CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Overview

- Information on the toxicokinetics of cis- and trans-1,2-dichloroethene comes mainly from inhalation studies conducted in rats.
- The cis- and trans- isomers have distinct toxicokinetics.

Absorption

- Studies conducted in rats indicate relatively rapid absorption of inhaled 1,2-dichloroethene with air:blood equilibrium occurring within 1–2 hours following initiation of a constant exposure.
- Continued inhalation absorption following equilibrium is driven by elimination of 1,2-dichloroethene, primarily by metabolism.
- The blood:air partition coefficient for cis-1,2-dichloroethene is higher than that of trans-1,2-dichloroethene.
- No studies were located that described amounts or kinetics of absorption of cis- or trans-1,2-dichloroethene following oral exposure.
- No studies were located that described amounts or kinetics of absorption of cis- or trans-1,2-dichloroethene following dermal exposure.

Distribution

- No studies were located regarding the distribution of cis- and trans-1,2-dichloroethene following exposure by any route.
- Tissue:air partition coefficients suggest that both isomers will enter most tissues and that the highest concentrations are likely to be observed in adipose.

Metabolism

- Metabolism is the primary mechanism of elimination of absorbed 1,2-dichloroethene.
- 1,2-Dichloroethene is metabolized by the microsomal CYP monooxygenase enzyme system in the liver.
- 1,2-Dichloroethene exhibits dose-dependent metabolic clearance resulting from substrate saturation and suicide inhibition of CYP.
- 1,2-Dichloroethene induces CYP isozymes in liver and these effects on CYP are sex-dependent in rats.

- trans-1,2-Dichloroethene is a more potent inhibitor of CYP than cis-1,2-dichloroethene and is more slowly metabolized than cis-1,2-dichloroethene.

- Zero-order metabolic elimination (saturation) occurs with exposures to 1,000 ppm cis-1,2-dichloroethene and 25 ppm trans-1,2-dichloroethene.

Excretion

- No studies were located regarding the excretion of 1,2-dichloroethene in humans or animals following exposure by any route.

Toxicokinetics Models

- A physiologically based pharmacokinetic (PBPK) model has been developed for simulating the kinetics of inhalation uptake, metabolic elimination, and inhibition of metabolism of cis- and trans-1,2-dichloroethene in rats.

- Generic PBPK models have been used to simulate steady-state blood 1,2-dichloroethene concentrations and blood concentration area under the curve (AUC) in humans.

3.1.1 Absorption

Closed chamber gas-uptake studies performed on rats have examined the kinetics of absorption and elimination of inhaled cis- and trans-1,2-dichloroethene (Andersen et al. 1980; Clewell and Andersen 1994; Filser and Bolt 1979). The kinetics of uptake from the chamber exhibited a rapid phase and a slow phase. The rapid phase of uptake, reflecting the kinetics of absorption and distribution, occurred within 1–2 hours. The slower phase, reflecting the kinetics of metabolism, exhibited saturation kinetics. The rate for the slower phase is dose-dependent, consistent with saturable metabolism and inhibition of metabolism (Clewell and Andersen 1994; Lilly et al. 1998).

Absorption of inhaled 1,2-dichloroethene will be governed, in part, by the blood:air partition coefficient. Several studies have measured blood:air partition coefficients for 1,2-dichloroethene (Gargas et al. 1988, 1989; Sato and Nakajima 1979). The blood:air partition coefficient for cis-1,2-dichloroethene is higher (approximately 20) than that of trans-1,2-dichloroethene (approximately 10) (Gargas et al. 1989).

No studies were located that described amounts or kinetics of absorption of cis- or trans-1,2-dichloroethene following oral or dermal exposure.

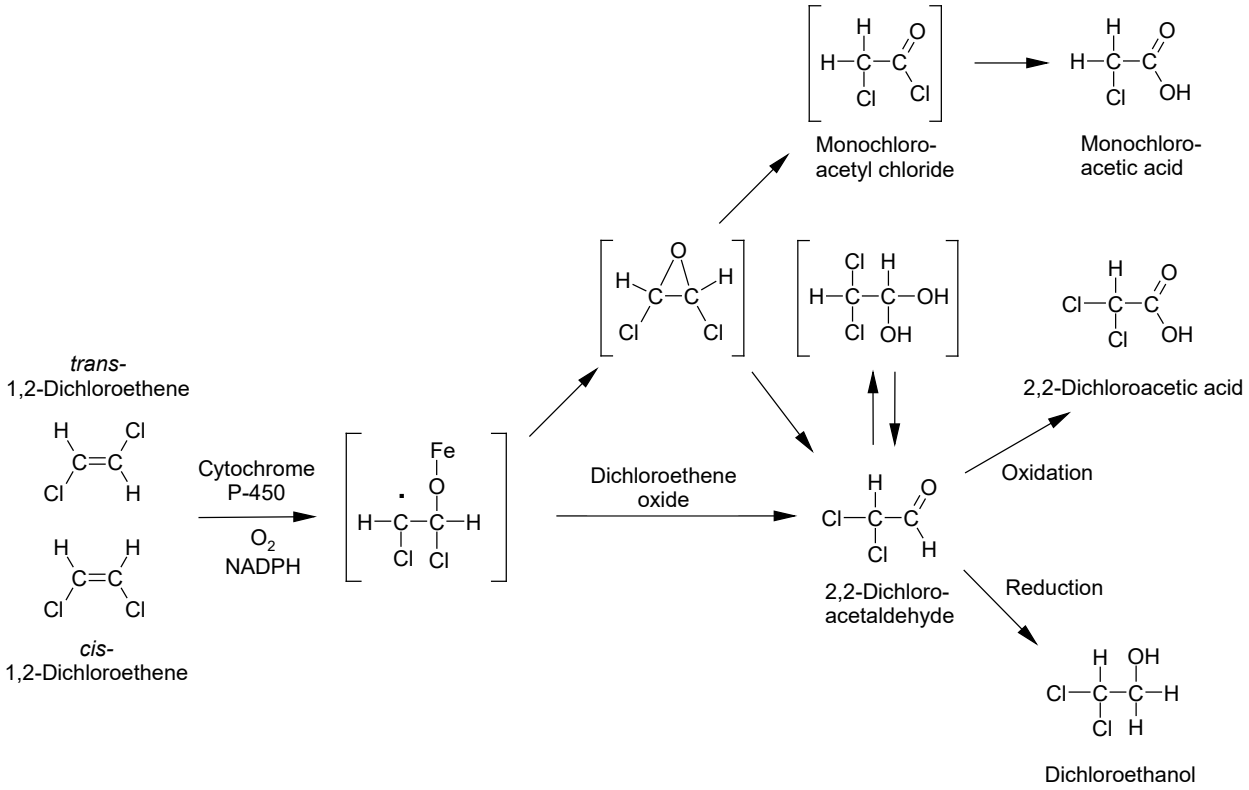
3.1.2 Distribution

No studies were located regarding the distribution of cis- and trans-1,2-dichloroethene following exposure by any route. However, tissue:air partition coefficients suggest that both isomers will enter most tissues and that the highest concentrations are likely to be observed in adipose. Gargas et al. (1988, 1989) determined rat tissue:air partition coefficients for cis- and trans-1,2-dichloroethene. The partition coefficients for cis-1,2-dichloroethene were as follows: blood 21.6 (±2.0), 0.9% saline 3.25 (±0.12), olive oil 278 (±6), fat 227 (±11), liver 15.3 (±11), and muscle 6.09 (±1.02). The coefficients for trans-1,2-dichloroethene were: blood 9.58 (±0.94), 0.9% saline 1.41 (±0.04), olive oil 178 (±6), fat 148 (±11), liver 8.96 (±0.61), and muscle 3.52 (±0.54).

3.1.3 Metabolism

Metabolism of 1,2-dichloroethene is initially catalyzed by hepatic microsomal CYP (Costa and Ivanetich 1982, 1984). The reaction is catalyzed by multiple isozymes, including the CYP2E1 and CYP34A (Costa and Ivanetich 1982; Lilly et al. 1998). Although there is no direct evidence, studies on the synthesis of epoxides suggest that metabolism involves epoxidation of the ethylene double bond, forming dichlorinated epoxides (Figure 3-1). Dichlorinated epoxides, in turn, can undergo a non-enzymatic rearrangement. Studies on the metabolism of 1,2-dichloroethene by hepatic microsomes and hepatocytes provide evidence to suggest that dichloroacetaldehyde is the predominant metabolite of microsomal CYP and that it, in turn, is extensively converted to dichloroethanol and dichloroacetate by cytosolic and/or mitochondrial aldehyde and alcohol dehydrogenases present in hepatocytes (Costa and Ivanetich 1982, 1984; Leibman and Ortiz 1977). Dechlorination of dichloroacetate is catalyzed by glutathione S-transferase (Costa and Ivanetich 1982). This is consistent with the report that both cis- and trans-1,2-dichloroethene were converted to dichloroethanol and dichloroacetic acid by perfused rat liver (Bonse et al. 1975).

Figure 3-1. Postulated Metabolic Scheme for 1,2-Dichloroethene



Source: Costa and Ivanetich 1982

Similarities and differences have been observed in the metabolism of cis- and trans-1,2-dichloroethene. Both isomers have been shown to bind to the active site of hepatic CYP (Costa and Ivanetich 1982). In addition, classic inhibitors of CYP have been shown to inhibit the production of dichloroacetaldehyde from both isomers. The binding and metabolism of 1,2-dichloroethene do not appear to be specific for any one form of CYP. cis-1,2-Dichloroethene had a 4-fold greater rate of turnover in hepatic microsomes *in vitro* than trans-1,2-dichloroethene. This is consistent with studies on isolated perfused rat livers, where metabolism of cis-1,2-dichloroethene occurred at a greater rate than metabolism of trans-1,2-dichloroethene (Bonse et al. 1975). In addition, differences between cis- and trans-1,2-dichloroethene in the rates of formation of dichloroethanol and dichloroacetic acid have been reported in rat hepatocytes (Costa and Ivanetich 1984).

Studies conducted in rats have shown that 1,2-dichloroethene can alter CYP levels and mixed-function oxidase activities. Effects observed in rats have included inhibition (Freundt and Macholz 1978; Hanioka et al. 1998; Lilly et al. 1998; McMillan 1986; Nakahama et al. 2000), decreased expression (Hanioka et al. 1998; Nakahama et al. 2000), and induction (Bronzetti et al. 1984; Hanioka et al. 1998; Paolini et al.

1992). These different effects are specific to CYP isozymes, dose levels, and sex. Treatment of male rats with cis- or trans-1,2-dichloroethene decreased expression of hepatic CYP3A, CYP2B, CYP2C, and CYP2E isoforms (Hanioka et al. 1998; Nakahama et al. 2000). Inhibition of CYP2E1 activity has been attributed to formation of reactive metabolites of 1,2-dichloroethene that disrupt the active site of the enzyme (*suicide inhibition*) (Lilly et al. 1998). trans-1,2-Dichloroethene was a more potent inhibitor of CYP2E1 than cis-1,2-dichloroethene in male rats (Lilly et al. 1998). cis-1,2-Dichloroethene decreased expression of hepatic CYP1A1/2 and CYP2B1/2, whereas trans-1,2-dichloroethene increased expression of these isozymes in male rats (Hanioka et al. 1998). Changes in CYP activities (increased or decreased) resulting from exposure to cis- or trans-1,2-dichloroethene were different in male and female rats (Hanioka et al. 1998). For example, cis-1,2-dichloroethene reduced expression of CYPA1/2 in male rats, but increased expression of the isozyme in female rats. Expression of CYP2E1 was increased in female rats but decreased in male rats. Freundt and Macholz (1978) demonstrated that cis-1,2-dichloroethene was a more potent inhibitor of metabolism of hexobarbital in rats. Inhibition of N- and O-demethylation by cis- and trans-1,2-dichloroethene was competitive and reversible in rat liver microsomes (Freundt and Macholz 1978).

The metabolic elimination of 1,2-dichloroethene has been described as a saturable, dose-dependent process (Andersen et al. 1980; Clewell and Andersen 1994; Filser and Bolt 1979). The primary basis for this conclusion is from observations made in rats of the kinetics of uptake of 1,2-dichloroethene from closed exposure chambers (Andersen et al. 1980; Clewell and Andersen 1994; Filser and Bolt 1979). An initial rapid “equilibrium” phase of uptake results from distribution of 1,2-dichloroethene into blood and tissues. This is followed by a slower “elimination” phase. The slow-phase kinetics has been used to estimate rates of metabolism of 1,2-dichloroethene, with the assumption that all slow-phase elimination results from metabolism. The rate of the elimination phase is dose-dependent; first-order at low exposure concentrations and zero-order and higher at “saturating” concentrations (e.g., 1,000 ppm) (Filser and Bolt 1979). Saturation is thought to reflect a combination of full occupancy of the enzyme coupled with inhibition of the enzyme from reactive intermediates (Lilly et al. 1998). Lilly et al. (1998) estimated the K_M, V_{max}, and inhibition constant (K_d) for metabolic elimination of cis- and trans-1,2-dichloroethene (Table 3-1). Based on the estimated K_M and V_{max}, 90% of the V_{max} was estimated to be achieved at an exposure concentration of 1,800 ppm for cis-1,2-dichloroethene and 800 ppm for trans-1,2-dichloroethene. However, Lilly et al. (1998) also concluded that trans-1,2-dichloroethene is a more potent inhibitor of CYP than cis-1,2-dichloroethene and, as a result, saturation of metabolism can occur at lower exposures to trans-1,2-dichloroethene than predicted from the K_M. If enzyme inhibition is not considered, the estimated V_{max} for trans-1,2-dichloroethene is substantially lower than cis-

1,2-dichloroethene (2.4 and 0.67 mg/kg/hour, respectively) (Csandy et al. 1995). The higher V_{max} for cis-1,2-dichloroethene is consistent with the higher rate of metabolism of cis-1,2-dichloroethene relative to trans-1,2-dichloroethene by rat liver microsomes (Costa and Ivanetich 1982) and by isolated perfused liver (Bonse et al. 1975).

Table 3-1. Optimized Values for Metabolism Parameters in the Lilly et al. (1998) Rat PBPK Model of 1,2-Dichloroethene[a]

	V_{max} (mg/hour/kg body weight)	K_M (mg/L, ppm)	K_d (mg/hour/hour)	K_{de} ($hour^{-1}$)
cis-1,2-Dichloroethene	4.53±0.12	0.19±0.01 (48±3)	2.07±0.05	0.025±0.001
trans-1,2-Dichloroethene	4.27±0.04	0.08±0.01 (20±3)	496±2.16	0.026±0.001

[a]Shown are mean ± standard deviation.

K_d = enzyme inhibition coefficient; K_{de} = enzyme degradation coefficient; K_M = half-saturation concentration; PBPK = physiologically based pharmacokinetic; V_{max} = maximum rate of metabolism

The importance of CYP2E1 and glutathione S-transferase in the metabolism of 1,2-dichloroethene raises the possibility of population genetic polymorphisms in these two enzyme systems contributing to variability in 1,2-dichloroethene metabolism in humans (Blackburn et al. 2000, 2001; Lipscomb et al. 1997).

3.1.4 Excretion

No studies were located regarding the excretion of 1,2-dichloroethene in humans or animals following exposure by any route.

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use

mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

Lilly et al. (1998) Model

Description. Lilly et al. (1998) developed a PBPK model for simulating the kinetics of inhalation uptake and elimination of cis- and trans-1,2-dichloroethene in rats. The model includes compartments representing lung, fat, liver, and lumped compartments representing all other rapidly perfused tissues and all other slowly perfused tissues. Exchange of 1,2-dichloroethene between air and blood and blood and tissues is assumed to be flow-limited and governed by tissue blood flow and tissue:blood partition coefficients. Elimination of 1,2-dichloroethene is simulated as a capacity limited metabolism (CYP2E1) governed by a V_{max} (mg/kg/hour) and K_M (mg/L). Inhibition of metabolism is simulated as a zero-order loss of activity (mg/hour/hour) and enzyme re-synthesis was simulated as a first-order order process ($hour^{-1}$).

Calibration and Evaluation. Blood:air and tissue:blood partition coefficients were from Gargas et al. (1989). Initial values for metabolism parameters, V_{max} and K_M, were estimated from closed chamber experiments (Gargas et al. 1988). Metabolic parameters were recalibrated to fit observations of rates of uptake of 1,2-dichloroethene by rats in closed exposure chambers (Lilly et al. 1998). Various approaches to modeling inhibition of metabolism were explored. The best fit to the closed chamber observations for both isomers was obtained with a model in which metabolism produces a reactive intermediate that binds to and inactivates the enzyme-substrate complex. The optimized values for V_{max} and K_M and the enzyme inhibition constant (K_d) are provided in Table 3-1. These values suggest that trans-1,2-dichloroethene is a more potent inhibitor of CYP2E1 than cis-1,2-dichloroethene.

Other Modeling Approaches

Aylward et al. (2010). Aylward et al. (2010) developed a model for simulating the steady-state concentration of cis- and trans-1,2-dichloroethene in rats and humans. The model was a steady-state solution to a generic multi-compartment PBPK model. At steady state, the multi-compartment model was reduced to parameters representing alveolar ventilation, cardiac output, liver blood flow, blood:air partition coefficient, liver:blood partition coefficient, and first order metabolism clearance coefficients (V_{max}/K_M). The rat model was extrapolated to humans by replacing the blood:air partition coefficient for humans (Gargas et al. 1989), and allometrically scaling the rat V_{max} by body weight ($BW^{0.7}$). Evaluation

of the human model was not reported. Aylward et al. (2010) applied the human model to predicting the blood/dose slope associated with continuous steady state exposures that would result in hepatic venous blood concentrations well below saturation of metabolism (i.e., $<0.1xK_M$). The predicted inhalation slopes were 3.3 μg/L per mg/m^3 (13 μg/L per ppm) for cis-1,2-dichloroethene and 1.9 μg/L per mg/m^3 (7.5 μg/L per ppm) for trans-1,2-dichloroethene. The predicted oral slopes were 17 μg/L per mg/kg/day (absorbed dose) for cis-1,2-dichloroethene and 3.4 μg/L per mg/kg/day for trans-1,2-dichloroethene.

Peyret and Krishnan (2012). Peyret and Krishnan (2012) utilized a generic PBPK model to predict the blood concentration AUC of cis- and trans-1,2-dichloroethene in humans. Blood:air and tissue:blood partition coefficients were based on Gargas et al. (1989). First-order metabolism clearance coefficients (V_{max}/K_M) were predicted from quantitative structure-activity relationship (QSAR) modeling of reported clearance coefficients estimated in rats for a set of 26 volatile organic compounds (VOCs), with the V_{max} allometrically scaled by body weight ($BW^{0.75}$). The clearance coefficients were used in the PBPK model to calculate the metabolic clearance of 1,2-dichloroethene in the liver at levels of hepatic venous blood concentrations well below saturation (e.g., inhalation exposures to 1 ppm). An evaluation of the model for predicting blood concentrations of 1,2-dichloroethene in humans was not reported.

3.1.6 Animal-to-Human Extrapolations

No studies were located regarding the toxicokinetics of 1,2-dichloroethene in humans. Toxicokinetic studies conducted in rats suggest that animal-to-human extrapolation of dose-response relationships should consider several factors: (1) dose-dependent metabolic clearance resulting from enzyme saturation and suicide inhibition; (2) production of reactive intermediates which may contribute to some forms of toxicity; and (3) sex-dependent effects on CYP mixed-function oxidase activities (see Section 3.1.3). Dose-dependent clearance is particularly important for animal-to-human extrapolation of dose-response relationships. Many studies conducted in animals have observed adverse effects at inhalation exposures predicted to be close to or above saturating levels for metabolic clearance of cis- and trans-1,2-dichloroethene (e.g., ≥800 ppm). Linear extrapolation of responses to lower dose levels, below saturation, would be highly uncertain and alternative approaches such as PBPK modeling would be needed to support such extrapolations. A PBPK model that simulates dose-dependent clearance of cis- and trans-1,2-dichloroethene in rats has been developed (see Section 3.1.5).

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to 1,2-dichloroethene are discussed in Section 5.7, Populations with Potentially High Exposures.

It is not known if children are more susceptible to toxic effects of 1,2-dichloroethene, and few studies have evaluated effects in immature offspring. No increase in the risk of birth defects (neural tube defect or oral cleft defects) or childhood hematopoietic cancers were observed in an epidemiological study in children born to women exposed to 1,2-dichlorethane in their drinking water during pregnancy (Ruckart et al. 2013). Gestational exposure of rats to inhaled trans-1,2-dichloroethene resulted in an increased number of resorptions and decreased fetal weight; no malformations or variations were identified (Hurtt et al. 1993). The significance of these findings to humans is unknown.

As discussed in Section 3.1.3 (Toxicokinetics, Metabolism), metabolism of 1,2-dichloroethene involves multiple CYP isozymes, including CYP2E1 and CYP34A (Costa and Ivanetich 1982, 1984; Lilly et al. 1998). Individuals with underlying liver disease may have a decreased capacity to metabolize 1,2-dichloroethene. In addition, CYP2E1 can be induced by fasting and diabetes (Rannug et al. 1995). In children, CYP enzymes may not have the same metabolic capacity as adults, leading to potentially higher blood levels; however, no information on metabolism of 1,2-dichloroethene isomers is available in infants, children, or immature animals. CYP2E1 and glutathione S-transferase zeta (GSTZ) exist in different polymorphic forms. Metabolic activity may vary between specific polymorphisms of these enzymes, resulting in altered blood levels of 1,2-dichloroethene and its metabolites.

No populations with unusual susceptibility to the health effects of 1,2-dichloroethene were identified. As discussed in Section 2.18 (Other Noncancer), small increases in serum glucose levels were observed in mice following a 90-day exposure to trans-1,2-dichloroethene in drinking water (Barnes et al. 1985); the increases did not exhibit dose-dependence. Although the toxicological significance of this finding is uncertain, an association between exposure to trans-1,2-dichloroethene and altered glucose metabolism cannot be ruled out. Therefore, individuals with diabetes may be more susceptible to trans-1,2-dichloroethene exposure. Studies have also shown that exposure to trans- and cis-1,2-dichloroethene can decrease erythrocyte counts (see Section 2.7, Hematological). Therefore, individuals with anemia may have increased susceptibility to 1,2-dichloroethene. Additionally, immunocompromised individuals may have increased susceptibility to 1,2-dichloroethene based on the findings of impaired immune response in mice exposed to trans-1,2-dichloroethene.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to 1,2-dichloroethene are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for 1,2-dichloroethene from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung

capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by 1,2-Dichloroethene are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

1,2-Dichloroethene can be measured in blood and expired air. Blood 1,2-dichloroethene levels have been used to quantify exposure in the U.S. general population (Ashley et al. 1994; CDC 2021). cis-1,2-Dichloroethene can be measured in expired air; however, its usefulness as a biomarker may be limited since a half-life of <30 minutes was estimated in a study of two volunteers (Pleil and Lindstrom 1997).

3.3.2 Biomarkers of Effect

There currently are no biomarkers of effect available to characterize effects specifically caused by 1,2-dichloroethene in humans. Effects observed following exposure to 1,2-dichloroethene can be observed with many chemicals, and there is not an effect that is unique to 1,2-dichloroethene.

3.4 INTERACTIONS WITH OTHER CHEMICALS

No studies were located regarding possible interactions between 1,2-dichloroethene and other chemicals that are likely to be found with 1,2-dichloroethene in the environment, workplace, or at hazardous waste sites.

CYP isozymes, glutathione s-transferases and glutathione are important for the metabolism of 1,2-dichlorethene (see Section 3.1.3). Chemicals that induce or inhibit CYP isozymes or decrease glutathione concentrations (e.g., ethanol) may alter metabolism and affect the toxicity of 1,2-dichloro-ethene. However, no *in vivo* studies were located investigating potential interactions. In an *in vitro* study, rat pancreatic tumor cells exposed to trans-1,2-dichloroethene alone or in combination with ethanol did not affect cell proliferation, viability, or fatty acid ethyl ester production of the cells (Bhopale et al. 2014).