, GSTZ1b-1b, GSTZ1c-1c, GSTZ1d-1d, and GSTZ1e-1e, respectively. Board et al. (2001) produced recombinant versions of variants GSTZ*A through GSTZ*D—corresponding to the variant alleles GSTZ1a-1a through GSTZ1d-1d—and tested their in vitro catalytic activities toward DCA. GSTZ*A had the highest activity with 1.61 µmol/minute/mg protein, followed by *B and *C each with 0.45, and *D with 0.3 µmol/minute/mg protein. Given the fact that only 9% of Caucasians carry the high-activity allele, the low-activity allelic variants may contribute to an increased susceptibility of at least 9 out of 10 Caucasians towards the toxic effects of DCA and, thus, also of cis- or trans-1,2-DCE.

5. DOSE-RESPONSE ASSESSMENT

5.1. ORAL REFERENCE DOSE

5.1.1. cis-1,2-DCE

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

The effects of oral exposure to cis-1,2-DCE in humans have not been investigated. McCauley et al. (1995, 1990) is the only published oral toxicity study of cis-1,2-DCE. Male and female Sprague-Dawley rats were administered 0, 32, 97, 291, or 872 mg/kg-day cis-1,2-DCE by corn oil gavage for 90 days. Terminal body weights in male rats at the two highest dose groups were lower than controls by 10–11%, but were not statistically significantly reduced. Relative liver weight was significantly increased in male and female rats at doses ≥97 mg/kg-day and relative kidney weight was significantly increased in male rats at all dose levels. Investigators reported no significant compound-related histopathological lesions of the liver or kidney. Statistically significant, but marginal, decreases in certain hematological parameters (primarily hemoglobin and hematocrit) were observed at doses ≥97 mg/kg-day. As discussed in Section 4.2.1.2.1, some errors and inconsistencies were identified upon examination of the unpublished (McCauley et al., 1990) and published (McCauley et al., 1995) versions of the study, principally related to the documentation of administered doses by the study authors, inconsistencies in reporting of methods, and some transcription or calculation errors in the unpublished report and published paper. These errors and inconsistencies suggest issues with the quality of the report writing, but not with the study findings themselves. As the only repeat-dose study of cis-1,2-DCE toxicity, this study was used as the basis for the oral RfD.

There is an overall increasing trend for both relative liver weight and relative kidney weight in rats exposed to cis-1,2-DCE. Lack of pathology for both the liver and the kidney indicate no overt signs of toxicity. Generally, increases in clinical chemistry parameters (such as BUN), may be indicative of liver and/or renal dysfunction. In the McCauley et al. (1995, 1990) study, BUN levels were statistically significantly decreased in high-dose (872 mg/kg-day) male rats but the values in females were similar to controls. Nevertheless, BUN levels cannot be used to determine a difference between kidney and liver toxicity because BUN levels can be impacted by both liver and kidney function. However, as discussed in Section 4.6.1.1, the most consistently observed finding, demonstrated in both male and female rats, was increased relative liver weight. Kidney weights were not significantly elevated in female rats and BUN and creatinine levels (indicators of renal dysfunction) were not elevated in any treatment groups. Hematological parameters were within normal ranges for Sprague-Dawley rats. Therefore, the kidney and hematology findings in McCauley et al. (1995, 1990) were not used for the derivation of the RfD.

As discussed in Section 4.6.1.1, increases in relative liver weight alone (up to 32 and 30% in high-dose male and female rats, respectively) are difficult to interpret. Liver weight changes occurred in the absence of compound-related changes in liver histopathology and AST, and measurements of other clinical chemistry indicators of liver function were not performed as part of this study. A review of acute and short-term toxicity studies on cis-1,2-DCE and the database for mixed isomers of 1,2-DCE provides support for the liver as a potential target of toxicity. Therefore, change in relative liver weight is identified as the critical effect and serves as the basis for the point of departure (POD) for the RfD for cis-1,2-DCE. This is based on the consistent findings of liver weight changes in both male and female rats in oral toxicity studies of varying durations of cis-1,2-DCE (McCauley et al., 1995, 1990).

5.1.1.2. *Methods of Analysis, Including Models*

Increased relative liver weight in male and female rats from the McCauley et al. (1995, 1990) study was selected as the critical effect for derivation of the RfD. Relative liver weight data are summarized in Table 5-1.

Table 5-1. Relative liver weights of rats exposed to cis-1,2-DCE by gavage for 90 days

		Dose (mg/kg-day)			
	Control	**32**	**97**	**291**	**872**
Males[a]	2.85 ± 0.26	3.15 ± 0.27	3.28 ± 0.18^{b}	3.34 ± 0.44^{b}	3.75 ± 0.20^{b}
Females[a]	2.82 ± 0.19	2.91 ± 0.18	3.21 ± 0.22^{b}	3.36 ± 0.18^{b}	3.67 ± 0.27^{b}

[a]Values are mean ± SD.
[b]Significantly different from control group; $p \leq 0.05$ by Tukey's multiple comparison test.

Source: McCauley et al. (1995).

Benchmark dose (BMD) modeling methodology (U.S. EPA, 2000b) was used to determine the POD. All of the models for continuous data in U.S. EPA's BMDS (version 1.4.1) (U.S. EPA, 2007) were fit to relative liver weight data in male and female rats. BMDS was used to calculate PODs for deriving the RfD by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower confidence limit ($BMDL_x$). A 10% change in relative liver weight compared with the control was selected as the benchmark response (BMR) level for this endpoint. A BMR of 10% change in relative liver weight was selected by analogy to body weight, for which a 10% change is generally recognized as a minimally biologically significant change (U.S. EPA, 2000b). In addition, consistent with EPA BMD guidance (U.S. EPA, 2000b), a BMR corresponding to a change in the mean response equal to one standard deviation from the control mean was also used to generate BMDs and BMDLs for comparison purposes.

In the female rat, only the Hill model (with the power parameter restricted to be greater than 1) adequately fit the relative liver weight data (test 4 χ^2 $p > 0.1$). The other two continuous models fit to these data, the first-degree polynomial and power models, exhibited significant lack of fit. Table B-1 in Appendix B presents the goodness-of-fit statistics and corresponding BMD and BMDL estimates for all three continuous models (i.e., first-degree polynomial, power, and Hill models) fit to these data. The Hill model predicted a BMD_{10} and $BMDL_{10}$ of 80.5 and 42.3 mg/kg-day, respectively. For comparison purposes, this same model was fit to these data using a BMR corresponding to a change in the mean response equal to one standard deviation from the control mean, and yielded BMD_{1SD} and $BMDL_{1SD}$ estimates of 53.2 and 28.8 mg/kg-day, respectively. In this particular case, one standard deviation from the control mean represented about a 7% change in relative liver weight.

For the male rat, only the Hill model (with power restricted to be greater than 1) adequately fit the relative liver weight data (test 4 χ^2 $p > 0.1$). The other two continuous models fit to these data, the first-degree polynomial and power models, exhibited significant lack of fit. Table B-2 in Appendix B presents the goodness-of-fit statistics and corresponding BMD and BMDL estimates for all three continuous models (i.e., first-degree polynomial, power, and Hill models) fit to these data. Modeling of the variance (i.e., the test 3 statistic in the BMDS output) was not satisfactory (test 3 χ^2 $p = 0.049$), but because the selected BMR is not expressed on a standard deviation basis, the impact on the POD is minimal. Therefore, the Hill model was considered to provide an adequate fit to the male rat relative liver weight data. This model predicted a BMD_{10} and $BMDL_{10}$ of 54.4 and 18.6 mg/kg-day, respectively. For comparison purposes, this same model was fit to these data using a BMR corresponding to a change in the mean response equal to one standard deviation from the control mean, and yielded BMD_{1SD} and $BMDL_{1SD}$ estimates of 40.4 and 13.0 mg/kg-day, respectively. In this particular case, one standard deviation from the control mean represented about a 9% change in relative liver weight. See Appendix B for further details regarding the BMD modeling of male and female rat relative liver weight data for cis-1,2-DCE.

Both male and female rats in the McCauley et al. (1995, 1990) study exhibited sensitivity to the effects of cis-1,2-DCE on the liver. Relative liver weight increases in high-dose male and female rats were essentially the same (i.e., 32% for males and 30% for females), and dose-response curves for relative liver weight in males and females were similar (see Appendix B, Section B.1). The $BMDL_{10}$ estimates for a 10% increase in relative liver weight in male and female rats were 18.6 and 42.3 mg/kg-day, respectively. The POD for the RfD for cis-1,2-DCE was chosen as 18.6 mg/kg-day, the lower of the male and female $BMDL_{10}$ values.

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

An RfD of 0.006 mg/kg-day for cis-1,2-DCE was derived by applying a composite uncertainty factor (UF) of 3,000 to the $BMDL_{10}$ of 18.6 mg/kg-day, as follows:

$$
\begin{aligned}
\text{RfD} \quad &= \quad \text{BMDL}_{10}/\text{UF} \\
&= \quad 18.6 \text{ mg/kg-day}/3{,}000 \\
&= \quad 0.006 \text{ mg/kg-day}
\end{aligned}
$$

The composite UF of 3,000 includes factors of 10 to protect susceptible individuals, 10 to extrapolate from animals to humans, 10 for use of a study of subchronic duration, and 3 to account for database deficiencies.

- An UF of 10 for intraspecies differences population (UF_H) was used to account for potentially sensitive human subpopulations in the absence of quantitative information on the variability of response to cis-1,2-DCE in the human population.

- An interspecies uncertainty factor (UF_A) of 10 was applied to account for the variability in extrapolating from laboratory animals to humans. No information was available to characterize the toxicokinetic or toxicodynamic differences between experimental animals and humans for cis-1,2-DCE.

- An UF of 1 was used to account for extrapolation from a LOAEL to a NOAEL (UF_L) because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% change in relative liver weight compared with the control was selected under an assumption that it represents a minimal biologically significant change.

- An UF of 10 was used to account for extrapolating from a POD for a subchronic exposure duration to estimate chronic exposure conditions (UF_S).

- An UF of 3 was used to account for database deficiencies (UF_D). The study used in this RfD derivation, McCauley et al. (1995, 1990), is the only study of repeat-dose toxicity available for cis-1,2-DCE. The database for this isomer is missing studies of reproductive, including a two-generation reproductive toxicity study, and developmental toxicity; however, the developmental toxicity potential for cis-1,2-DCE is informed by a series of range-finding studies of the developmental toxicity of a mixture of 1,2-DCE isomers (composition of isomers unknown) (NTP, 1991a, b, c). No evidence of developmental toxicity was observed in mice or rats based on the parameters evaluated in these range-finding studies (gravid uterus weight, fetal body weight, number of fetuses [live/dead], implantation sites, and resorptions).

5.1.1.4. *Previous Oral Assessment*

An oral RfD for cis-1,2-DCE was not previously available on IRIS.

5.1.2. trans-1,2-DCE

5.1.2.1. *Choice of Principal Studies and Critical Effects—with Rationale and Justification*

The effects of oral exposure to trans-1,2-DCE in humans have not been investigated. No chronic studies of trans-1,2-DCE in experimental animals are available. There are four subchronic studies of oral exposure to trans-1,2-DCE (NTP, 2002; Hayes et al., 1987; Barnes et al., 1985; Shopp et al., 1985). Table 4-12 presents a summary of these studies.

In a 14-week gavage study, NTP (2002) examined the effects of trans-1,2-DCE in both sexes of F344/N rats and B6C3F$_1$ mice. Doses ranged from 190 to 3,210 mg/kg-day in male rats; 190 to 3,245 mg/kg-day in female rats; 480 to 8,065 mg/kg-day in male mice; and 450 to 7,925 mg/kg-day in female mice. Both untreated and vehicle controls were used. Rats exhibited a decrease (approximately 6%) in final mean body weight and body weight gain, a minimal (no greater than 13%) but transient decrease of ALP activity that was not considered by the authors to be toxicologically relevant, a significant increase in relative liver weight (up to approximately 10% increase in females), a significant decrease in kidney weight (up to 7% decrease in males), but no gross or histological lesions. The relative liver weight changes in treated female rats were statistically significantly increased about 6-10% compared with controls at doses of ≥395 mg/kg-day; male rats exhibited slight increases (<6%). Similarly in mice, there were generally no exposure-related alterations in clinical chemistry parameters and no exposure-related deaths. Mice exhibited an approximate 4–7% decrease in final mean body weight and body weight gain, a significant increase in relative liver weight (9-15% increase at doses ≥1,900 mg/kg-day in males: approximately 11% doses at ≥3,760 mg/kg-day in females), but no gross or histological lesions.

Barnes et al. (1985) and Hayes et al. (1987) are 90-day drinking water studies. The most prominent effect observed by Barnes et al. (1985) was a statistically significant increase in serum ALP levels of 62 and 33% in male mice at the 175 and 387 mg/kg-day doses, respectively. These increases showed no dose-response relationship, were not found in the female mice, and were within the normal range for this strain of mouse. The only treatment-related effects observed by Hayes et al. (1987) were small but statistically significant increases in absolute kidney weight (8–9%) in female rats at doses of 1,257 and 2,809 mg/kg-day.

Immunotoxicity of trans-1,2-DCE in CD-1 mice was assessed in a 90-day drinking water study by Shopp et al. (1985). A dose-related suppression of sRBC-specific AFCs was observed in the spleens of male mice treated with trans-1,2-DCE. Shopp et al. (1985) reported marked suppression in humoral immune status in male mice at 175 and 387 mg/kg-day as indicated by the significantly decreased number of AFCs in these mice (when expressed as AFCs per 10^6 cells).

The subchronic studies by NTP (2002), Hayes et al. (1987), and Barnes et al. (1985) provide limited evidence for effects of trans-1,2-DCE on other organs. Overall, however, the evidence does not support a conclusion that the lung (NTP, 2002; Hayes et al., 1987; Barnes et

al., 1985) or thymus (NTP, 2002; Hayes et al., 1987; Barnes et al., 1985) are targets of trans-1,2-DCE toxicity. Although there are some positive hematological findings associated with trans-1,2-DCE exposure (NTP, 2002; Hayes et al.,1987; Barnes et al.,1985), changes in these parameters were not dose-related or consistent across sexes except for decreases in RBC counts (NTP, 2002). Decreases in the RBC count were small and are not considered biologically significant. Therefore, the available evidence does not support consideration of changes in hematological parameters as a critical effect for trans-1,2-DCE. Body weights were dose-dependently reduced in male rats (NTP, 2002) by about 6%. Such reductions were not observed in other oral studies of trans-1,2-DCE.

Decreased number of AFCs against sRBCs (Shopp et al., 1985) and increased liver weight (NTP, 2002) were considered for derivation of potential PODs to serve as the basis of the trans-1,2-DCE RfD. The immunological response reported in Shopp et al. (1985) is regarded as a biologically significant response and was observed at relatively low doses (≥175 mg/kg-day) of trans-1,2-DCE. A review of the subchronic toxicity studies for trans-1,2-DCE provides support for the liver as a target of toxicity. Liver weight changes were observed in female rats and male and female mice exposed to trans-1,2-DCE in NTP (2002). The female rats exposed to trans-1,2-DCE (190-3,245 mg/kg-day) exhibited statistically significant increases in liver weight at doses ≥395 mg/kg-day. Liver weights increased in both male and female mice exposed to trans-1,2-DCE (450-8,065 mg/kg-day), although the male mice were more sensitive with significant increases at doses ≥1,900 mg/kg-day compared with the increases in females at doses ≥3,760 mg/kg-day.

5.1.2.2. *Methods of Analysis-- Including Models*

A benchmark dose (BMD) approach (U.S. EPA, 2000b) was employed to determine the POD for the two endpoints considered as candidates for the critical effect—decreased number of AFCs against sRBCs (Shopp et al., 1985) and increased liver weight (NTP, 2002).

AFC response to sRBCs

Decreased antibody production directed against sRBCs in male mice from the Shopp et al. (1985) study was considered as a candidate critical effect for the derivation of the RfD for trans-1,2-DCE. Data for humoral immune response to sRBCs in CD-1 mice exposed to trans-1,2-DCE are summarized in Table 5-2.

Table 5-2. Humoral immune response to sRBCs in CD-1 mice exposed to trans-1,2-DCE in drinking water for 90 days (Day 4)

Exposure group	Spleen weight (mg)	AFCs per spleen ($\times 10^{-5}$)	AFCs per 10^6 cells
Males[a]			
Control	202 ± 30	4.48 ± 0.32	$2,200 \pm 125$
0.1 mg/mL	164 ± 13	3.28 ± 0.28[b]	$2,048 \pm 152$
1.0 mg/mL	178 ± 6	3.34 ± 0.39[b]	$1,625 \pm 136$[b]
2.0 mg/mL	173 ± 10	2.87 ± 0.37[b]	$1,618 \pm 226$[b]
Females[a]			
Control	228 ± 13	4.38 ± 0.37	$1,765 \pm 110$
0.1 mg/mL	176 ± 11[b]	2.97 ± 0.49[b]	$1,478 \pm 211$
1.0 mg/mL	230 ± 12	4.51 ± 0.24	$1,967 \pm 89$
2.0 mg/mL	191 ± 13[b]	3.47 ± 0.50	$1,518 \pm 184$

[a]Values are mean ± SE for 12 mice in the control group and 8 mice in treatment groups, measured on day 4 after antigen presentation.
[b]Values differ significantly from control group, $p < 0.05$.

Source: Shopp et al. (1985).

The EPA's benchmark dose software (BMDS), version 1.4.1c (U.S. EPA, 2007), was used to estimate a POD for deriving an RfD for trans-1,2-DCE. All of the available continuous models in BMDS (i.e., polynomial, power, and Hill models) were fit to the decrease in IgM AFCs (i.e., AFCs per 10^6 spleen cells) observed in male CD-1 mice exposed to trans-1,2-DCE in drinking water for 90 days (Shopp et al., 1985). These continuous models were used to estimate the effective dose at a specified level of response (BMD$_x$), and its 95% lower bound (BMDL$_x$). For continuous endpoints, the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b) states that a minimal level of change in an endpoint that is generally considered to be biologically significant may be used to define the specified level of response, or BMR. However, little information exists concerning the biological significance of particular changes in AFC levels in rodents, and what these changes would correspond to in humans. Therefore, as recommended for continuous data in the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), a change in the mean response equal to one standard deviation from the control mean was used to facilitate a consistent basis of comparison across assessments for this endpoint in the absence of information regarding the level of change considered to be biologically significant. In this case, a BMR of 1 standard deviation corresponds to a 20% decrease in AFCs per 10^6 spleen cells.

The BMDL$_{1SD}$ based on AFCs per 10^6 spleen cells was identified by evaluating the BMDS outputs from each of the fitted models, i.e., polynomial, power, and Hill. Adequacy of each model fit was determined by examining the chi-square goodness-of-fit statistic, with χ^2 p-values less than 0.1 indicative of statistically significant lack of fit. Table B-6 in Appendix

B presents the goodness-of-fit statistics and corresponding BMD and BMDL estimates for all four models fit to these data (i.e., second-degree polynominal, first-degree polynomial, power, and Hill models). The best-fitting model was chosen from those models exhibiting adequate fit by selecting the model with the lowest Akaike Information Criteria (AIC) value, as well as evaluating how well each model visually fit the data, especially in the region of the curve near the BMD (see Appendix B for further details). Based on the model selection procedure outlined above, a second-degree polynomial model provided the best fit to these data, yielding a BMD_{1SD} of 125.6 mg/kg-day and a $BMDL_{1SD}$ of 65.0 mg/kg-day. Based on a 1 standard deviation decrease from the control in IgM antibody-forming cells, the $BMDL_{1SD}$ of 65.0 mg/kg-day was identified as a candidate POD for the trans-1,2-DCE RfD.

Relative liver weight

Increased relative liver weight in male and female mice and female rats from the NTP (2002) study was also considered in the selection of a critical effect for development of the RfD. The changes in relative liver weight in treated male rats were not significantly elevated over controls and this male rat data set was not further considered. Relative liver weight data are summarized in Table 5-3.

Table 5-3. Relative liver weights in male and female mice and rats exposed to trans-1,2-DCE in the feed for 14 weeks

	Mice - males					
	Dose (mg/kg-day)					
	0	480	920	1,900	3,850	8,065
Relative liver weights (mean ± SE)[a]	4.347 ± 0.056	4.552 ± 0.113	4.597 ± 0.115	4.745 ± 0.084[b]	4.736 ± 0.079[b]	4.979 ± 0.111[b]
	Mice - females					
	Dose (mg/kg-day)					
	0	450	915	1,830	3,760	7,925
Relative liver weights (mean ± SE)[a]	4.621 ± 0.07	4.738 ± 0.068	4.970 ± 0.127	4.813 ± 0.05	5.115 ± 0.139[b]	5.117 ± 0.08[b]
	Rats - males					
	Dose (mg/kg-day)					
	0	190	380	770	1,540	3,210
Relative liver weights (mean ± SE)[a]	3.465 ± 0.058	3.538 ± 0.032	3.658 ± 0.099	3.524 ± 0.050	3.492 ± 0.048	3.634 ± 0.056
	Rats - females					
	Dose (mg/kg-day)					
	0	190	395	780	1,580	3,245
Relative liver weights (mean ± SE)[a]	2.937 ± 0.038	3.040 ± 0.052	3.220 ± 0.066[b]	3.100 ± 0.051[b]	3.132 ± 0.052[b]	3.216 ± 0.051[b]

[a]Ten animals per group.
[b]Statistically significant, $p \leq 0.01$.

Source: NTP (2002).

All of the models for continuous data in U.S. EPA's BMDS (version 1.4.1) (U.S. EPA, 2007) were fit to the relative liver weight data from NTP (2002) in female rats and male and female mice. BMDS was used to calculate PODs for deriving the RfD by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower confidence limit ($BMDL_x$). A 10% change in relative liver weight compared with the control was selected as the BMR for this endpoint. A BMR of 10% change in relative liver weight was selected by analogy to body weight, for which a 10% change is generally recognized as a minimally biologically significant change (U.S. EPA, 2000c).

Only the male mouse relative liver weight data could be adequately modeled by the continuous models in BMDS. The Hill model (with the power parameter restricted to be greater than 1) and two other continuous models, the first-degree polynomial and power models, did not exhibit significant lack of fit (based on χ^2 p-value >0.1). The Hill model exhibited the best fit of

the data based on the AIC value, which was lower than the AIC values for the other models. Table B-3 in Appendix B presents the goodness-of-fit statistics and corresponding BMD and BMDL estimates for all three continuous models fit to these data (i.e., first-degree polynomial, power, and Hill models). The Hill model predicted a BMD_{10} and $BMDL_{10}$ of 3,241.9 and 867.3 mg/kg-day, respectively. Consistent with EPA guidance (U.S. EPA, 2000b), for comparison purposes, this same model was fit to these data using a BMR corresponding to a change in the mean response equal to one standard deviation from the control mean, and yielded BMD_{1SD} and $BMDL_{1SD}$ estimates of 1,348.7 and 395.9 mg/kg-day, respectively. In this case, one standard deviation from the control mean represented about a 4% change in relative liver weight. See Appendix B for further details regarding the BMD modeling of male mouse relative liver weight data for trans-1,2-DCE. The $BMDL_{10}$ of 867.3 mg/kg-day based on a 10% increase in relative liver weight in male mice was identified as a candidate POD for the trans-1,2-DCE RfD.

Increased relative liver weight was observed in female mice and rats (Table 5-3), although these data sets were not amenable to BMD modeling because at least one of the mid-level dose groups exhibited a decrease in relative liver weight yielding a non-monotonically increasing dose-response function. Therefore, a NOAEL/LOAEL approach was applied. In female mice, the NOAEL was 1,830 mg/kg-day and the LOAEL was 3,760 mg/kg-day, based on statistically significant increases in relative liver weight. In female rats, the NOAEL was 190 mg/kg-day and the LOAEL was 395 mg/kg-day, based on statistically significant increases in relative liver weight. The NOAEL of 190 mg/kg-day was identified as a candidate POD for the trans-1,2-DCE RfD.

The dose-response analysis of the immune and liver endpoints suggests that the immune system is more sensitive to the effects of trans-1,2-DCE. Therefore, suppression of the humoral immune system, as measured by spleen cell antibody production directed against sRBCs, was selected as the critical effect for the trans-1,2-DCE RfD, and the Shopp et al. (1985) study was identified as the principal study. The $BMDL_{1SD}$ of 65.0 mg/kg-day was selected as the POD for deriving the RfD for trans-1,2-DCE.

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

To derive an RfD for trans-1,2-DCE, the $BMDL_{1SD}$ of 65.0 mg/kg-day (Shopp et al., 1985) was divided by a composite UF of 3,000. Therefore, the RfD for trans-1,2-DCE is calculated as follows:

$$
\begin{aligned}
\text{RfD} \quad &= \text{BMDL}_{1SD} \div \text{UF} \\
&= 65.0 \text{ mg/kg-day} \div 3,000 \\
&= 0.02 \text{ mg/kg-day}
\end{aligned}
$$

The composite UF of 3,000 includes factors of 10 to protect sensitive individuals, 10 to extrapolate from animals to humans, 10 for use of a study of subchronic duration, and 3 to account for database deficiencies.

- An UF of 10 for intraspecies differences (UF_H) was used to account for potentially sensitive human subpopulations in the absence of quantitative information on the variability of response to trans-1,2-DCE in the human population.

- An interspecies uncertainty factor of 10 (UF_A) was applied for variability in extrapolating from laboratory animals to humans. No information was available to characterize the toxicokinetic or toxicodynamic differences between experimental animals and humans for trans-1,2-DCE.

- An UF of 1 was used to account for extrapolation from a LOAEL to a NOAEL (UF_L) because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of 1 standard deviation in spleen cell antibody production was selected under an assumption that it represents a minimal biologically significant change.

- An UF of 10 was used to account for extrapolating from a POD for a subchronic exposure duration to estimate chronic exposure conditions (UF_S).

- An UF of 3 was used to account for database deficiencies (UF_D). There are several subchronic oral studies of trans-1,2-DCE (NTP, 2002; Hayes, 1987; Barnes, 1985; Shopp, 1985). There is one study that investigated developmental toxicity of trans-1,2-DCE via inhalation (DuPont, 1988a) that showed few developmental parameters to be affected by treatment. In this study developmental toxicity was manifest only in high-dose groups. Developmental toxicity potential for trans-1,2-DCE is informed by a series of oral range-finding studies of the developmental toxicity of a mixture of 1,2-DCE isomers (composition of isomers unknown) (NTP, 1991a, b, c). No evidence of developmental toxicity was observed in mice or rats based on the parameters evaluated in these range-finding studies (gravid uterus weight, fetal body weight, and number of fetuses [live/dead], implantation sites, and resorptions). The database for trans-1,2-DCE is missing studies of reproductive toxicity, including a two-generation reproductive toxicity study.

5.1.2.4. *Previous Oral Assessment*

The previous RfD of 0.02 mg/kg-day for trans-1,2-DCE was based on the 90-day subchronic drinking water study in mice (Barnes et al., 1985). The critical effect was increased serum ALP in male mice. The LOAEL-NOAEL approach was used to derive the RfD. A POD of 17 mg/kg-day (NOAEL) was identified and a combined UF of 1,000 was applied, resulting in an RfD of 0.02 mg/kg-day. The UF of 1,000 accounts for the uncertainty in the extrapolation of dose levels from laboratory animals to humans ($UF_A = 10$), uncertainty in the threshold for sensitive humans ($UF_H = 10$), and uncertainty in extrapolating from subchronic to chronic exposure ($UF_S = 10$), but did not account for database deficiencies. The current assessment uses a

different principal study and a different approach for the derivation of the RfD from the previous oral assessment. The Shopp et al. (1985) study was selected as the principal study. A decrease in spleen cell antibody production directed against sRBCs was identified as the critical effect. BMD modeling was used to analyze the data from the Shopp et al. (1985) study; a POD of 65 mg/kg-day was derived. A composite UF of 3,000 was applied to the POD to derive the RfD for trans-1,2-DCE of 0.02 mg/kg-day. The UF of 3,000 accounts for the uncertainty in the extrapolation of dose levels from laboratory animals to humans ($UF_A = 10$), uncertainty in the threshold for sensitive humans ($UF_H = 10$), uncertainty in extrapolating from subchronic to chronic exposure ($UF_S = 10$), and deficiencies in the database (UF = 3). The previous RfD did not include a UF for database deficiencies.

5.2. INHALATION REFERENCE CONCENTRATION

5.2.1. cis-1,2-DCE

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

There are no human, chronic, or subchronic inhalation studies for cis-1,2-DCE. There is only an acute study by DuPont (1999). This is a 4-hour inhalation LC_{50} study in which groups of five male and five female Crl:CD®BR rats were exposed to cis-1,2-DCE. The LC_{50} was calculated to be 54,200 mg/m^3. During exposure, the rats were prostrate, with eyes open, but unresponsive to alerting stimuli. Other clinical signs included weakness and irregular respiration immediately after exposure. Slight to severe weight loss occurred for one day after exposure, but normal weight gain was seen thereafter.

5.2.1.2. *Method of Analysis*

In the absence of a long-term inhalation study, no critical effect was defined for the derivation of an RfC for cis-1,2-DCE.

5.2.1.3. *RfC Derivation*

No inhalation data are available for cis-1,2-DCE to support an RfC derivation.

5.2.1.4. *Previous Inhalation Assessment*

An inhalation assessment for cis-1,2-DCE was not previously developed for the IRIS database.

5.2.2. trans-1,2-DCE

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

No epidemiological studies of the effects of inhalation exposure to trans-1,2-DCE in humans are available, and case reports involving acute exposure to 1,2-DCE do not provide data useful for derivation of an RfC. There are two 90-day or longer duration studies using trans-

1,2-DCE (Kelly et al., 1999; Freundt et al., 1977). The Kelly et al. (1999) study exists as an abstract only and the Freundt et al. (1977) subchronic study is a one-concentration study over several exposure durations. According to the abstract by Kelly et al. (1999), there were no notable effects on body weight or on food consumption and clinical symptoms were not remarkable. Additionally, no exposure-related effects were seen in clinical or anatomic pathology parameters or on liver cell proliferation, indicating a low degree of toxicity at the exposure concentrations used (0, 792, 3,960, or 15,800 mg/m^3). Considering these study findings were reported as an abstract only, the information on the study methods were not available to evaluate this study.

Freundt et al. (1977) exposed six rats/group for 8 hours/day, 5 days/week to air containing 792 mg/m^3 trans-1,2-DCE for 1, 2, 8, and 16 weeks. As shown in Table 4-7, histological changes included slight to severe fatty accumulation in the liver lobules and Kupffer cells. After exposure to 792 mg/m^3 for 1 week, slight fat accumulation in liver lobules and Kupffer cells occurred in two of the six rats but not in any of the controls. After exposure for 2 weeks, slight fat accumulation in liver lobules and Kupffer cells occurred in four of the six rats but not in any of the controls. With an exposure duration of 8 weeks, three of the six rats showed evidence of fat accumulation; slight changes in liver lobules and severe changes in the Kupffer cells. Severe changes were also noted in the Kupffer cells in one of the six controls. When the exposure duration was for 16 weeks, slight fat accumulation in the Kupffer cells and severe fat accumulation in liver lobules were noted in three of the six exposed rats. Slight fat accumulation in both the Kupffer cells and liver lobules also occurred in another two of the treated animals in this 16-week exposure group, for a total of five of the six rats showing some liver effect in this exposure group. However, slight fat accumulation in both the Kupffer cells and in liver lobules also occurred in two of the control animals in this 16-week exposure group. For each of the exposure durations (1, 2, 8, and 16 weeks) there was no statistically significant difference between the controls and the exposed groups with respect to the incidence of liver effects (fat accumulation). However, in general, the incidence and severity of fat accumulation increased with increasing exposure duration.

The Freundt et al. (1977) study was chosen as the principal study since it was the only peer-reviewed studied available on the inhalation toxicity of trans-1,2-DCE and it demonstrated effects in the liver. Fatty accumulation in liver lobules and Kupffer cells is considered the critical effect. There was no evidence of a functional change in livers of rats in this study. The choice of this endpoint as the critical effect is supported by an acute inhalation study by the same investigators and oral studies indicating changes in liver weight.

5.2.2.2. *Method of Analysis—NOAEL/LOAEL Approach*

A NOAEL/LOAEL approach was used to derive the POD for trans-1,2-DCE based on the LOAEL of 792 mg/m^3 for fatty accumulation in liver lobules and Kupffer cells identified by

Freundt et al. (1977) in rats. BMD modeling was not utilized because the single-concentration study is not amenable to modeling.

Because the RfC is a metric that addresses continuous human exposure for a lifetime, adjustments need to be made to animal data obtained from intermittent and/or less-than-lifetime exposure scenarios, as supported in the *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b). The first step is adjustment of the intermittent inhalation exposure to continuous exposure (7 days/week, 24 hours/day), based on the assumption that the product of exposure concentration and exposure time is constant, in the absence of information to the contrary (U.S. EPA, 2002). In Freundt et al. (1977), animals were exposed for 8 hours/day, 5 days/week. Therefore, the POD adjusted for continuous exposure (LOAEL$_{ADJ}$) for inhalation of trans-1,2-DCE is as follows:

$$
\begin{aligned}
\text{LOAEL}_{ADJ} \quad &= \text{LOAEL} \times (8 \text{ hours})/(24 \text{ hours}) \times (5 \text{ days})/(7 \text{ days}) \\
&= 792 \text{ mg/m}^3 \times 0.33 \times 0.71 \\
&= 188 \text{ mg/m}^3
\end{aligned}
$$

EPA guidance for RfC derivation provides procedures for determining an HEC from the POD$_{ADJ}$ obtained from animal data (U.S. EPA, 1994b). The approach considers the physicochemical characteristics of the gas or vapor in question as well as the toxicological specifics of the target tissue (respiratory versus systemic and, in the former case, extrathoracic, thoracic, tracheobronchial, or pulmonary) and separates gases into three categories. Freundt et al. (1977) reported alveolar septal distension of the lungs and pulmonary capillary hyperemia in rats exposed to trans-1,2-DCE, although these lung effects were considered to be slight. Systemic effects, fatty degeneration in the liver, were also observed in treated rats and serve as the critical effect for derivation of the reference value. Trans-1,2-DCE qualifies as a category 2 gas: moderately water soluble, reactive in respiratory tissue, and toxicologically active at remote sites (U.S. EPA, 1994b). For category 2 gases, HEC values are calculated by using methods for category 1 gases for portal-of-entry effects and category 3 methods for systemic effects (U.S. EPA, 1994b). The critical effect selected for RfC derivation is a systemic effect (fatty degeneration in the liver); thus, the methods for category 3 were applied.

The HEC was calculated by applying a dosimetric adjustment factor (DAF). A DAF is a ratio of animal and human physiologic parameters. The specific DAF used depends on the nature of the contaminant (particle or gas) and the target site (e.g., respiratory tract or remote to the portal of entry). The DAF for an extra-respiratory effect of a gas is the ratio of the animal/human blood: air partition coefficients [($H_{b/g\text{-animal}}$)/($H_{b/g\text{-human}}$)]. The human and rat blood partition coefficients for trans-1,2-DCE are reported by Gargas et al. (1989) as 9.58 in rats and 6.04 in humans. In situations where the ratio of the partition coefficients is greater than one, the default of 1 is used. The LOAEL$_{HEC}$ is therefore derived as follows:

$$
\begin{aligned}
\text{LOAEL}_{\text{HEC}} \quad &= \text{LOAEL}_{\text{ADJ}} \ (\text{mg/m}^3) \times (\text{H}_{\text{b/g-animal}})/(\ \text{H}_{\text{b/g-human}}) \\
&= \text{LOAEL}_{\text{ADJ}} \ (\text{mg/m}^3) \times 1 \\
&= 188 \ \text{mg/m}^3
\end{aligned}
$$

Thus, application of the inhalation dosimetry methods to the POD for fatty accumulation in the liver results in a LOAEL$_{\text{HEC}}$ of 188 mg/m^3.

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

The composite UF for trans-1,2-DCE that would be used to derive an RfC is 10,000, consisting of five areas of uncertainty, including three areas of maximum uncertainty. As described further below, these UFs include 10 for intraspecies extrapolation, 10 for subchronic to chronic extrapolation, and 10 for extrapolation from a LOAEL to NOAEL, 3 for interspecies variation, and 3 for database deficiencies.

- An UF of 10 for intraspecies differences (UF$_H$) was used to account for potentially sensitive human subpopulations in the absence of quantitative information on the variability of response to trans-1,2-DCE in the human population.

- An UF of 3 was used to account for laboratory animal-to-human interspecies differences (UF$_A$). This UF is adopted by convention where an HEC adjustment has been incorporated. Application of a full uncertainty factor of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed by the determination of an HEC as described in the RfC methodology (U.S. EPA, 1994b). An UF of 3 is retained to account for the toxicodynamic uncertainty.

- An UF of 10 was used to account for extrapolating from a POD for a subchronic exposure duration to trans-1,2-DCE to estimate chronic exposure conditions (UF$_S$).

- An UF of 10 was used to account for extrapolating from a LOAEL to a NOAEL.

- An UF of 3 was used to account for database deficiencies (UF$_D$). There is only one subchronic inhalation study available for evaluation of toxicity associated with trans-1,2-DCE exposure (Freundt et al., 1977), and one subchronic inhalation study presented in abstract form only (Kelly et al., 1999). In addition, there is one study that investigated developmental toxicity of trans-1,2-DCE via inhalation (DuPont, 1988a) which showed few parameters to be affected by treatment. In this study, developmental toxicities were only observed in high-concentration groups, and in the presence of maternal toxicity. A two-generation reproductive toxicity study is not available.

In the report, *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), the RfD/RfC technical panel concluded that, in cases where maximum uncertainty exists in four or more areas of uncertainty, or when the total uncertainty factor is

10,000 or more, it is unlikely that the database is sufficient to derive a reference value. Therefore, consistent with the recommendations in U.S. EPA (2002), the available inhalation data for trans-1,2-DCE were considered insufficient to support reference value derivation and an RfC for trans-1,2-DCE was not derived.

5.2.2.4. *Previous Inhalation Assessment*

No inhalation assessment for trans-1,2-DCE was previously included in the IRIS database.

5.3. UNCERTAINTIES IN THE ORAL REFERENCE DOSE (RfD)

Risk assessments need to describe associated uncertainty. The following discussion identifies uncertainties associated with the RfDs for cis- and trans-1,2-DCE. RfC values were not derived for cis- or trans-1,2-DCE in this assessment. As presented earlier in this section, the uncertainty factor approach, following EPA practices and RfD and RfC guidance (U.S. EPA, 2002b; 1994b) was applied to POD ($BMDL_{10}$) values for the cis- and trans-1,2-DCE RfDs. Using this approach, the POD was divided by a set of factors to account for uncertainties associated with a number of steps in the analysis, including extrapolation from responses observed in animal bioassays to humans, extrapolation of data from subchronic exposure to chronic exposure, to account for a diverse population of varying susceptibilities, and to account for database deficiencies. Because information specific to cis- and trans-1,2-DCE was limited, default factors were generally applied for these extrapolations.

The human database for 1,2-DCE is limited to two early studies (from the 1930s) involving acute inhalation exposure; one of the two was a case report of 1,2-DCE (of unknown isomeric composition) and the other a human subject study of trans-1,2-DCE with only two subjects. The animal database available to assess cis-1,2-DCE hazard is limited, consisting of limited acute oral and inhalation studies and a 14-day and 90-day toxicity study. The database for the trans-isomer, which includes multiple studies of acute and subchronic toxicity, developmental toxicity, and immunotoxicity, is more extensive (see Section 4). Uncertainties associated with gaps in the databases for both 1,2-DCE isomers are more fully discussed below.

Selection of the critical effect for reference value determination. The selection of the critical effect is a source of uncertainty for the oral RfD for both cis- and trans-1,2-DCE. For both cis- and trans-1,2-DCE, liver effects were noted. The most consistent finding across isomers, species, and routes of exposure was increased relative liver weight. In most instances, liver weight changes were not accompanied by gross or histopathological lesions of the liver, increases in serum levels of liver enzymes, or by other evidence of functional impairment. This absence of supporting evidence for liver toxicity makes interpretation of the liver weight findings difficult and the biological relevance of increased liver weight uncertain. NTP (2002) concluded

that the histopathology and clinical chemistry data combined with organ and body weight data revealed that the MTD had not been reached in this ingestion study of microencapsulated trans-1,2-DCE. EPA determined that increased relative liver weight may be an early indicator of liver toxicity, and that more overt liver toxicity could occur at higher exposures or with longer durations of exposure.

The critical effect for the RfD for trans-1,2-DCE is based on decreased antibody production directed against sRBCs in male mice (Shopp et al., 1985). EPA determined that the 26% suppression in the number of sRBC-specific AFCs per 10^6 spleen cells of male mice in Shopp et al. (1985) is a biologically significant measure indicating suppressed antibody response associated with oral exposure to trans-1,2-DCE that is not contradicted by a lack of observed change in the hemagglutination assay to sRBCs or proliferative response to LPS. Suppression of T-cell-dependent antibody response as determined by the AFC assay to sRBCs is a well validated endpoint that is highly predictive for immunotoxicity (Herzyk and Holsapple, 2007; Luster et al., 1992).

Dose-response modeling. BMD modeling was used to estimate the POD for the cis- and trans-1,2-DCE RfDs. BMD modeling has advantages over a POD based on a NOAEL/LOAEL approach because the latter is a reflection of the particular doses (and dose spacing) selected in the principal study. The NOAEL/LOAEL approach lacks characterization of the dose-response curve and for this reason is less informative than a POD obtained from BMD modeling. The selected models used to derive the cis- and trans-1,2-DCE PODs provided the best mathematical fits to the experimental data sets, but do not represent all possible models one might fit. Other models could be selected to yield more extreme results, both higher and lower than those used to derive the cis- and trans-isomer RfDs in the current assessment.

Animal to human extrapolation. Extrapolating dose-response data from animals to humans is another source of uncertainty. The effect and magnitude at the POD in rodents are extrapolated to human response. Uncertainty in interspecies extrapolation can be separated into two general areas—toxicokinetic and toxicodynamic. In the absence of information to quantitatively assess either toxicokinetic or toxicodynamic differences between animals and humans, a 10-fold UF was used to account for uncertainty in extrapolating from laboratory animals to humans in the derivation of the RfDs for cis- and trans-1,2-DCE. Toxicokinetic and toxicodynamic information for the isomers of 1,2-DCE is not available to inform potential magnitude of over- or underestimation of this UF. A PBPK model adequately parameterized for both animals and humans could reduce uncertainty in the pharmacokinetic portion of interspecies extrapolation; however, such a model is not available for cis- or trans-1,2-DCE.

Intrahuman variability. Heterogeneity among humans is another source of uncertainty. In the absence of cis- and trans-1,2-DCE-specific data on human variation in response to exposure to these isomers, a default UF of 10 was used to account for uncertainty associated with human variation in the derivation of the RfDs. Human variations may be larger or smaller; however, 1,2-DCE-specific data to examine the potential magnitude of over- or underestimation is unavailable.

Subchronic to chronic exposure extrapolation. Because no chronic toxicity studies for the cis- or trans-isomers of 1,2-DCE are available, a factor was applied to extrapolate data obtained from studies of subchronic exposure to chronic exposure. This factor is based on the assumption that an effect seen at a shorter duration will also be seen after a lifetime of exposure, but with greater severity or at a lower exposure level. In the absence of information to inform this extrapolation, a default UF of 10 was applied. The magnitude of uncertainty associated with this extrapolation and UF cannot be quantified.

Data gaps. The cis- and trans-1,2-DCE database lacks a multigenerational study of reproductive toxicity by any route of exposure, and the cis-1,2-DCE database lacks studies of developmental toxicity. The absence of these studies introduces uncertainty in the RfDs. Uncertainty resulting from gaps in developmental toxicity data specific to the cis- and trans-1,2-DCE isomers was reduced by developmental toxicity studies of mixed 1,2-DCE isomers. The magnitude of the uncertainty associated with database deficiencies for these chemicals cannot be quantified. However, a database uncertainty factor of 3 was used to account for the lack of reproductive and developmental toxicity studies.

5.4. CANCER ASSESSMENT

The lack of human studies and chronic animal studies precludes a cancer assessment for cis- or trans-1,2-DCE. Accordingly, both isomers provide inadequate information to assess their carcinogenic potential.

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

6.1. HUMAN HAZARD POTENTIAL

1,2-DCE exists as two isomers, the cis- and the trans-forms, with a molecular mass of 96.95. Both are colorless, flammable liquids that are heavier than water, with a chloroform-like, sweet, pungent smell. With boiling points between 50 and 60°C, they are volatile. At approximately 5 g/L, both are moderately water soluble. Their oil:water partition coefficients, at around 100, suggest that these chemicals will preferentially partition into lipophilic media. The two isomers may be used in their pure forms or as a mixture of varying isomer composition, typically a 60:40 cis-/trans-mixture.

The trans-isomer is the most commonly used form of 1,2-DCE. It is an effective degreasing agent and is also frequently used as a solvent for polymers and rubber. The wide use of both isomers gives rise to environmental contamination, and they are also formed by degradation of drinking water disinfection by-products. Their moderate water solubility allows them to be carried into bodies of water, from where they tend to volatilize rapidly. Both isomers are degraded quite effectively by microorganisms in water, the cis-isomer more rapidly than the trans-isomer. After evaporation into air, they are degraded more slowly—cis-1,2-DCE with a half-life of about 12 days, trans-1,2-DCE with a half-life of about 5 days.

Little information is available regarding the potential toxicity of cis- or trans-1,2-DCE in humans by either the oral or the inhalation route of exposure. Acute effects described for trans-1,2-DCE in humans include eye irritation, drowsiness, nausea, vertigo, narcosis, and death. No long-term effects are known. There are no chronic exposure studies in animals. Several subchronic oral exposure studies in animals exist, including a 90-day gavage study of the cis-isomer in rats (McCauley et al., 1995, 1990), 90-day drinking water studies of the trans-isomer in rats (Hayes et al., 1987) and mice (Barnes et al., 1985), and a 90-day feed study in rats and mice (NTP, 2002). Studies of inhalation exposure consist of two 90-day studies of the trans-isomer (Kelly et al., 1999; Freundt et al., 1977) and one study of a mixture of cis- and trans-isomers (Dow, 1962) in rats. Although the liver was consistently observed as the organ most affected by exposure to relatively high doses of 1,2-DCE, there is limited evidence for any specific pathological event (NTP, 2002; McCauley et al., 1995, 1990; Hayes et al., 1987; Freundt et al., 1977). A general overview of toxicity studies conducted with cis- or trans-1,2-DCE or their mixture indicates that both compounds display low toxicity.

Only one subchronic oral study (McCauley et al. 1995, 1990) was conducted with cis-1,2-DCE. Statistically significant increases in liver weight in both male and female rats, increases in kidney weight in male rats, and inconsistent hematological responses were noted in this study (McCauley et al., 1995, 1990). The biological significance of the effects on relative

liver weight is unknown. Hepatic effects observed in studies of acute and short-term (14-day) exposure durations and in toxicity studies of closely related chemicals (i.e., trans-1,2-DCE and 1,1-DCE) provide some evidence that liver damage (i.e., fatty accumulation in liver lobules and Kupffer cells) might be observed if studies were conducted at higher doses or for longer duration.

The subchronic oral toxicity of trans-1,2-DCE has been investigated by NTP (2002), Hayes et al. (1987), Barnes et al. (1985), and Shopp et al. (1985). Hayes et al. (1987) found no significant differences in body weight or body weight gain in either male or female rats or effects on any of the hematological, serological, or urinary parameters evaluated. In addition, there were no significant changes in organ weights or relative organ weights in males, and only a significant elevation in absolute kidney weights and kidney weights relative to brain weights in females, with no evidence of microscopic histopathological changes in the female kidney. In the Barnes et al. (1985) study, changes in relative organ weights were few, sporadic, and not believed by the authors to be treatment-related. Few changes in hematological parameters were seen in this study, and slight changes in several clinical chemistry parameters were observed. Although some values were significantly different from those of the controls, there were no consistent trends or deviations from historical control values. A statistically significant increase in relative liver weight at the mid-dose (175 mg/kg-day), but not at the highest dose, was seen in male mice in the Barnes et al. (1985) study, and statistically significant changes in liver function enzymes, including LDH, AST (SGOT), and ALP activities, in male mice occurred. Significant increases of 62 and 33% in serum ALP levels were reported at the 175 and 387 mg/kg-day doses, respectively. These increases showed no dose-response relationship, were within the normal range for the CD-1 mouse strain, and were not observed in female mice. The findings of Barnes et al. (1985) provide no evidence that trans-1,2-DCE induces hepatotoxicity in mice at doses up to 387 mg/kg-day in males and 452 mg/kg-day in females.

In NTP (2002), the final mean body weight and body weight gain of male rats exposed to trans-1,2-DCE were reduced by about 6% below controls (a statistically significant decrease). In general, no exposure-related alterations in clinical chemistry parameters in rats were observed. NTP (2002) reported statistically significant changes in absolute and relative liver weights in rats for the females only. The relative liver weights of female rats exposed to ≥395 mg/kg-day were significantly higher, by about 6–10%. No gross or histological lesions were observed in rats that were attributed to exposure to trans-1,2-DCE. In mice, statistically significant, dose-dependent increases in relative liver weights in both sexes were observed in the NTP (2002) study. The maximum changes in liver weights in mice were increases of 15 and 11% at the highest dose for males and females, respectively. No gross or histological lesions were observed in mice. The liver effects may be early indicators or precursors of liver toxicity, and it is not possible to determine whether overt liver damage would occur at higher doses or in studies of longer exposure duration (i.e., chronic studies).

Shopp et al. (1985) reported a dose-related suppression of the humoral immune status in male mice treated with trans-1,2-DCE as indicated by a reduction in sRBC-specific AFCs in the spleen. When expressed as AFCs per 10^6 spleen cells, the number of AFCs were reduced by 26% in male mice at doses of 175 and 387 mg/kg-day (significantly different at $p < 0.05$ from control mice). Suppression of T-cell-dependent antibody response as determined by the AFC response to sRBCs is a well validated endpoint that is one of the most predictive assays for chemical immunotoxicity (Herzyk and Holsapple, 2007; Luster et al., 1992). EPA concluded that the reduced number of sRBC-specific AFCs per 10^6 spleen cells of male mice in Shopp et al. (1985) is a biologically significant measure indicating suppressed antibody response associated with oral exposure to trans-1,2-DCE.

For the inhalation route of exposure, there are no human, chronic, or subchronic studies for the cis-isomer and only three 90-day or longer duration studies using trans-1,2-DCE (Kelly et al., 1999; Freundt et al., 1977) or the isomer mixture (Dow, 1962). The Kelly et al. (1999) study exists as an abstract only. The Dow (1962) report is an unpublished study and reported only minor effects. The Freundt et al. (1977) subchronic inhalation study is a one-concentration study over several exposure durations. This study demonstrated liver effects (fatty accumulation in liver lobules and Kupffer cells) that were also seen in some of the controls. For each of the exposure durations (1, 2, 8, and 16 weeks), the incidence and severity of fat accumulation increased with increasing exposure duration; however, these increases were not statistically significantly different from the controls.

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *inadequate information to assess the carcinogenic potential* of cis- or trans-1,2-DCE. This description reflects the lack of human epidemiological investigations or chronic animal bioassays.

6.2. DOSE RESPONSE

6.2.1. Noncancer – Oral Exposure

6.2.1.1. *cis-1,2- DCE*

McCauley et al. (1995, 1990) conducted the only available subchronic study of cis-1,2-DCE. This 90-day gavage study was used as the basis for the oral RfD. An increase in relative liver weight in male and female rats was selected as the critical effect for derivation of the RfD. Relative liver weights were increased by up to 32% in males and 30% in females. There were no histopathological changes of the liver or changes in serum levels of AST (the only liver enzyme measured); however, the observation of hepatic effects (fatty accumulation) in studies of acute and short-term (14-day) exposures to cis-1,2-DCE and in toxicity studies of closely related chemicals (i.e., trans-1,2-DCE and 1,1-DCE) provides support for use of relative liver weight increases as the critical effect. A 10% change in relative liver weight compared with the control was selected as the BMR level for this endpoint. BMD modeling was used to

calculate the POD by estimating the effective dose at a specified level of response (BMD_{10}) and its 95% lower confidence limit ($BMDL_{10}$). All of the continuous models in U.S. EPA's BMDS (version 1.4.1) (U.S. EPA, 2007) were fit to the relative liver weight data. For both male and female rat relative liver weight data, the Hill model (restricted) provided the best fit of the data, yielding a BMD_{10} and $BMDL_{10}$ of 54.4 and 18.6 mg/kg-day, respectively, in males, and 80.5 and 42.3 mg/kg-day, respectively, in females. The POD for the RfD for cis-1,2-DCE was chosen as 18.6 mg/kg-day, the lower of the male and female $BMDL_{10}$ values. Applying a composite UF of 3,000 to the POD of 18.6 mg/kg-day yields an RfD of 0.006 mg/kg-day. The composite UF of 3,000 includes factors of 10 to protect sensitive individuals, 10 to extrapolate from animals to humans, 10 for use of a study of subchronic duration, and 3 to account for database deficiencies. Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between experimental animals and humans (applied a factor of 10) or the potential variability in human susceptibility (applied a factor of 10) to cis-1,2-DCE. In the absence of any chronic toxicity studies, a UF of 10 was used to account for extrapolating from a subchronic study to estimate chronic exposure conditions. An UF of 3 was used to account for deficiencies in the database, including lack of reproductive and developmental toxicity data for the cis-isomer. The potential for developmental toxicity of cis-1,2-DCE, however, is informed by a series of oral range-finding studies of the developmental toxicity of a mixture of 1,2-DCE isomers (composition of isomers unknown) (NTP, 1991a, b, c). No evidence of developmental toxicity was observed in mice or rats based on the parameters evaluated in these range-finding studies (gravid uterus weight, fetal body weight, and number of fetuses [live/dead], implantation sites, and resorptions).

Confidence in the principal study (McCauley et al. 1995, 1990) is low to medium. The 90-day oral gavage study (McCauley et al. 1995, 1990) is well-conducted and uses four dose groups plus a control. The study measured multiple parameters: body weight, clinical chemistries, liver weight, kidney weight, and blood parameters. McCauley et al. (1995, 1990) reported statistically significant increases in relative liver weights in rats but no histopathological changes in the liver or changes in serum AST levels at any dose in either sex. The biological significance of the relative liver weight findings are difficult to interpret in the absence of compound-related histopathological changes in the liver, the absence of AST changes, and the lack of measurements of other clinical chemistry indicators of liver function in the McCauley et al. (1995, 1990) study. Similarly, the absence of compound-related histopathological changes in the kidney in the McCauley et al. (1995, 1990) study raises questions about the biological significance of the relative kidney weight findings. BUN and creatinine, two clinical chemistry parameters that are indicators of kidney function, were measured in McCauley et al. (1995, 1990) and did not provide supporting evidence for functional damage to the kidney. There are no oral studies of chronic, reproductive, or developmental toxicity of cis-1,2-DCE. The McCauley et al. (1995, 1990) study is the only available subchronic study of cis-1,2-DCE and

was used as the basis for the oral RfD. Thus, the confidence in the database is low. Taking into account the confidence in the principal study (low to medium) and the database (low), the overall confidence in the RfD is low to medium.

6.2.1.2. *trans-1,2- DCE*

There are a number of oral subchronic studies that have evaluated the toxicity of trans-1,2-DCE (NTP, 2002; Hayes et al., 1987; Barnes et al., 1985; Shopp et al., 1985). Changes in body weight, liver and kidney weights, and hematological parameters were observed across species, sexes, and routes of administration. In addition, immunotoxicity was indicated by observed suppression of AFCs in the spleens of male CD-1 mice exposed to trans-1,2-DCE (Shopp et al., 1985).

The Shopp et al. (1985) study was chosen as the principal study. Shopp et al. (1985) reported a statistically significant dose-related suppression of sRBC-specific AFCs in the spleen in male mice exposed to trans-1,2-DCE in drinking water for 90 days. The authors of this study reported marked suppression in humoral immune status in male mice as indicated by the significantly decreased number of AFCs. As described in more detail in Section 4.6.1.2, EPA concludes that the 26% suppression in the number of sRBC-specific AFCs per 10^6 spleen cells of male mice in Shopp et al. (1985) is a biologically significant measure indicating suppressed antibody response associated with oral exposure to trans-1,2-DCE.

BMD modeling methods were used to calculate the POD by estimating the effective dose at a specified level of response (BMDx) and its 95% lower confidence limit (BMDLx). A BMR of 1 standard deviation from the control mean number of AFCs per 10^6 spleen cells was used. All of the continuous models in U.S. EPA's BMDS (version 1.4.1c) (U.S. EPA, 2007) were fit to the data on numbers of AFCs (i.e., AFCs per 10^6 spleen cells) observed in male CD-1 mice. A second-degree polynomial model provided the best fit to these data, yielding a $BMDL_{1SD}$ of 65.0 mg/kg-day. Thus, the POD for the RfD for trans-1,2-DCE was the $BMDL_{1SD}$ of 65.0 mg/kg-day. Applying a composite UF of 3,000 to the POD of 65 mg/kg-day yields an RfD of 0.02 mg/kg-day. The composite UF of 3,000 includes a UF of 10 for intraspecies variability, a UF of 10 for interspecies variability, a UF of 10 for extrapolation from a subchronic to a chronic study, and a UF of 3 for database uncertainties. Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between experimental animals and humans (applied a factor of 10) or the potential variability in human susceptibility (applied a factor of 10) to trans-1,2-DCE. In the absence of any chronic toxicity studies, a UF of 10 was used to account for extrapolating from a subchronic study to estimate chronic exposure conditions. An UF of 3 was used to account for deficiencies in the database, including lack of a multigeneration reproductive toxicity study.

Confidence in the principal study (Shopp et al., 1985) is medium. This 90-day study of oral exposure to trans-1,2-DCE (administered in drinking water) is a well-conducted, peer

reviewed study. It was performed to evaluate the immunotoxic effects of trans-1,2-DCE in male and female CD-1 mice. The Shopp et al. (1985) study included three dose groups as well as a vehicle control group. Animals were evaluated for humoral immune status as measured by the ability of spleen cells from these mice to produce splenic IgM AFCs against sRBC, hemagglutination titers to sRBC, and by spleen cell response to LPS. Confidence in the study findings from Shopp et al. (1985) is limited because this is the only available study evaluating the antibody response to a T-cell-dependent antigen in mice or rats exposed to trans-1,2-DCE for 90 days or more and therefore the immunotoxicity findings reported in this study have not been corroborated by other investigators. Differences in the interpretation of the Shopp et al. (1985) study findings reached by the study investigators and by the EPA are acknowledged in the consideration of confidence in this study. The basis for these differences is addressed in Section 4.6.1.2.

Confidence in the oral database is medium. Four subchronic studies were considered in the evaluation of oral exposure to trans-1,2-DCE (NTP, 2002; Hayes, et al., 1987; Barnes et al., 1985; Shopp et al., 1985). These studies evaluated a wide range of toxicity endpoints, including hematology, urinalysis, clinical chemistry, histopathology, and immune system function. Developmental toxicity potential for trans-1,2-DCE is informed by a series of oral range-finding studies of the developmental toxicity of a mixture of 1,2-DCE isomers (composition of isomers unknown) (NTP, 1991a, b, c). No evidence of developmental toxicity was observed in mice or rats based on the parameters evaluated in these range-finding studies (gravid uterus weight, fetal body weight, and number of fetuses [live/dead], implantation sites, and resorptions). There are no chronic studies of trans-1,2-DCE toxicity. Taking into account the confidence in the principal study (medium) and the confidence in the database (medium), the overall confidence in the RfD is medium.

6.2.2. Noncancer – Inhalation Exposure

6.2.2.1. *cis-1,2-DCE*

There are no human studies, nor chronic or subchronic inhalation studies in animals for cis-1,2-DCE. In the absence of a long-term inhalation study, no RfC was derived.

6.2.2.2. *trans-1,2-DCE*

The available inhalation data for trans-1,2-DCE were considered insufficient to support reference value derivation. An RfC for trans-1,2-DCE was not derived.

6.2.3. Cancer

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for both cis- and trans-1,2-DCE is *inadequate to assess the human carcinogenic potential*. There

are no studies that examine the potential carcinogenicity of cis- and trans-1,2-DCE in humans, nor are any chronic studies in experimental animals available.

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APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW, PUBLIC COMMENTS, AND DISPOSITION

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APPENDIX B: BENCHMARK DOSE MODELING RESULTS AND OUTPUTS

B.1. RfD for cis-1,2-DCE

Relative liver weight, female rat (McCauley et al., 1995, 1990)
BMR = 10% change in mean relative liver weight relative to the control mean
Constant variance

**Table B-1. BMDS modeling summary of relative liver weights in female
rats exposed to cis-1,2-DCE by gavage for 90 days**

Model	Test 3 *p*-Value	Test 4 *p*-Value	AIC	BMD (mg/kg-d)	BMDL (mg/kg-d)
Linear, Polynomial (restricted)	0.6325	0.0014	-84.9916	339.0	278.3
Power (≥1)	0.6325	0.0014	-84.9916	339.0	278.3
Hill (≥1)	**0.6325**	**0.3208**	**-96.2572**	**80.5**	**42.3**

Only the Hill model [restricted] adequately described the data (test 4 χ^2 $p > 0.1$).

Hill Model (Power parameter restricted to be greater than 1)

```
=====================================================================
      Hill Model. (Version: 2.12;  Date: 02/20/2007)
      Input Data File: G:\IRIS_CHEMICALS\DCE\BMD -- MCCAULEY\RELLIVERWTFEMALE.(d)
      Gnuplot Plotting File:  G:\IRIS_CHEMICALS\DCE\BMD -- MCCAULEY\RELLIVERWTFEMALE.plt
                                      Wed Aug 29 15:30:30 2007
=====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = intercept + v*dose^n/(k^n + dose^n)


  Dependent variable = MEAN
  Independent variable = Dose(mg/kg/d)
  rho is set to 0
  Power parameter restricted to be greater than 1
  A constant variance model is fit

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


              Default Initial Parameter Values
                    alpha =    0.0446279
                      rho =           0   Specified
                intercept =        2.82
                        v =        0.85
```

```
                          n =        0.3078
                          k =        439.733


            Asymptotic Correlation Matrix of Parameter Estimates

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

                     alpha       intercept           v             k

        alpha            1      -4.5e-008      -7.8e-009      -2.9e-008

    intercept    -4.5e-008             1         -0.021           0.6

            v    -7.8e-009        -0.021             1            0.7

            k    -2.9e-008           0.6           0.7             1



                            Parameter Estimates

                                               95.0% Wald Confidence Interval
         Variable        Estimate      Std. Err.    Lower Conf. Limit    Upper Conf. Limit
            alpha       0.0419186      0.0085566          0.025148           0.0586893
        intercept         2.81563      0.0589669           2.70006             2.93121
                v         1.04577       0.140777           0.76985             1.32169
                n               1             NA
                k         218.547        103.222            16.235             420.858

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



         Table of Data and Estimated Values of Interest

     Dose        N     Obs Mean     Est Mean   Obs Std Dev   Est Std Dev    Scaled Res.
     ------     ---    --------     --------   -----------   -----------    ----------


         0      10        2.82         2.82          0.19         0.205         0.0675
        32       9        2.91         2.95          0.18         0.205         -0.574
        97       9        3.21         3.14          0.22         0.205          1.07
       291      10        3.36         3.41          0.18         0.205         -0.817
       872      10        3.67         3.65          0.27         0.205          0.281



  Model Descriptions for likelihoods calculated


  Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

  Model  R:        Yi = Mu + e(i)
           Var{e(i)} = Sigma^2
```

```
                    Likelihoods of Interest

          Model        Log(likelihood)   # Param's       AIC
           A1            53.265523           6         -94.531046
           A2            54.549420          10         -89.098840
           A3            53.265523           6         -94.531046
         fitted          52.128602           4         -96.257205
            R            23.670875           2         -43.341750


                    Explanation of Tests

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

                    Tests of Interest

   Test     -2*log(Likelihood Ratio)   Test df        p-value

   Test 1             61.7571             8          <.0001
   Test 2              2.56779            4           0.6325
   Test 3              2.56779            4           0.6325
   Test 4              2.27384            2           0.3208
```

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data.

          Benchmark Dose Computation

Specified effect =   0.1   1

Risk Type   =   Relative risk   Standard deviation

Confidence level =   0.95   0.95

          BMD =   80.5212   53.2

          BMDL =   42.3183   28.7

Hill Model with 0.95 Confidence Level

15:30 08/29 2007

Relative liver weight, female rat (McCauley et al., 1995, 1990)

BMR = change in the mean response equal to 1 standard deviation from the control mean

```
====================================================================
        Hill Model. (Version: 2.12;  Date: 02/20/2007)
         Input Data File: G:\DCE DOSE-RESPONSE MODELING\FEMALE_RAT_REL_LIVER_WEIGHT.(d)
         Gnuplot Plotting File:  G:\DCE DOSE-RESPONSE MODELING\FEMALE_RAT_REL_LIVER_WEIGHT.plt
                                        Tue Feb 26 11:30:23 2008
 ====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

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



                  Default Initial Parameter Values
                        alpha =    0.0446279
                          rho =            0   Specified
                    intercept =         2.82
                            v =         0.85
                            n =       0.3078
                            k =      439.733


           Asymptotic Correlation Matrix of Parameter Estimates

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

                   alpha     intercept           v           k

       alpha           1     -4.5e-008    -7.8e-009    -2.9e-008

   intercept   -4.5e-008            1       -0.021          0.6

           v   -7.8e-009       -0.021            1          0.7

           k   -2.9e-008          0.6          0.7            1



                          Parameter Estimates

                                            95.0% Wald Confidence Interval
           Variable      Estimate       Std. Err.     Lower Conf. Limit    Upper Conf. Limit
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          intercept       2.81563       0.0589669               2.70006             2.93121
                  v       1.04577        0.140777               0.76985             1.32169
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      implied by some inequality constraint and thus
      has no standard error.
```

```
     Table of Data and Estimated Values of Interest

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------    ---   --------     --------   -----------   -----------   ----------

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   97      9      3.21         3.14         0.22          0.205        1.07
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  872     10      3.67         3.65         0.27          0.205        0.281



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 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:        Yi = Mu + e(i)
          Var{e(i)} = Sigma^2


                   Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1          53.265523           6       -94.531046
             A2          54.549420          10       -89.098840
             A3          53.265523           6       -94.531046
           fitted        52.128602           4       -96.257205
              R          23.670875           2       -43.341750



                      Explanation of Tests

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

   Test    -2*log(Likelihood Ratio)   Test df        p-value

   Test 1          61.7571               8          <.0001
   Test 2          2.56779               4          0.6325
   Test 3          2.56779               4          0.6325
   Test 4          2.27384               2          0.3208

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =          1

Risk Type      =      Estimated standard deviations from the control mean
```
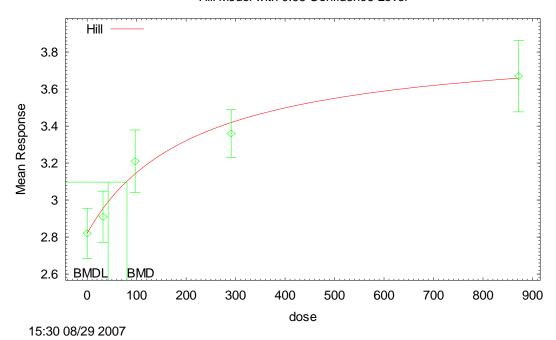
```
Confidence level =              0.95
           BMD =        53.2031
           BMDL =        28.7628
```

Hill Model with 0.95 Confidence Level

11:30 02/26 2008

Relative liver weight, male rat (McCauley et al., 1995, 1990)

BMR = 10% change in mean relative liver weight relative to the control mean

Constant variance

<p align="center">Table B-2. BMDS modeling summary of relative liver weights in
male rats exposed to cis-1,2-DCE by gavage for 90 days</p>

Model	Test 3 *p*-Value	Test 4 *p*-Value	AIC	BMD (mg/kg-d)	BMDL (mg/kg-d)
Linear, Polynomial (restricted)	0.04879	0.04268	-54.8404	379.4	281.1
Power (≥1)	0.04879	0.04268	-54.8404	379.4	281.1
Hill (≥1)	**0.04879**	**0.1662**	**-57.4185**	**54.4**	**18.6**

Only the Hill model [restricted] adequately described the data (test 4 χ^2 $p > 0.1$). Modeling of the variance (i.e., test 3 statistic in BMDS output) was not adequate (i.e., test 3 χ^2 $p>0.1$), but since BMR is not on a standard deviation basis, fitting a homogeneous variance model is not essential.

Hill Model (Power parameter restricted to be greater than 1)

```
=====================================================================
        Hill Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\IRIS_CHEMICALS\DCE\BMD -- MCCAULEY\RELLIVERWTMALE.(d)
        Gnuplot Plotting File:  G:\IRIS_CHEMICALS\DCE\BMD -- MCCAULEY\RELLIVERWTMALE.plt
                                        Thu Aug 30 12:01:20 2007
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = intercept + v*dose^n/(k^n + dose^n)


  Dependent variable = MEAN
  Independent variable = Dose(mg/kg/d)
  Power parameter restricted to be greater than 1
  The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

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




              Default Initial Parameter Values
                  lalpha =      -2.56356
                     rho =             0
               intercept =          2.85
                       v =           0.9
                       n =      0.109937
                       k =       420.333


     Asymptotic Correlation Matrix of Parameter Estimates
```

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

                    lalpha         rho    intercept           v           k

      lalpha             1          -1        0.056        0.25        0.23

         rho            -1           1        -0.06       -0.25       -0.23

   intercept         0.056       -0.06            1       -0.06        0.65

           v          0.25       -0.25        -0.06           1        0.62

           k          0.23       -0.23         0.65        0.62           1


                              Parameter Estimates

                                                   95.0% Wald Confidence Interval
            Variable        Estimate      Std. Err.    Lower Conf. Limit    Upper Conf. Limit
              lalpha        -5.64025        3.22635            -11.9638             0.683284
                 rho         2.59276        2.74992            -2.79698             7.98251
           intercept         2.88261      0.0845329             2.71693             3.04829
                   v        0.827803       0.173635            0.487485             1.16812
                   n               1             NA
                   k          101.77        82.8267             -60.567              264.108

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


      Table of Data and Estimated Values of Interest

  Dose        N     Obs Mean     Est Mean   Obs Std Dev  Est Std Dev    Scaled Res.
  ------      ---   --------     --------   -----------  -----------    ----------


     0         9       2.85         2.88         0.26         0.235         -0.416
    32        10       3.15         3.08         0.27         0.256          0.856
    97        10       3.28         3.29         0.18         0.279         -0.0746
   291         7       3.34          3.5         0.44         0.302          -1.37
   872         6       3.75         3.62          0.2         0.316          0.976


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:        Yi = Mu + e(i)
          Var{e(i)} = Sigma^2


                    Likelihoods of Interest

            Model     Log(likelihood)   # Param's       AIC
             A1          35.496626           6        -58.993253
             A2          39.438593          10        -58.877185
             A3          35.503884           7        -57.007768
          fitted         33.709241           5        -57.418482
              R          20.057106           2        -36.114211


            Explanation of Tests
```

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

                    Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1            38.763              8           <.0001
   Test 2           7.88393              4           0.09592
   Test 3           7.86942              3           0.04879
   Test 4           3.58929              2            0.1662
```

The p-value for Test 1 is less than .05. There appears to be a difference between response
and/or variances among the dose levels. It seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be
appropriate.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

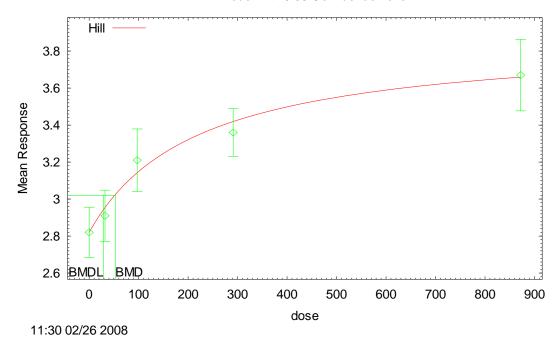The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the
data.

```
         Benchmark Dose Computation

Specified effect =        0.1                          1

Risk Type        =     Relative risk          Standard deviation

Confidence level =        0.95                        0.95

          BMD =         54.3727                       40.4

          BMDL =        18.5549                       13.0
```

Hill Model with 0.95 Confidence Level

12:01 08/30 2007

Relative liver weight, male rat (McCauley et al., 1995, 1990)

BMR = change in the mean response equal to 1 standard deviation from the control mean

```
====================================================================
         Hill Model. (Version: 2.12;  Date: 02/20/2007)
         Input Data File: G:\DCE DOSE-RESPONSE MODELING\MALE_RAT_REL_LIVER_WEIGHT.(d)
         Gnuplot Plotting File:  G:\DCE DOSE-RESPONSE MODELING\MALE_RAT_REL_LIVER_WEIGHT.plt
                                         Tue Feb 26 10:56:27 2008
====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

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



                  Default Initial Parameter Values
                        lalpha =      -2.56356
                           rho =             0
                     intercept =          2.85
                             v =           0.9
                             n =      0.109937
                             k =       420.333


           Asymptotic Correlation Matrix of Parameter Estimates

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

                    lalpha          rho    intercept            v            k

    lalpha               1           -1        0.056         0.25         0.23

       rho              -1            1        -0.06        -0.25        -0.23

 intercept           0.056        -0.06            1        -0.06         0.65

         v            0.25        -0.25        -0.06            1         0.62

         k            0.23        -0.23         0.65         0.62            1



                           Parameter Estimates

                                              95.0% Wald Confidence Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          lalpha         -5.64025          3.22635             -11.9638            0.683284
             rho          2.59276          2.74992             -2.79698             7.98251
       intercept          2.88261        0.0845329              2.71693             3.04829
               v         0.827803         0.173635             0.487485             1.16812
               n                1               NA
               k           101.77          82.8267              -60.567             264.108

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



      Table of Data and Estimated Values of Interest

  Dose      N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
 ------    ---   --------     --------   -----------  -----------   ----------
```

```
     0     9     2.85      2.88      0.26     0.235     -0.416
    32    10     3.15      3.08      0.27     0.256      0.856
    97    10     3.28      3.29      0.18     0.279     -0.0746
   291     7     3.34       3.5      0.44     0.302      -1.37
   872     6     3.75      3.62       0.2     0.316      0.976
```

Model Descriptions for likelihoods calculated

 Model A1:   Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:   Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:   Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model R: Yi = Mu + e(i)
          Var{e(i)} = Sigma^2

                  Likelihoods of Interest

          Model   Log(likelihood)   # Param's   AIC
           A1   35.496626   6   -58.993253
           A2   39.438593   10   -58.877185
           A3   35.503884   7   -57.007768
         fitted   33.709241   5   -57.418482
            R   20.057106   2   -36.114211

                  Explanation of Tests

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

   Test   -2*log(Likelihood Ratio)   Test df   p-value

   Test 1   38.763   8   <.0001
   Test 2   7.88393   4   0.09592
   Test 3   7.86942   3   0.04879
   Test 4   3.58929   2   0.1662

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1. You may want to consider a
different variance model

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

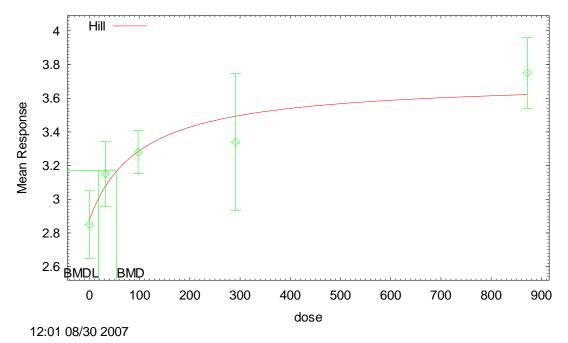   Benchmark Dose Computation

Specified effect =   1

Risk Type   =   Estimated standard deviations from the control mean

Confidence level =   0.95

          BMD =   40.3732

          BMDL =   12.9953

Hill Model with 0.95 Confidence Level

10:56 02/26 2008

B.2. RfD for trans-1,2-DCE

B.2.1. Relative Liver Weight (NTP, 2002)

Relative liver weight, male mouse (NTP, 2002)

BMR = 10% change in mean relative liver weight relative to the control mean

Constant variance

Table B-3. BMDS modeling summary of relative liver weights in male mice exposed to trans-1,2-DCE in the feed for 14 weeks

Model	Test 3 *p*-Value	Test 4 *p*-Value	AIC	BMD (mg/kg-d)	BMDL (mg/kg-d)
Linear, Polynomial (restricted)	0.2228	0.2974	-79.0425	7,063.8	5,109.8
Power (≥1)	0.2228	0.2974	-79.0425	7,063.8	5,109.8
Hill (≥1)	**0.2228**	**0.6152**	**-80.1465**	**3,241.9**	**867.3**

All models used in the evaluation of relative liver weight in male mice produced outputs with χ^2 $p > 0.1$. The Hill model, with the lowest AIC values, provided the best fit of the data.

Hill Model (Power parameter restricted to be greater than 1)

```
=====================================================================
        Hill Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\IRIS_CHEMICALS\DCE\BMD-- NTP\REL_LIVER_WT_MOUSEM.(d)
        Gnuplot Plotting File:  G:\IRIS_CHEMICALS\DCE\BMD-- NTP\REL_LIVER_WT_MOUSEM.plt
                                        Tue Sep 11 10:27:29 2007
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = intercept + v*dose^n/(k^n + dose^n)


  Dependent variable = MEAN
  Independent variable = Dose(mg/kg-d)
  rho is set to 0
  Power parameter restricted to be greater than 1
  A constant variance model is fit

  Total number of dose groups = 6
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


              Default Initial Parameter Values
                    alpha =    0.0912885
                      rho =            0   Specified
                intercept =        4.347
                        v =        0.632
                        n =     0.479874
                        k =      2442.97
```

```
              Asymptotic Correlation Matrix of Parameter Estimates

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

                    alpha      intercept          v            k

         alpha          1     -2.5e-009      2e-008     9.8e-009

      intercept   -2.5e-009          1         -0.02         0.68

           v       2e-008        -0.02           1          0.65

           k     9.8e-009         0.68         0.65           1


                             Parameter Estimates

                                             95.0% Wald Confidence Interval
              Variable       Estimate      Std. Err.    Lower Conf. Limit    Upper Conf. Limit
                 alpha      0.0846603      0.0154568         0.0543656            0.114955
             intercept        4.36928        0.09406           4.18493             4.55364
                   v         0.679896       0.178916          0.329227             1.03057
                   n               1             NA
                   k         1802.79        1800.91          -1726.93             5332.51

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


         Table of Data and Estimated Values of Interest

      Dose       N     Obs Mean      Est Mean    Obs Std Dev   Est Std Dev    Scaled Res.
      ------     ---    --------      --------    -----------   -----------    ----------


          0      10        4.35          4.37         0.177         0.291         -0.242
        480      10        4.55          4.51         0.357         0.291          0.432
        920      10         4.6           4.6         0.364         0.291        -0.0219
       1900      10        4.75          4.72         0.266         0.291          0.292
       3850      10        4.74          4.83          0.25         0.291          -1.05
       8065      10        4.98          4.92         0.351         0.291          0.587


   Model Descriptions for likelihoods calculated


   Model A1:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma^2

   Model A2:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma(i)^2

   Model A3:        Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma^2
       Model A3 uses any fixed variance parameters that
       were specified by the user

   Model  R:        Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                     Likelihoods of Interest

             Model     Log(likelihood)   # Param's       AIC
              A1           44.972729          7        -75.945458
              A2           48.458304         12        -72.916608
              A3           44.972729          7        -75.945458
           fitted         44.073247          4        -80.146494
               R          33.552988          2        -63.105977
```

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

                    Tests of Interest

   Test   -2*log(Likelihood Ratio)   Test df   p-value

   Test 1   29.8106   10   0.0009199
   Test 2   6.97115   5   0.2228
   Test 3   6.97115   5   0.2228
   Test 4   1.79896   3   0.6152

The p-value for Test 1 is less than .05. There appears to be a difference between response
and/or variances among the dose levels. It seems appropriate to model the data.

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be
appropriate here.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the
data.

        Benchmark Dose Computation

Specified effect =   0.1

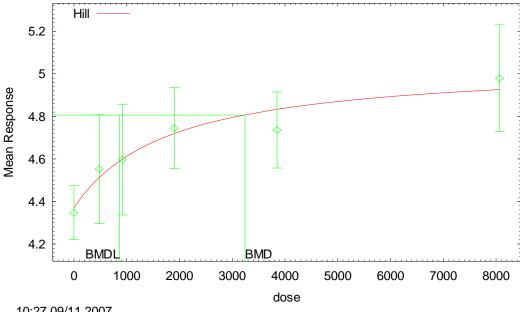Risk Type   =   Relative risk

Confidence level =   0.95

         BMD =   3241.95

        BMDL =   867.264

Hill Model with 0.95 Confidence Level

10:27 09/11 2007

Relative liver weight, male mouse (NTP, 2002)

BMR = change in the mean response equal to 1 standard deviation from the control mean

```
=====================================================================
        Hill Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\DCE DOSE-RESPONSE MODELING\MALE_MOUSE_REL_LIVER_WEIGHT.(d)
        Gnuplot Plotting File:  G:\DCE DOSE-RESPONSE MODELING\MALE_MOUSE_REL_LIVER_WEIGHT.plt
                                        Tue Feb 26 11:49:57 2008
=====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                        alpha =    0.0912885
                          rho =            0   Specified
                    intercept =        4.347
                            v =        0.632
                            n =     0.479874
                            k =      2442.97


          Asymptotic Correlation Matrix of Parameter Estimates

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

                   alpha     intercept           v            k

       alpha           1    -2.5e-009        2e-008     9.8e-009

   intercept   -2.5e-009            1         -0.02         0.68

           v       2e-008        -0.02             1         0.65

           k     9.8e-009         0.68          0.65            1




                       Parameter Estimates

                                            95.0% Wald Confidence Interval
        Variable        Estimate        Std. Err.    Lower Conf. Limit    Upper Conf. Limit
           alpha       0.0846603        0.0154568            0.0543656             0.114955
       intercept         4.36928          0.09406              4.18493              4.55364
               v        0.679896         0.178916             0.329227              1.03057
               n               1               NA
               k         1802.79          1800.91             -1726.93              5332.51

 NA - Indicates that this parameter has hit a bound
      implied by some inequality constraint and thus
```

```
     has no standard error.



       Table of Data and Estimated Values of Interest

    Dose      N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
   ------    ---   --------     --------   -----------  -----------   ----------


       0      10      4.35        4.37        0.177        0.291        -0.242
     480      10      4.55        4.51        0.357        0.291         0.432
     920      10       4.6         4.6        0.364        0.291        -0.0219
    1900      10      4.75        4.72        0.266        0.291         0.292
    3850      10      4.74        4.83         0.25        0.291        -1.05
    8065      10      4.98        4.92        0.351        0.291         0.587



  Model Descriptions for likelihoods calculated


  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

  Model  R:        Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                    Likelihoods of Interest

           Model      Log(likelihood)   # Param's       AIC
            A1          44.972729            7        -75.945458
            A2          48.458304           12        -72.916608
            A3          44.972729            7        -75.945458
          fitted        44.073247            4        -80.146494
            R           33.552988            2        -63.105977



                     Explanation of Tests

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

                     Tests of Interest

    Test    -2*log(Likelihood Ratio)   Test df        p-value

   Test 1            29.8106               10         0.0009199
   Test 2            6.97115                5         0.2228
   Test 3            6.97115                5         0.2228
   Test 4            1.79896                3         0.6152

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here
```

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

        Benchmark Dose Computation

Specified effect =   1

Risk Type   =   Estimated standard deviations from the control mean

Confidence level =   0.95

          BMD =   1348.69

         BMDL =   395.878

Hill Model with 0.95 Confidence Level

11:49 02/26 2008

Relative liver weight, female mouse (NTP, 2002)

BMR = 10% change in mean relative liver weight relative to the control mean

Nonhomogeneous variance

Table B-4. BMDS modeling summary of relative liver weights in female mice exposed to trans-1,2-DCE in the feed for 14 weeks

Model	Test 3 p-Value	Test 4 p-Value	AIC	BMD (mg/kg-d)	BMDL (mg/kg-d)
Linear, Polynomial (restricted)	0.1553	0.001521	-74.100992	8,457.39	5,488.88
Polynomial, 2nd degree (unrestricted)	0.1553	0.007562	-77.688156	3,807.76	2,027.13
Power (≥1)	0.1553	0.001521	-74.100992	8,457.39	5,488.88
Power (unrestricted)	0.1553	0.007744	-77.739382	5,224.74	1,603.23
Hill (≥1)	0.1553	0.003766	-76.473258	6,158.22	BMDL computation failed
Hill (unrestricted)	0.1553	0.003766	-76.473259	6,158.23	BMDL computation failed

None of the models in BMDS (version 1.4.1) provided an adequate fit of the data for relative liver weight in female mice from the NTP (2002) study.

Relative liver weight, male rat (NTP, 2002)

BMD methods were not applied to male rat data because relative liver weights were not significantly elevated over controls.

Relative liver weight, female rat (NTP, 2002)

BMR = 10% change in mean relative liver weight relative to the control mean
Constant variance

Table B-5. BMDS modeling summary of relative liver weights in female rats exposed to trans-1,2-DCE in the feed for 14 weeks

Model	Test 3 p-Value	Test 4 p-Value	AIC	BMD (mg/kg-d)	BMDL (mg/kg-d)
Linear, Polynomial (restricted)	0.7	0.01169	-143.4885	5,447.8	3,362.9
Polynomial, 2nd degree (unrestricted)[a]	0.7	0.008974	-142.8260	1,971.2	1,027.2
Power (≥1)	0.7	0.01169	-143.4885	5,447.8	3,362.9
Power (unrestricted)	0.7	0.09092	-147.9366	6,165.2	539.0
Hill (≥1)	0.7	0.2317	-150.1137	10% BMR is not in the range of the fitted model	
Hill (unrestricted)	0.7	0.2317	-150.1137	10% BMR is not in the range of the fitted model	

[a]BMR = 8%, maximum within range of data.

None of the models in BMDS (version 1.4.1) provided an adequate fit of the data for relative liver weight in female rats from the NTP (2002) study. The Hill model (with power parameter restricted to be greater than 1) provided an adequate statistical fit of the relative liver weight data (i.e., χ^2 $p>0.1$), but the curve generated by the model was not supported biologically (i.e., the curve was essentially a step function, with almost no transition between the dose at which no effect was observed and the dose causing a maximum effect). This data set did not demonstrate as sensitive a response as the others.

B.2.2. Decreased Antibody Directed Against sRBC (Shopp et al., 1985)

BMR = change in the mean response equal to 1 standard deviation from the control mean

Table B-6. BMDS modeling summary of decreased antibody directed against sRBC in male mice exposed to trans-1,2-DCE in drinking water for 90 days

Model	Test 3 *p*-Value	Test 4 *p*-Value	AIC	BMD (mg/kg-d)	BMDL (mg/kg-d)
Polynomial, 2nd degree (unrestricted)	0.4558	0.7077	483.818	125.55	65.04
Polynomial, 1st degree (unrestricted)	0.4558	0.2596	484.375	309.20	195.01
Power (≥1)	0.4558	0.2596	484.375	309.20	195.01
Hill (>1)	0.4558	NA	485.678	45.98	13.32

```
====================================================================
      Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
      Input Data File: M:\NCEA\IRIS\DCE\BMDS RUNS\MALE_MICE_AFC_SHOPP_AG.(d)
      Gnuplot Plotting File:  M:\NCEA\IRIS\DCE\BMDS RUNS\MALE_MICE_AFC_SHOPP_AG.plt
                                         Wed Mar 04 09:40:13 2009
====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


  Dependent variable = MEAN
  Independent variable = DOSE
  rho is set to 0
  Signs of the polynomial coefficients are not restricted
  A constant variance model is fit

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



              Default Initial Parameter Values
                    alpha =            1
                      rho =            0   Specified
                   beta_0 =      2164.22
                   beta_1 =     -4.53587
                   beta_2 =   0.00808257


        Asymptotic Correlation Matrix of Parameter Estimates

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

               alpha        beta_0        beta_1        beta_2

     alpha         1       0.00028      -0.00047       0.00046

    beta_0   0.00028             1         -0.53           0.4
```

```
     beta_1      -0.00047          -0.53              1           -0.97

     beta_2       0.00046           0.4            -0.97              1




                          Parameter Estimates

                                          95.0% Wald Confidence Interval
       Variable      Estimate      Std. Err.   Lower Conf. Limit   Upper Conf. Limit
         alpha         202454       47770.9             108825             296084
        beta_0        2172.39       106.683             1963.3            2381.49
        beta_1       -4.61897       1.97169            -8.48342          -0.754516
        beta_2     0.00824479     0.00506139          -0.00167535        0.0181649



      Table of Data and Estimated Values of Interest

  Dose      N    Obs Mean      Est Mean    Obs Std Dev   Est Std Dev   Scaled Res.
 ------    ---   --------      --------    -----------   -----------   ----------

      0    12   2.2e+003     2.17e+003          433           450         0.213
     17     8   2.05e+003     2.1e+003          430           450        -0.303
    175     8   1.63e+003    1.62e+003          385           450         0.053
    387     8   1.62e+003    1.62e+003          639           450        -0.0105



 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:        Yi = Mu + e(i)
          Var{e(i)} = Sigma^2



                     Likelihoods of Interest

            Model      Log(likelihood)    # Param's       AIC
             A1         -237.839020            5       485.678039
             A2         -236.534278            8       489.068557
             A3         -237.839020            5       485.678039
           fitted       -237.909292            4       483.818585
              R         -243.158365            2       490.316730



                       Explanation of Tests

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

                        Tests of Interest

   Test    -2*log(Likelihood Ratio)   Test df        p-value

   Test 1            13.2482              6          0.03926
   Test 2            2.60948              3          0.4558
   Test 3            2.60948              3          0.4558
   Test 4            0.140546             1          0.7077
```

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

                Benchmark Dose Computation

Specified effect =   1

Risk Type   =   Estimated standard deviations from the control mean

Confidence level =   0.95

        BMD =   125.55

      BMDL =   65.0386

Polynomial Model with 0.95 Confidence Level

09:40 03/04 2009