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

3.1 TOXICOKINETICS

Data regarding toxicokinetics of 1,1-dichloroethene in humans are not available. Toxicokinetic studies in animals indicate the following:

- Inhaled or ingested 1,1-dichloroethene is readily absorbed through the lung and by the gastrointestinal tract.
- Following absorption, 1,1-dichloroethene and its metabolites are rapidly distributed by the blood; particular toxicity targets include the liver and kidney.
- 1,1-Dichloroethene is metabolized by the hepatic microsomal cytochrome P450 system to reactive metabolites; detoxification of reactive metabolites occurs primarily via epoxide hydrolase/hydrase-catalyzed hydrolysis and conjugation with GSH.
- Excretion of metabolites occurs primarily via the urine and exhaled air; unmetabolized parent compound may also be eliminated in exhaled air.

3.1.1 Absorption

No studies were located regarding absorption of 1,1-dichloroethene in humans.

Studies in laboratory animals demonstrate that inhaled 1,1-dichloroethene is rapidly absorbed (Dallas et al. 1983; McKenna et al. 1978a). The observation that rats exhibited a higher body burden than mice following similar exposure to 1,1-dichloroethene vapor indicates that species-specific differences may exist in the rate and/or extent of absorption (McKenna et al. 1977). No studies were located that described transport mechanisms for 1,1-dichloroethene absorption. Since 1,1-dichloroethene is a small organic molecule with chemical and physical properties similar to those of lipid soluble anesthetics, it is expected to penetrate pulmonary membranes easily and to enter the bloodstream rapidly. Substantial levels of the parent compound were found in the venous blood of rats within 2 minutes after inhalation exposure (Dallas et al. 1983). Absorption of 1,1-dichloroethene was duration- and dose-dependent. The percentage of systemic uptake decreased with time from the onset of exposure until an equilibrium was reached within 1 hour. Once equilibrium was reached, percentage uptake varied inversely with dose. The cumulative uptake of 1,1-dichloroethene following inhalation exposure was linear for levels ≤150 ppm. However, at 300 ppm, a steady state was never achieved. This finding indicates that 1,1-dichloroethene

absorption following inhalation exposure was saturable at high levels, and the kinetics at these levels are best described by a cubic curve (Dallas et al. 1983).

Studies in animals clearly indicate that oral doses of 1,1-dichloroethene (in corn oil) ranging from 10 to 100 mg/kg are rapidly and almost completely absorbed from the gastrointestinal tract of rats and mice (Jones and Hathway 1978a; Putcha et al. 1986). Rapid absorption occurred following oral administration of 200 mg/kg in an aqueous emulsion, as evidenced by the observation that the largest percentage of the dose was exhaled during the initial 15-minute period (Chieco et al. 1981). Peak blood levels were achieved in rats within 2–8 minutes after oral administration (Putcha et al. 1986). When 0.5–50 mg/kg of radiolabeled 1,1-dichloroethene was given to female rats, approximately 10% of the parent compound was recovered in the expired air by 1 hour after exposure, indicating that oral absorption was rapid (Reichert et al. 1979). After oral administration to rats of 1,1-dichloroethene labeled with radioactive carbon (^{14}C), 81–99.8% of the administered radioactivity was recovered within 72 hours (Reichert et al. 1979). Studies have shown that 9–21% was recovered in the expired air, 53.9% in urine, 14.5% in feces, 2.8% in the carcass, and 7.5% in the cage rinse following oral administration of 1 or 5 mg ^{14}C-1,1-dichloroethene/kg (McKenna et al. 1978b; Reichert et al. 1979). After a dose of 50 mg ^{14}C-1,1-dichloroethene/kg, 19 and 29% of the parent compound was excreted via lungs in nonfasted and fasted rats, respectively (McKenna et al. 1978b).

No studies were located regarding absorption in animals after dermal exposure to 1,1-dichloroethene. Nonetheless, the physical/chemical properties of 1,1-dichloroethene indicate that dermal absorption of 1,1-dichloroethene is probable. 1,1-Dichloroethene is a small organic molecule with properties similar to that of lipid-soluble anesthetics. Thus, liquid 1,1-dichloroethene is expected to readily penetrate the skin, which is a lipid-rich tissue. However, with a vapor pressure of 600 mmHg at 25°C, the rate of evaporation would be rapid leaving only a short time for skin penetration.

3.1.2 Distribution

No studies were located regarding distribution of 1,1-dichloroethene in humans.

Following inhalation exposure of rats to 10 or 200 ppm of ^{14}C-labeled 1,1-dichloroethene, the highest level of radioactivity was found in the liver and kidneys after 72 hours, with only very small amounts present in other tissues (McKenna et al. 1978a). These authors found that the tissue burden/g of tissue (mg equivalents of ^{14}C-1,1-dichloroethene/g of tissue/total mg equivalents recovered per rat) in the liver,

kidneys, and lungs of fasted rats was significantly greater than the tissue burden in nonfasted rats at both exposure levels, even though the total accumulation of ^{14}C in fasted rats was less than that of nonfasted rats. The results of this study suggest the nonrandom retention of parent compound and/or metabolites in specific target tissues of fasted animals.

Preferential accumulation of radioactivity was reported in the kidney and liver of rats exposed to radiolabeled 1,1-dichloroethene vapor for 2 hours at 2,000 ppm (Jaeger et al. 1977). Higher levels of radioactivity were noted in liver and kidney from fasted rats than from nonfasted rats. Examination of ^{l4}C activity at the subcellular level in these two tissues revealed that significantly more water-soluble ^{14}C activity was present in the cytosolic fractions of fasted rats. This observation suggests that distribution pathways for metabolism differ according to the amount of food ingested.

1,1-Dichloroethene was rapidly distributed to all tissues examined following a single oral dose of the ^{14}C-labeled compound to rats (Jones and Hathway 1978c). The highest amount of radioactivity was found in the liver and kidneys within 30 minutes of administration. More general redistribution throughout the soft tissues of the body followed.

No studies were located regarding distribution after dermal exposure to 1,1-dichloroethene.

In a study by Okine et al. (1985) in which mice were administered a single intraperitoneal injection of 125 mg/kg of ^{14}C-1,1-dichloroethene, radioactivity was distributed to some extent to all examined tissues, with peak levels seen 6 hours after administration. The highest levels of radioactivity were found in the kidney, liver, and lung, with lesser amounts in the skeletal muscle, heart, spleen, and gut.

3.1.3 Metabolism

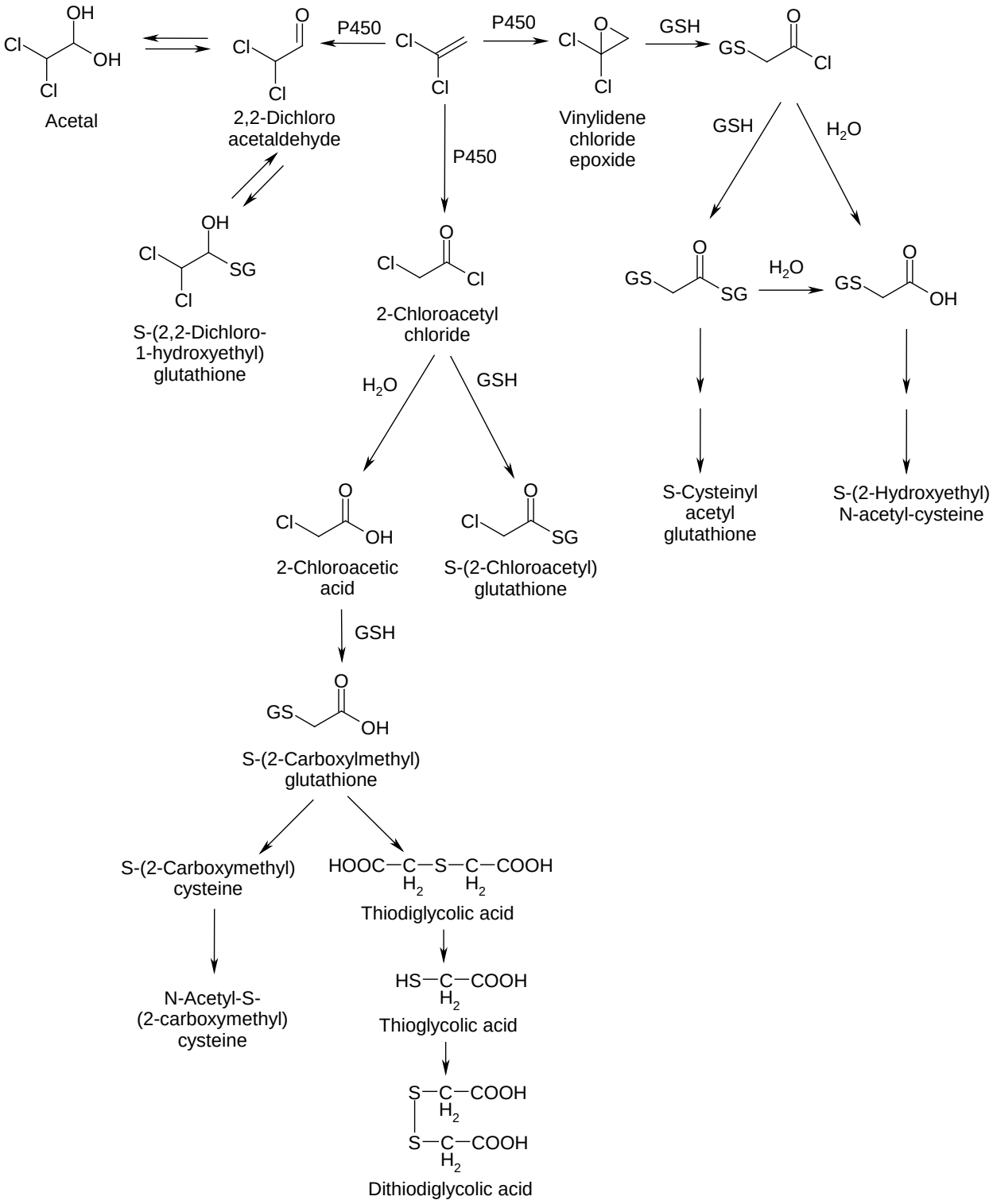
The metabolism of 1,1-dichloroethene following oral administration in rats has been extensively studied (Jones and Hathway 1978a, 1978c; McKenna et al. 1978b; Reichert et al. 1979). These studies demonstrate that 1,1-dichloroethene undergoes extensive biotransformation, and several metabolites have been identified. Results from *in vitro* assays using human tissues provide evidence for similarities between animals and humans in 1,1-dichloroethene metabolism (Dowsley et al. 1999). The cytochrome P450 CYP2E1 isozyme has been demonstrated to catalyze the formation of the 1,1-dichloroethene epoxide in both animal and human tissues (Dowsley et al. 1996; Speerschneider and Dekant 1995). The finding that liver cells from a human subject, together with Arochlor-pretreated S9-activated

1,1-dichloroethene, induced unspecified mutagenic metabolites in a *S. typhimurium* assay (Jones and Hathway 1978b) suggests that reactive metabolites may be produced in humans.

Proposed metabolic pathways for 1,1-dichloroethene are presented in Figure 3-1. According to the metabolic scheme, 1,1-dichloroethene undergoes P450-catalyzed epoxidation or oxidation to form the electrophilic metabolites 1,1-dichloroethene epoxide, 2-chloroacetyl chloride, and 2,2-dichloro-acetaldehyde. In rats, the oxidative metabolism of 1,1-dichloroethene reached saturation at an inhalation exposure level of approximately 200 ppm and an oral exposure of 10–50 mg/kg (Andersen et al. 1979; Dallas et al. 1983; D'Souza and Andersen 1988; McKenna et al. 1977). The reactive metabolites of 1,1-dichloroethene undergo hydrolysis and react with glutathione and cellular macromolecules. The observation that GSH is depleted in the liver following exposure to 1,1-dichloroethene suggests the importance of GSH conjugation as a major pathway in the detoxification of the reactive 1,1-dichloro-ethene metabolites (Jaeger et al. 1974; Reichert et al. 1978; Reynolds et al. 1980). Reynolds et al. (1980) reported a linear relationship in rats between intraperitoneally administered 1,1-dichloroethene and GSH depletion over the range of 20–100 mg/kg; above this level, GSH depletion reached a plateau. The maximum reduction (70%) occurred 4 hours after treatment, with a subsequent gradual recovery to normal levels within 24 hours. These findings led several investigators to suggest that 1,1-dichloro-ethene-induced hepatotoxicity is related to the depletion of hepatic GSH levels, thereby permitting the reactive intermediate to covalently bind to and alkylate hepatic macromolecules instead of being detoxified, ultimately leading to cell death (Jaeger et al. 1974; McKenna et al. 1977, 1978b; Reynolds et al. 1980).

Several 1,1-dichloroethene urinary and biliary metabolites have been identified in animal studies (Costa and Ivanetich 1982; Dowsley et al. 1995; Forkert 1999a, 1999b; Jones and Hathway 1978a, 1978c; Jones et al. 2003; Liebler et al. 1985, 1988; Okine and Gram 1986; Okine et al. 1985; Simmonds et al. 2004). Urinary metabolites include *N*-acetyl-*S*-(2-hydroxyethyl) cysteine, *S*-(cysteinyl acetyl) glutathione, *N*-acetyl-*S*-(2-carboxymethyl) cysteine, thiodiglycolic acid, dithioglycolic acid, dithiodiglycolic acid, and chloroacetic acid. Biliary metabolites include *S*-(2-carboxymethyl) glutathione, *S*-(cysteinyl acetyl) glutathione, several carboxymethylated proteins, and a product of the intramolecular rearrangement of the metabolite, *S*-(2-chloroacetyl)glutathione.

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



Source: NTP 2015a

The pathways of 1,1-dichloroethene metabolism in the mouse were generally similar to pathways in the rat, although several differences were observed. The rate of metabolism was greater in the mouse (Dowsley et al. 1995; Jones and Hathway 1978a). A predominant urinary metabolite of 1,1-dichloroethene in the mouse was *N*-acetyl-*S*-(2-carboxymethyl) cysteine; this metabolite was not detected in rat urine. The mouse produced a higher proportion of urinary S-(2-hydroxyethyl)-N-acetyl cysteine (a product of the reaction between 1,1-dichloroethene epoxide and GSH), suggesting a greater rate and/or extent of formation of 1,1-dichloroethene epoxidation in the mouse. Quantitatively greater amounts of other (water-soluble) urinary metabolites were present in the mouse urine (and consequently less parent compound in the expired air), attesting to a greater metabolic capacity. Furthermore, β-thionase activity was more pronounced in the mouse since more dithioglycolic acid was found than thiodiglycolic acid (Jones and Hathway 1978a). Oesch et al. (1983) suggested that 1,1-dichloroethene may have different effects on cytosolic GSH transferase activity and that this difference may contribute to species differences in 1,1-dichloroethene metabolism.

Forkert and Boyd (2001) evaluated hepatic metabolism of 1,1-dichloroethene in three different strains of mice (A/J, CD-1, and C57Bl/6). The A/J strain exhibited the highest level of hepatic CYP2E1, the greatest extent of covalent binding of 1,1-dichloroethene to liver proteins, and the highest level of 1,1-dichloroethene epoxide-derived glutathione conjugate in liver cytosol. These findings correlated well with the greater degree of 1,1-dichloroethene-induced centrilobular necrosis in the A/J strain compared to the CD-1 and C57Bl/6 strains.

1,1-Dichloroethene metabolism has been studied in a variety of *in vitro* tests. 1,1-Dichloroethene epoxide was the major metabolite produced in rat liver and mouse liver and lung microsomal incubations; minor metabolites included 2,2-dichloroacetaldehyde and 2-chloroacetylchloride (Costa and Ivaneitch 1982; Dowsley et al. 1995, 1996; Forkert 2001; Liebler and Guengerich 1983; Liebler et al. 1985; Simmonds et al. 2004). Secondary reactions included oxidation, glutathione conjugation, and hydrolysis. Dowsley et al. (1999) demonstrated that human lung and liver microsomal preparations exposed to 1,1-dichloroethene produced 1,1-dichloroethene epoxide as a major metabolite and that the reaction was catalyzed by CYP2E1. Simmonds et al. (2004) evaluated 1,1-dichloroethene metabolism in incubated mouse lung microsomes, and recombinant rat and human CYP2E1, mouse CYP2F2, goat CYP2F3, and rat CYP2F4. The recombinant rat CYP2E1 exhibited the greatest affinity and catalytic efficiency for 1,1-dichloroethene, suggesting a possible basis for species-specific differences in sensitivity to 1,1-dichloroethene liver and kidney toxicity.

1,1-Dichloroethene covalently binds preferentially to liver and kidney tissues following administration, which may provide a basis for the toxic effects seen in these organs (Jaeger et al. 1977; McKenna et al. 1977, 1978b). A linear increase in the amount of covalently bound radioactivity in the liver of rats exposed to 10–200 ppm ^{14}C-1,1-dichloroethene by inhalation for 6 hours was reported by McKenna et al. (1977). However, GSH depletion approached saturation at about 200 ppm. Therefore, the actual amount of reactive metabolite formed and available for binding was probably determined by a combination of both activation and detoxification pathways.

The increased severity of nephrotoxic effects induced by 1,1-dichloroethene in the mouse, compared to the rat, may be partially explained by the observation that the level of covalently bound reactive material in the mouse kidney was 6 times higher than the level in the rat kidney following similar inhalation exposure of both species (McKenna et al. 1977). This observation might also explain the increased severity of hepatic effects in the mouse compared to the rat. Similar results were reported by Short et al. (1977a) when a single dose of ^{14}C-1,1-dichloroethene was injected intraperitoneally into mice. The highest level of covalently bound radioactivity was seen in the mouse kidney. The study authors found that pretreatment with disulfiram also reduced the amount of covalent binding. The study authors speculated that disulfiram may reduce the activation of 1,1-dichloroethene and increase the extent of its detoxification. The inhibition of CYP2E1 by disulfiram has been demonstrated by Guengrich et al. (1991) and Yamazaki et al. (1992). Thus, conjugation of reactive intermediates of 1,1-dichloroethene with GSH is a major detoxification mechanism in laboratory animals because it reduces the amount of reactive material available to covalently bind to cellular macromolecules.

3.1.4 Excretion

No studies were located regarding excretion in humans exposed to 1,1-dichloroethene. Available animal data demonstrate that elimination is relatively rapid following inhalation or oral exposure.

Following inhalation exposure of rats to low levels of 1,1-dichloroethene elimination is rapid, mostly as metabolites in the urine, and very little (1% of the administered dose) eliminated as the unchanged parent compound in the expired air (McKenna et al. 1977). After exposure to low levels (25–150 ppm) of 1,1-dichloroethene, steady-state levels of 1,1-dichloroethene in the expired air are achieved within 30–45 minutes, indicating that elimination is first-order at low levels of exposure (Dallas et al. 1983). Steady-state levels of 1,1-dichloroethene in expired air are never reached when exposure levels approach 200–300 ppm because metabolic processes are saturated. When metabolic processes become saturated,

increased amounts of 1,1-dichloroethene can easily be eliminated unchanged via the expired air because 1,1-dichloroethene is volatile (vapor pressure of 600 mmHg at 25°C) and relatively insoluble in blood. Following cessation of exposure, concentrations of 1,1-dichloroethene in both blood and breath were observed to fall rapidly (Dallas et al. 1983). Similar results were reported by McKenna et al. (1978a).

1,1-Dichloroethene exhibited a biphasic elimination profile following inhalation exposure in rats (McKenna et al. 1977, 1978a). For the first phase, elimination half-lives were about 20 minutes for unchanged 1,1-dichloroethene in breath and 3 hours for water-soluble metabolites in urine. For the second phase, elimination half-lives were about 4 hours in breath and 20 hours in urine. The bulk of the material was eliminated in both the breath and the urine during the rapid first phase. Fasting did not appear to affect the elimination kinetics of 1,1-dichloroethene following inhalation exposure in rats (McKenna et al. 1978a). Bruckner et al. (2010) evaluated the plasma kinetics of 1,1-dichloroethene in fasted Sprague-Dawley rats. Following inhalation exposure at 300 ppm, the elimination half-life was 50 minutes.

Information is limited on elimination in mice following inhalation exposure to 1,1-dichloroethene. However, McKenna et al. (1977) reported that at low levels of exposure (10 ppm for 6 hours), somewhat smaller amounts of unchanged 1,1-dichloroethene were eliminated in the expired air of mice and larger amounts of water-soluble metabolites were found in the urine of mice compared to levels observed in rats. This indicates that mice metabolize 1,1-dichloroethene at a greater rate than rats.

Elimination of 1,1-dichloroethene and its metabolites following oral administration in rats is very similar to that seen following inhalation exposure. Following oral administration of 1 mg/kg ^{14}C-1,1-dichloroethene in corn oil, <1% of the administered dose was excreted unchanged in the expired air; another 8–14% was recovered as ^{14}C-carbon dioxide. Most of the radioactivity (44–80% of the administered dose) was eliminated in the urine within 3 days, most within the first 24 hours. Smaller amounts of water-soluble metabolites (8–16% of the administered dose) were found in the feces (Jones and Hathway 1978c; McKenna et al. 1978b; Reichert et al. 1979). Following the oral administration of higher doses to rats (50 mg/kg ^{14}C-1,1-dichloroethene), a higher proportion of unchanged parent compound (16–30% of the administered dose) was excreted in the breath, with a concomitant reduction in the amount of expired carbon dioxide (3–6% of the administered dose) and urinary metabolites (35–42% of the administered dose) (Jones and Hathway 1978c; McKenna et al. 1978b; Reichert et al. 1979). Similar, but more marked, trends were observed at even higher doses (Chieco et al. 1981; Jones and Hathway 1978c). Thus, metabolic processes become saturated at rather low dose levels.

The elimination of orally administered 1,1-dichloroethene is triphasic according to Putcha et al. (1986); however, McKenna et al. (1978a) and Reichert et al. (1979) reported that elimination is biphasic. The first phase identified by Putcha et al. (1986) occurred almost immediately, within the first few minutes after exposure, and the subsequent two phases corresponded to those observed by the other investigators.

The amount of food ingested in the previous 24 hours slightly modifies the elimination of 1,1-dichloroethene by rats after oral administration. It was found that 19 and 29% of a 50 mg/kg dose was excreted unchanged via the lungs of nonfasted and fasted rats, respectively (McKenna et al. 1978a). This finding provides suggestive evidence that unchanged 1,1-dichloroethene may be eliminated to a greater extent from fasted rats. However, elimination of nonvolatile metabolites was slightly greater in nonfasted animals than in fasted animals, indicating a reduced capacity for metabolism in fasted rats.

At comparable doses, mice eliminate more 1,1-dichloroethene as water-soluble metabolites in the urine than rats (Jones and Hathway 1978a). These results indicate that orally administered 1,1-dichloroethene is metabolized to a greater extent in mice than rats.

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.

D'Souza and Andersen (1988) developed PBPK models for inhalation and oral exposure of rats to 1,1-dichloroethene. There is no validated model for humans. Allometric scaling was employed to estimate amounts of epoxide formed in rats and humans. Cardiac output and ventilation rates were scaled by body weight. For oral exposures at <5 mg/kg, the model estimated comparable amounts of epoxide formation for rats and humans. At inhalation exposure levels <100 ppm, estimates of epoxide formation

for humans were 5-fold higher than those in those for rats. This model is not useful for human health risk assessment due to the lack of a validated human model.

El-Masri et al. (1996a, 1996b) assessed the potential for competitive inhibition between trichloroethylene and 1,1-dichloroethene using results from gas uptake experiments in rats and physiologically based pharmacodynamic (PBPD) modeling. The model descriptions of hepatic GSH kinetics were calibrated against published data and gas uptake experiments. The model was used to identify the critical time point at which GSH is at a minimum. According to the combination of gas uptake experiments and PBPD modeling, 1,1-dichloroethene, but not trichloroethylene, was capable of significantly depleting hepatic GSH. At exposure concentrations higher than 100 ppm (but not <100 ppm), trichloroethylene obstructed the ability of 1,1-dichloroethene to deplete hepatic GSH. Thus, trichloroethylene exposure concentrations >100 ppm are predicted to competitively inhibit GSH-mediated metabolism of 1,1-dichloroethene to its epoxide. This model is not useful for human health risk assessment.

3.1.6 Animal-to-Human Extrapolations

Studies in rats and mice demonstrate rapid absorption following inhalation or oral exposure to 1,1-dichloroethene (Dallas et al. 1983; Jones and Hathway 1978a; McKenna et al. 1978a; Putcha et al. 1986). Absorbed 1,1-dichloroethene, its metabolites, and covalently bound derivatives are found in the liver and kidney (Jaeger et al. 1977; Jones and Hathway 1978c; McKenna et al. 1978a). In animals, 1,1-dichloroethene is rapidly oxidized by CYP2E1 to three initial metabolites (1,1-dichloroethene epoxide, 2-chloroacetyl chloride, and 2,2-dichloroacetaldehyde) (Jones and Hathway 1978a, 1978c; McKenna et al. 1978b; Reichert et al. 1979). The extent of similarities between animals and humans regarding metabolism of 1,1-dichloroethene is not known. However, human and rodent *in vitro* microsomal preparations form the same initial 1,1-dichloroethene metabolites, including 1,1-dichloroethene epoxide (the metabolite of major cytotoxic and mutagenic concern) (Dowsley et al. 1996, 1999).

The toxicity of 1,1-dichloroethene has been studied in acute-, intermediate-, and chronic-duration inhalation and oral studies. Critical targets of toxicity in rats and mice exposed by inhalation are nasal epithelium, liver, and kidney, as demonstrated in intermediate- and chronic-duration studies of both species (NTP 2015a). Rats appear to be more sensitive than mice to nasal and hepatic effects; mice appear to be more sensitive than rats to kidney effects. Limited data regarding species differences in

sensitivity to 1,1-dichloroethene toxicity do not indicate significant species differences in 1,1-dichloroethene toxicity following oral exposure.

Human toxicokinetic and toxicity data for 1,1-dichloroethene are lacking. Available rat and mouse data indicate significant species differences. There are insufficient data to assess which species would represent the best model for human toxicity. Therefore, for purposes of human health hazard assessment, the species exhibiting the most sensitive endpoint considered relevant to humans is considered the most conservative approach to derivation of MRLs for 1,1-dichloroethene.

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 1,1-dichloroethene are discussed in Section 5.7, Populations with Potentially High Exposures.

Specific information regarding human subpopulations, including infants and children, that are unusually susceptible to the toxic effects of 1,1-dichloroethene were not located. However, results from animal studies suggest that certain populations may exhibit increased sensitivity to 1,1-dichloroethene toxicity.

The liver mixed function oxidase (MFO) activity in fasted animals or animals kept on a low carbohydrate diet was enhanced when exposed to 1,1-dichloroethene, compared to that in similarly exposed control (carbohydrate-fed) animals (McKenna et al. 1978b; Nakajima et al. 1982). Fasting prior to 1,1-dichloro-ethene exposure resulted in an earlier appearance of hepatic lesions, a more extensive distribution of

lesions, and a reduced ability to metabolize high doses of 1,1-dichloroethene when compared to control (nonfasted) rats (Jaeger et al. 1974; McKenna et al. 1978b; Reynolds and Moslen 1977).

Sex differences in the toxic response to 1,1-dichloroethene were observed in animals. For example, in a chronic inhalation exposure study in rats, hepatotoxic effects occurred at lower 1,1-dichloroethene concentrations in female rats than in male rats (25 and 75 ppm, respectively) (Quast et al. 1986). Fasted male animals, particularly young males, appear to be more susceptible to the toxic effects of 1,1-dichloroethene than fasted females, as evidenced by their enhanced responses at lower doses of 1,1-dichloroethene.

Individuals with high levels of CYP2E1 such as abusers of ethanol and those routinely exposed to other substances that induce CYP2E1 might be at increased risk of 1,1-dichloroethene toxicity. Individuals with low levels of GSH (e.g., individuals malnourished or fasting and those taking acetaminophen) might also be at increased risk of 1,1-dichloroethene toxicity. Phenobarbital, even though somewhat protective against 1,1-dichloroethene-generated liver damage (Carlson and Fuller 1972), sensitized the heart to 1,1-dichloroethene-induced arrhythmias (Siletchnik and Carlson 1974). Since phenobarbital is sometimes used as a soporific, and by those with various forms of epilepsy or seizure disorders, people who are taking this medication or those with pre-existing arrhythmic heart conditions should not be exposed to high levels of 1,1-dichloroethene. Thyroidectomy, either chemical or surgical, can protect against the hepatotoxicity associated with inhalation of 1,1-dichloroethene. Conversely, thyroxine treatment to replace or supplement normal thyroid function increases the amount of liver damage upon subsequent exposure to 1,1-dichloroethene in animals (Szabo et al. 1977). Individuals with liver or kidney disease or those with an acute hypersensitivity to 1,1-dichloroethene should avoid exposure to 1,1-dichloroethene.

Specific data concerning teratogenicity in humans exposed to 1,1-dichloroethene were not found in the literature. 1,1-Dichloroethene has been described as a possible teratogen responsible for soft-tissue anomalies in rats and skeletal defects in mice, rats, and rabbits, often at levels that produced clear evidence of toxicity in the dam.

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, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to 1,1-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 1,1-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 1,1-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

There are limited data on biomarkers of 1,1-dichloroethene exposure. Halogenated solvents, including 1,1-dichloroethene, measured in blood reflect recent exposure (CDC 2017). Blood 1,1-dichloroethene levels were used to evaluate exposure in samples collected from National Health and Nutrition Examination Survey (NHANES) participants. Toxicokinetic studies in animals have identified 1,1-dichloroethene and a number of 1,1-dichloroethene metabolites in blood and urine and parent compound in expired air (Dallas et al. 1983; McKenna et al. 1977, 1978a). Since 1,1-dichloroethene and

its metabolites are rapidly eliminated from the body (Bruckner et al. 2010; Dallas et al. 1983; McKenna et al. 1978a), measurements of biomarker levels would only be indicative of recent exposure. Furthermore, some of the metabolites may be formed following exposure to other chlorinated substances as well.

3.3.2 Biomarkers of Effect

The liver and kidney are primary target organs for 1,1-dichloroethene exposure. Inhalation exposure to 50 ppm 1,1-dichloroethene was associated with minimal rates of DNA alkylation in liver and kidney cells of laboratory rats and mice (Reitz et al. 1980). Exposure to 1,1-dichloroethene (depending on dose and duration of exposure) increases serum levels of certain liver enzymes such as AST, ALT, and others, which is taken as an indication of liver injury. However, these effects are caused by other halogenated alkenes as well, such as vinyl chloride, and cannot be considered as a specific indicator of 1,1-dichloroethene effects.

3.4 INTERACTIONS WITH OTHER CHEMICALS

As discussed in previous sections, it is apparent that the toxicity of 1,1-dichloroethene is largely due to the formation of toxic intermediates during metabolism *in vivo*. The production and biotransformation of toxic metabolic intermediates of 1,1-dichloroethene can be greatly influenced by various metabolic inhibitors and inducers, and by the availability of precursors of compounds involved in detoxification, such as GSH.

Microsomal MFOs are a group of enzymes involved in the biotransformation and detoxication of xenobiotics such as 1,1-dichloroethene. Inhibitors of some microsomal MFOs include the compound SKF-525-A, disulfiram, and other dithiocarbamates, such as thiram and diethyldithiocarbamate. These compounds reduce the toxic effects of 1,1-dichloroethene in the liver, probably by inhibiting the enzymes responsible for the formation of reactive toxic intermediates (Masuda and Nakayama 1983; Short et al. 1977b). Pretreatment with intracellular cysteine precursor, L-2-oxothiazolidine-4-carboxylate, is also used to protect against 1,1-dichloroethene toxicity (Moslen et al. 1989a). Cysteine precursors enhance GSH levels, thus promoting detoxification of toxic 1,1-dichloroethene intermediates. Inhibitors of metabolic enzymes responsible for the breakdown of reactive 1,1-dichloroethene intermediates may also enhance the toxicity of 1,1-dichloroethene. For example, 1,1,1-trichloropropane and other inhibitors of epoxide hydrolase can potentiate the toxicity of 1,1-dichloroethene (Jaeger 1977). It should be noted, however, that substances such as 1,1,1-trichloropropene-2,3-oxide may inhibit CYP450 isozymes

involved in biotransformation and detoxification as well (Ivanetich et al. 1982). Other chemicals that reduce the activity of metabolic enzymes and show some protective effects against the toxicity of 1,1-dichloroethene include pyrazole, and 3-aminotriazol (Andersen et al. 1978).

Pretreatment of rats with acetaminophen greatly increased lethality and the hepatotoxic effects of 1,1-dichloroethene (Wright and Moore 1991). Although the depletion of GSH was not discussed, the study authors concluded that acetaminophen produces alterations that make hepatocytes more susceptible to 1,1-dichloroethene injury.

Enzyme inducers may either protect against or exacerbate the toxicity of 1,1-dichloroethene. Induction of enzymes involved in the formation of toxic intermediates potentiates 1,1-dichloroethene-induced toxicity following 1,1-dichloroethene exposure; conversely, induction of enzymes responsible for the biodegradation of the toxic intermediate(s) decreases toxicity. Examples of compounds that induce MFOs and increase toxic effects upon exposure to 1,1-dichloroethene include ethanol and acetone (Charbonneau et al. 1991; Hewitt and Plaa 1983; Kainz et al. 1993; Sato et al. 1983). In acetone-pretreated rats, mixtures containing chloroform or carbon tetrachloride plus 1,1-dichloroethene increased hepatotoxic responses additively (Charbonneau et al. 1991).

Many inducers of MFO enzymes do not increase the hepatotoxicity of 1,1-dichloroethene, apparently because they stimulate enzyme systems not involved in the metabolism of 1,1-dichloroethene. An example of a P450 inducer is phenobarbital (Carlson and Fuller 1972). Jenkins et al. (1972) found that pretreatment of rats with phenobarbital followed by oral administration with 1,1-dichloroethene had a protective effect against liver damage, while Carlson and Fuller (1972) found that pretreatment of rats with phenobarbital followed by inhalation exposure to 1,1-dichloroethene increased mortality but had no effect on hepatotoxicity. This discrepancy may be due to differences in routes of administration and indicators of toxicity examined.

Thyroidectomy protected rats from the hepatotoxic effects of 1,1-dichloroethene, probably by increasing the amount of hepatic GSH (Szabo et al. 1977). Other studies have also reported increases in hepatic GSH in thyroidectomized rats (e.g., Teare et al. 1993). Thyroxine replacement in thyroidectomized rats exacerbated the liver damage seen upon subsequent exposure to 1,1-dichloroethene (Szabo et al. 1977).

Pretreatment of animals with compounds that deplete GSH levels (such as buthionine sulfoximine) increased the amount of liver damage caused by 1,1-dichloroethene exposure (Reichert et al. 1978).

Conversely, pretreatment of animals with supplements containing high concentrations of the amino acids cysteine and/or methionine, both of which are metabolic contributors of the sulfhydryl group required for GSH biosynthesis, had a protective effect against the toxicity of 1,1-dichloroethene (Short et al. 1977a).