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

3.1   TOXICOKINETICS

Information on the toxicokinetics of 1,2-dichloroethane is available from a limited number of human studies and several animal studies:

- 1,2-Dichloroethane is well absorbed after inhalation exposure through the lungs, through the gastrointestinal tract after oral exposure, and through the skin after dermal exposure in humans. In animal studies, equilibrium blood concentrations of 1,2-dichloroethane were obtained 2– 3 hours after inhalation exposure, 15–60 minutes after oral exposure, and 1–2 hours after aqueous dermal exposure. Absorption probably occurs by passive diffusion for all three routes of exposure.

- Upon absorption, 1,2-dichloroethane is widely distributed within the body. Experiments in animals exposed orally or by inhalation showed that the highest concentrations of 1,2-dichloro-ethane (7–17 times that of the blood) were found in adipose tissue. The liver and lung were shown to have lower equilibrium levels of 1,2-dichloroethane than the blood.

- 1,2-Dichloroethane is readily metabolized in the body. The primary metabolic pathways for this chemical are oxidation by CYP and glutathione conjugation. Oxidative metabolites include 2-chloroacetaldehyde, 2-chloroethanol, and 2-chloroacetic acid. CYP metabolism of 1,2-dichloroethane appears to be saturable at oral gavage doses ~25 mg/kg and inhalation concentrations of ~150 ppm, both of which correspond to blood levels of 5–10 µg/mL. Glutathione conjugation becomes relatively more important at higher doses, and increased metabolism by this pathway may be responsible for the toxic effects noted at these high doses.

- Excretion of 1,2-dichloroethane and its metabolites is rapid; in animal studies, excretion was essentially complete 48 hours after acute-duration exposure. Following inhalation exposure to 1,2-dichloroethane, excretion of 1,2-dichloroethane was primarily in the form of metabolites (thiodiglycolic acid and thiodiglycolic acid sulfoxide) in the urine (84%), and as carbon dioxide (CO_2) in the exhaled air (7%). Following oral exposure to 1,2-dichloroethane, the largest percentage of the administered dose (29%) was excreted as unchanged 1,2-dichloroethane in the exhaled air, which may reflect metabolic saturation.

3.1.1   Absorption

1,2-Dichloroethane is readily absorbed through the lungs following inhalation exposure in both humans and experimental animals. This is expected, based on 1,2-dichloroethane's high vapor pressure of 78.9 mmHg at 20°C and high serum/air partition coefficient of 19.5 (Gargas et al. 1989). Thus, absorption occurs most likely via passive diffusion across alveolar membranes. Gargas et al. (1989) estimated a blood:air partition coefficient of 19.5±0.7 for humans, and a blood:air partition coefficient of 30.4±1.2 for F-344 rats. Nursing women exposed to 15.6 ppm of 1,2-dichloroethane in the workplace air

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(with concurrent dermal exposure) accumulated the chemical in breast milk (Urusova 1953). The concentration of the chemical in milk gradually increased, reaching the maximum level 1 hour after work ended, although the validity of the results could not be assessed because of a lack of sufficient detail in reported methods and because the sample size was not provided. EPA (1980) also found 1,2-dichloroethane in the milk of lactating women. These results indicate that 1,2-dichloroethane is absorbed through the lungs by humans and accumulates (because of its high lipid-water partition coefficient) in the breast milk of nursing women. Concurrent levels of 1,2-dichloroethane in blood were not measured (EPA 1980; Urusova 1953).

Nouchi et al. (1984) reported a fatal case of 1,2-dichloroethane poisoning in a man exposed to 1,2-dichloroethane vapors for approximately 30 minutes in an enclosed space (concentration in air not specified), providing indirect evidence that this chemical undergoes absorption from inhalation exposure in humans. However, adverse effects were seen at 20 hours post-exposure, prompting the study authors to suggest that the formation of reactive metabolites is a necessary first step in the expression of 1,2-dichloroethane-induced toxicity. An alternative explanation is that the 1,2-dichloroethane is, in part, slowly released from adipose tissue or other compartments (see Section 3.1.3).

The rapid absorption of 1,2-dichloroethane following inhalation exposure has also been demonstrated in experimental animals. Reitz et al. (1980, 1982) found that peak blood levels reached a near-steady state concentration of 8 µg/mL 1–2 hours after the onset of a 6-hour inhalation exposure to 150 ppm of 1,2-dichloroethane in rats. Similar results were obtained by Spreafico et al. (1980) at inhalation exposures of 50 ppm of 1,2-dichloroethane. However, at 250 ppm of 1,2-dichloroethane, equilibrium was not achieved until 3 hours from the start of exposure. It is likely that as the concentration of inspired 1,2-dichloroethane increases, the time required to reach an equilibrium concentration of 1,2-dichloro-ethane in the blood also increases. Repeated exposure of rats to 200 ppm 1,2-dichloroethane for 6 hours/day for 5 days (nose-only) showed similar steady-state blood concentrations within an hour of exposure and no accumulation in the blood of rats sacrificed after multiple exposures (Saghir et al. 2006). In rats that had been exposed to 1,2-dichloroethane vapor (50 ppm) intermittently for 2 years, blood levels of 1,2-dichloroethane 15 minutes after the end of a 7-hour exposure to 50 ppm were 0.26–0.28 µg/mL (Cheever et al. 1990). Blood levels were not increased, but rather only slightly reduced after an additional 2 hours, which suggests that equilibrium had been reached during the exposure period. In mice exposed to 25, 87, or 185 ppm 1,2-dichloroethane for 6 hours, maximal blood concentrations were achieved within 2 hours and remained constant until the end of the exposure period (C_{max} values of 208.06±34.79, 551.86±61.76, and 1,334.41±201.72 µg/L, respectively) (Zhong et al. 2022). 1,2-Dichloroethane was

rapidly eliminated from mouse blood with elimination half-life ($t_{1/2}$) values of 0.48±0.18, 0.43±0.012, and 0.37±0.11 hours, respectively (Zhong et al. 2022).

No studies were located regarding absorption in humans following oral exposure to 1,2-dichloroethane. However, it can be inferred from case studies, which described toxic effects (including death) after accidental (Hueper and Smith 1935) or intentional (Lochhead and Close 1951; Yodaiken and Babcock 1973) ingestion of 1,2-dichloroethane by humans. It is likely that 1,2-dichloroethane is rapidly absorbed into the systemic circulation following exposure by the oral route. 1,2-Dichloroethane is lipophilic, with a log K_{ow} of 1.48, and is expected to be absorbed largely via passive diffusion across the mucosal membranes of the gastrointestinal tract.

Studies in experimental animals indicate that the oral absorption of 1,2-dichloroethane is rapid, complete, and essentially linear (Reitz et al. 1980, 1982; Spreafico et al. 1980). Reitz et al. (1982) reported that peak blood levels of 1,2-dichloroethane were reached within 15 minutes after oral administration of 150 mg/kg by gavage in corn oil to male Osborne-Mendel rats, attesting to the rapid nature of oral absorption. These investigators reported complete recovery of orally administered radioactivity (from [^{14}C]-1,2-dichloroethane) in exhaled air, urine, and carcass, thereby demonstrating that absorption of 1,2-dichloroethane from the gastrointestinal tract of rats is virtually complete (Reitz et al. 1980). The percentage of radioactivity recovered in the feces following inhalation or oral exposure to [^{14}C]-1,2-dichloroethane was 1.7–2.1%; 7.0–7.7% of the administered dose was recovered in the expired air following exposure by either route (Reitz et al. 1980). This implies that at least 90% of the inhaled or orally administered 1,2-dichloroethane was absorbed at 150 ppm and 150 mg/kg, respectively.

Data reported by Spreafico et al. (1980) supported the observation that absorption of 1,2-dichloroethane is rapid and complete. In Sprague-Dawley rats, peak blood levels were achieved within 30–60 minutes of oral administration at doses of 25, 50, and 150 mg/kg in corn oil. One-half of the low dose was absorbed within 3.3 minutes, and one-half of the high dose was absorbed within 6.4 minutes (Spreafico et al. 1980). Peak blood levels achieved were proportional to the dose administered, thus providing evidence that 1,2-dichloroethane is absorbed by passive transport across the gastrointestinal tract. Furthermore, comparison of blood levels attained after intravenous (i.e., reflective of 100% absorption) and oral administration of 1,2-dichloroethane in rats indicates that oral absorption is 100%, if first-pass effects through the liver and lung are taken into consideration (Spreafico et al. 1980).

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The vehicle used in oral administration studies appears to play a role in the time course of absorption. Withey et al. (1983) found that 1,2-dichloroethane is absorbed more readily by the gastrointestinal tract when administered in water than in corn oil. Peak blood concentrations (C_{max}) of 1,2-dichloroethane were about 4 times higher following oral administration in water than when given in corn oil. Furthermore, the time taken to reach peak levels was approximately 3 times longer when administered in corn oil, compared to water. Similar findings were reported in rats given gavage doses of 150 mg/kg/day in corn oil (C_{max} at 30 minutes) or 43 mg/kg/day in water (C_{max} at 15 minutes) (Saghir et al. 2006). Repeated gavage exposure for 5 days did not result in accumulation in the blood for either vehicle; however, elimination from blood was faster in rats given water (within 2 hours) compared to rats given corn oil (by 8–16 hours after exposure) (Saghir et al. 2006). This may have important implications with regard to human exposure to 1,2-dichloroethane. Since animal data and the available information in humans indicate that oral absorption of 1,2-dichloroethane in aqueous solutions is rapid and complete, ingestion of water contaminated with high levels of 1,2-dichloroethane is of particular concern and could result in adverse health effects in humans. However, no unequivocal information was available concerning health effects in humans after long-term exposure to low levels of 1,2-dichloroethane in drinking water.

Urusova (1953) reported a gradual increase in the concentration of 1,2-dichloroethane in the breast milk of nursing women following both dermal and inhalation exposure to 1,2-dichloroethane at the workplace. Maximum levels were reached within 1 hour (2.8 mg/100 mL of milk) after skin contact and decreased over time. Eighteen hours later, the concentration of 1,2-dichloroethane in milk ranged between 0.195 and 0.63 mg/100 mL of milk. The findings of Urusova (1953) indicate that percutaneous absorption via contact with contaminated water or the chemical itself may be a potential route of exposure to 1,2-dichloroethane in humans. However, no details of analytical methodology were reported, and the sample size was not provided; thus, the reliability of these results cannot be assessed. A more recent study conducted by Gajjar and Kasting (2014) found that the majority of all applied doses of 1,2-dichloroethane to *in vitro* human skin evaporated from the skin's surface. Specifically, 0.21% of the lowest administered dose of 7.9 mg/cm^2 1,2-dichloroethane was absorbed by the skin, while 0.13% of the highest administered dose of 63.1 mg/cm^2 was absorbed, over the course of a 24-hour period.

Studies in animals have shown that 1,2-dichloroethane is well absorbed through the skin following dermal exposure. Male rats exposed to 2 mL of 1,2-dichloroethane under cover on a shaved area of the back had blood 1,2-dichloroethane levels of 25 µg/mL after 30 minutes (Morgan et al. 1991). After 24 hours, blood levels were 135 µg/mL, and a total of 1.08 mL had been absorbed. The continued build-up of blood levels throughout the 24-hour exposure period shows that the rate of absorption exceeded that of

distribution and elimination throughout this entire period. When the experiment was repeated using solutions of 1,2-dichloroethane in water, blood levels peaked after 1–2 hours (at concentrations of 0.35–1.4 µg/mL, depending on degree of saturation of the applied solution) and then declined to control levels within 24 hours. Analysis of the aqueous solutions remaining in the exposure chamber after 24 hours showed that they contained <1% of the initial 1,2-dichloroethane concentration. This result suggests that 1,2-dichloroethane in water was rapidly and completely absorbed from solution, thus allowing elimination processes to reduce blood concentration to control levels within the 24-hour exposure period. 1,2-Dichloroethane was among the best absorbed of the 14 volatile organic compounds (VOCs) tested in this experiment. It should be noted that some degree of uncertainty exists with results from Morgan et al. (1991), as the shaving of the animals' backs abrades the stratum corneum (Hamza et al. 2015), which in turn removes a main barrier to the percutaneous absorption of VOCs like 1,2-dichloroethane. Thus, this shaving could have affected the levels of dermal absorption of 1,2-dichloroethane in the study in a way that would not be applicable in a naturally occurring setting.

Supporting data for the time course of absorption following neat exposure were obtained by Jakobson et al. (1982), who studied the dermal absorption of 1,2-dichloroethane in anesthetized guinea pigs. Blood concentrations rose rapidly during the first half-hour after application, followed by steadily increasing blood levels throughout the 12-hour exposure period. Tsuruta (1975) estimated the rate of percutaneous absorption of 1,2-dichloroethane. After a 15-minute exposure, the absorption rate through the abdominal skin of mice was 480 nmol/minute/cm^2. In contrast to the results of Morgan et al. (1991), comparisons of this absorption rate with those of other chlorinated hydrocarbons tested in the same study did not support the conclusion that 1,2-dichloroethane is among the more rapidly absorbed of these chemicals.

In an *in vitro* study of dermal absorption and lag time using hairless guinea pig skin carried out by two separate laboratories, Frasch et al. (2007) estimated mean steady-state fluxes (J_{ss}) of neat 1,2-dichloroethane of 6,280 and 3,842 µg/cm^2-hour by each laboratory, respectively. Compared with neat fluxes measured in the same laboratory, flux from saturated aqueous solution was slightly lower. An *in vitro* study using static diffusion cells and pig skin reported a J_{ss} value of 1,360 µg/cm^2-hour and an apparent partition coefficient (K_p) of 1.9x10^{-3} cm/hour (Schenk et al. 2018).

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3.1.2 Distribution

1,2-Dichloroethane was detected in the breath (14.3 ppm) and breast milk (0.54–0.64 mg % [per 100 mL]) of nursing mothers 1 hour after leaving factory premises containing 15.6 ppm 1,2-dichloro-ethane in the air (Urusova 1953). This observation suggests a possible rapid distribution of 1,2-dichloro-ethane in humans following inhalation exposure, although these workers could have been exposed to the chemical over a number of days prior to and up to this observation.

The distribution of 1,2-dichloroethane in rats following a 6-hour inhalation exposure to 50 or 250 ppm occurred readily throughout body tissues; levels achieved in tissues were dose-dependent (Spreafico et al. 1980). The investigators measured 1,2-dichloroethane in blood, liver, lung, and fat, and found that blood and tissue levels reached equilibrium by 2 hours after exposure to 50 ppm and 3 hours after exposure to 250 ppm. Concentrations of 1,2-dichloroethane in liver and lung were lower than those in blood. The highest concentration of 1,2-dichloroethane was found in fat (8–9 times that seen in blood). A similar study exposed male rats to 160 ppm of 1,2-dichloroethane vapor for 6 hours and also found that the highest concentration of the chemical was distributed in abdominal fat (Take et al. 2013). 1,2-Dichloro-ethane was found in maternal blood (83.6±20.2 mg %), placental tissue (43.0±9.6 mg %), amniotic fluid (55.5±11.1 mg %), and fetal tissue (50.6±11.5 mg %) after inhalation exposure of female rats to 247±10 ppm 1,2-dichloroethane during pregnancy (Vozovaya 1977), but the reliability of the data is unclear. The geometric mean concentration of 1,2-dichloroethane in maternal blood and in fetuses of rats that inhaled 150–2,000 ppm for 5 hours increased linearly with increasing exposure level (Withey and Karpinski 1985), indicating transplacental distribution of 1,2-dichloroethane. The slope and intercept of the relation between fetal concentration of 1,2-dichloroethane ($\mu g/g$) and exposure level were 0.035 and -3.95, respectively, and for concentration in maternal blood ($\mu g/g$), they were 0.092 and -10.4, respectively. However, details of the methods used to detect 1,2-dichloroethane and quantify its concentration in tissues were not provided in Withey and Karpinski (1985), so the validity of the results cannot be confirmed.

No studies were located regarding distribution in humans after oral exposure to 1,2-dichloroethane. However, the wide variety of effects noted in humans following oral exposure suggest a wide distribution.

1,2-Dichloroethane was distributed readily throughout the body following oral administration of single doses to rats (Spreafico et al. 1980). As was seen following inhalation exposure, peak tissue levels were dose dependent. Spreafico et al. (1980) reported that 1,2-dichloroethane absorbed through the

gastrointestinal tract reached peak concentrations in the liver within 10 minutes. Again, equilibrium levels in liver and lung (achieved by 2 hours post-exposure) were lower than in blood, while levels in fat were 7–17 times greater than in blood. This difference in tissue levels decreased with increasing dose. Thus, there appears to be little difference between oral and inhalation exposure with regard to tissue distribution in animals, and specific target organ toxicity cannot be explained by differential distribution of 1,2-dichloroethane.

Payan et al. (1995) evaluated [^{14}C]-1,2-dichloroethane distribution in maternal rats following a single bolus oral dose of approximately 160 mg/kg on GD 12. At 1 hour after exposure, 50% of the orally administered dose was in gastrointestinal tract tissues, falling to 0.2% of the administered dose by 48 hours after exposure, while <1% was accounted for in the feces. Aside from the absorptive tissues, the liver and kidney accounted for most of the distributed radioactivity throughout the 48-hour post-exposure observation period, although adipose tissue and brain and spinal cord tissues, possible sites of accumulation, were not included in the evaluation. The highest tissue concentrations were found in the liver, ovary, and kidney. Transplacental distribution of radiocarbon was demonstrated by the presence of radioactivity in the developing conceptus at 1-hour post-exposure, with the highest amount in the conceptus (0.057% of administered dose) occurring at approximately 4 hours post-exposure. At 48 hours post-exposure, most of the residual radioactivity was located in the liver (0.215% of administered dose). When 160 mg/kg was administered on GD 18, the pattern of distribution was similar, except that greater accumulation occurred in the developing fetus and placenta. At 48 hours post-exposure (the 20th day of gestation), most of the residual radioactivity burden was located in the fetus (0.167% of administered dose) and the liver (0.156% of administered dose).

Spreafico et al. (1980) studied the distribution of 1,2-dichloroethane in rats following repeated oral administration (11 daily doses). They demonstrated that there was no difference between blood or tissue levels following either single or repeated exposure. This finding suggests that bioaccumulation of 1,2-dichloroethane does not occur with repeated oral exposure.

1,2-Dichloroethane was detected in the breast milk of nursing mothers following dermal exposure (with probable concurrent inhalation exposure) to liquid 1,2-dichloroethane at the workplace (Urusova 1953). The concentration in milk gradually increased, with the maximum level (2.8 mg %) reached 1 hour after work ended. Eighteen hours later, the levels in milk ranged from 0.195 to 0.63 mg %. This study did not report the dermal exposure concentration of 1,2-dichloroethane. Because of the lack of details on methodology, the validity of these findings cannot be assessed.

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No studies regarding distribution in animals following dermal exposure to 1,2-dichloroethane were located. Since the tissue distribution of this chemical did not appear to be route-dependent after either inhalation or oral exposure, and since it is well absorbed through the skin, the distribution pattern of 1,2-dichloroethane following percutaneous application may possibly resemble that observed following exposure via other routes.

No studies were located regarding distribution in humans after parenteral exposure to 1,2-dichloroethane.

Mice exposed to radiolabeled 1,2-dichloroethane by a single intravenous injection had high levels of tightly bound radioactivity in the nasal mucosa and tracheobronchial epithelium within 1 minute of exposure; these levels persisted throughout the 4-day observation period (Brittebo et al. 1989). Lower levels of radioactivity were bound to epithelia of the upper alimentary tract, eyelid, and vagina, as well as the liver, kidney, adrenal cortex, and submaxillary gland. The bound radioactivity was considered to represent nonvolatile reactive metabolites formed in the tissues where it was found. A study of tissue kinetics of 1,2-dichloroethane in rats after a single intravenous dose of 15 mg/kg reported preferential initial distribution to fat (Withey and Collins 1980) and first-order elimination from each tissue studied (except blood). The estimated initial concentration in fat was 36.9 µg/g, while for other soft tissues (including heart, lung, liver, spleen, kidney, and brain), the initial concentrations were relatively uniform, with estimates ranging from 4.2 to 9.2 µg/g. The study also showed that distributed 1,2-dichloroethane remained in fat longer than in other soft tissues, as indicated by a lower estimated elimination coefficient in fat (0.0088 minute^{-1}) relative to other tissues (ranged from 0.0226 to 0.0514 minute^{-1}).

In vitro tissue:air partition coefficients were measured using rat tissue homogenates and the vial equilibration method (Saghir et al. 2006). Mean tissue:air partition coefficients in the brain, kidney, testis, and ovary were 39.50 ± 2.89, 44.89 ± 6.77, 31.14 ± 7.98, and 74.59 ± 9.82, respectively.
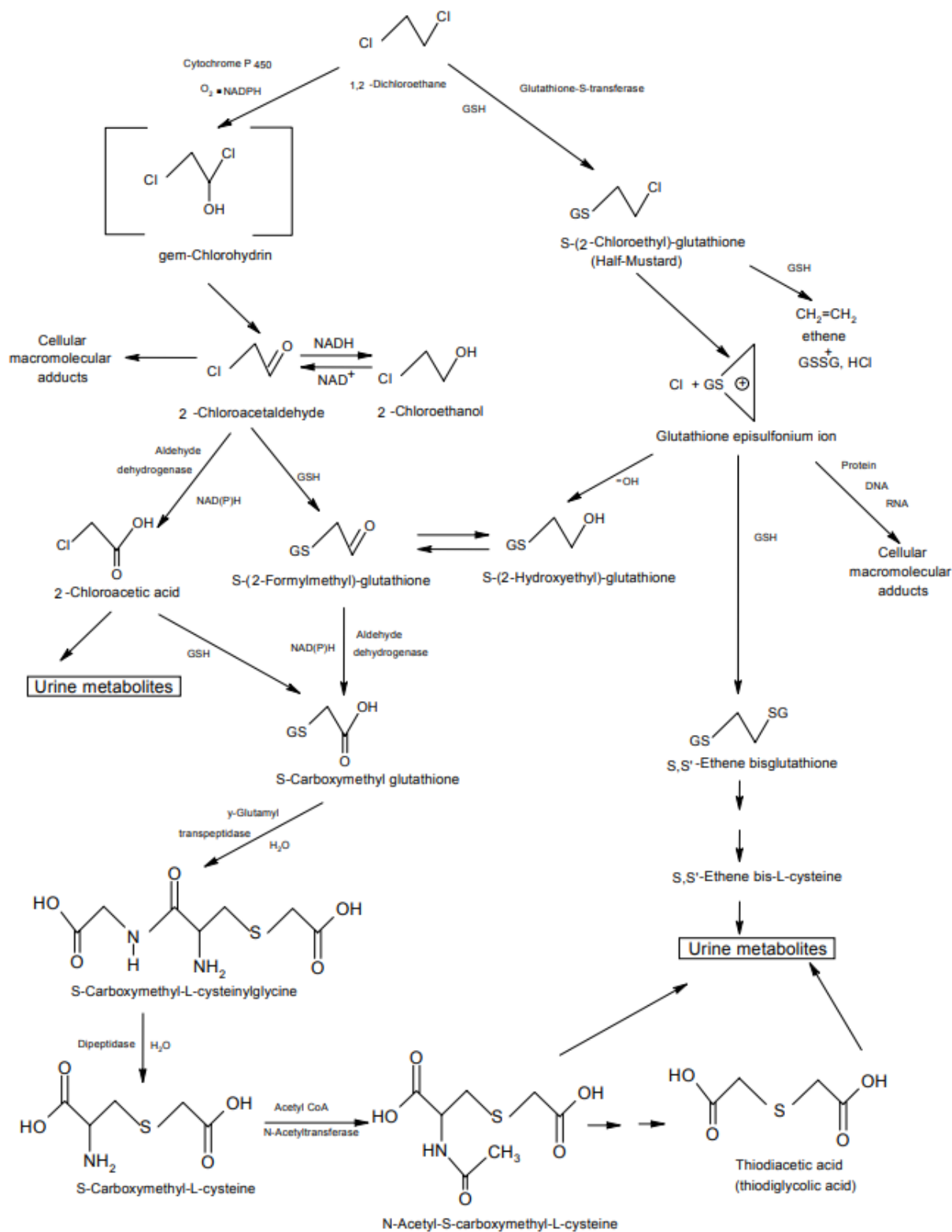
3.1.3 Metabolism

No studies regarding metabolism in humans following inhalation, oral, or dermal exposure to
1,2-dichloroethane were located. The biotransformation of 1,2-dichloroethane has been studied
extensively in rats and mice both *in vivo* and *in vitro*. Proposed metabolic pathways for 1,2-dichloro-
ethane are shown in Figure 3-1. The results of the *in vivo* studies indicate that 1,2-dichloroethane is
readily metabolized in the body, the primary route of biotransformation involves conjugation with
glutathione to yield nonvolatile urinary metabolites, and the enzymes involved in the biotransformation of
1,2-dichloroethane are saturable at approximately 25 mg/kg/day (gavage) and 150 ppm (inhalation) in rats
(D'Souza et al. 1988; Reitz et al. 1982; Spreafico et al. 1980). Metabolic saturation appears to occur at
lower concentrations after gavage administration than after inhalation exposure. A physiological
pharmacokinetic model explains the route-of-exposure difference in quantifying the amount of
1,2-dichloroethane-glutathione conjugate produced in target organs after oral and inhalation exposures
(D'Souza et al. 1987, 1988).

Zeng et al. (2019) attributed hepatic apoptosis to down-regulation of an anti-apoptosis insulin growth
factor *in vitro*. The researchers hypothesized this was due to 2-chloroacetaldehyde, an oxidative
metabolite of 1,2-dichloroethane. 2-Chloroacetaldehyde is a very potent mutagen *in vitro* (McCann et al.
1975). Several researchers have also presented *in vitro* evidence that 1,2-dichloroethane is activated to a
mutagen by glutathione conjugation (Rannug et al. 1978; van Bladeren et al. 1979). Electrophilic
episulfonium ions formed via the glutathione pathway are believed to bind to DNA and cause genetic
damage (Guengerich et al. 1987). Kramer et al. (1987) described the role of glutathione-generated
episulfonium ions in 1,2-dichloroethane-induced nephrotoxicity in rats. Results of relatively recent
research indicate that oxidative metabolites of 1,2-dichloroethane are also responsible for kidney injury.

Reitz et al. (1982) studied the metabolism of 1,2-dichloroethane in male rats following a 6-hour exposure
to 150 ppm of [^{14}C]-1,2-dichloroethane. The exact metabolic pathways were not determined, but an
observed depression of hepatic nonprotein sulfhydryl groups may indicate that glutathione plays a major
role in the metabolism of 1,2-dichloroethane following inhalation exposure. Saturation of
biotransformation enzymes was not apparent at this dose since 84% of the administered ^{14}C was
recovered as urinary metabolites and only 2% of the administered ^{14}C was recovered as parent compound
in the expired air. However, the data of Spreafico et al. (1980) suggest that saturation does occur after
inhalation exposure in rats, since peak blood levels of 1,2-dichloroethane rose 22-fold when the exposure
concentration was increased from 50 to 250 ppm. Based on the data of these two groups of investigators,

Figure 3-1. Proposed Pathways for 1,2-Dichloroethane Metabolism

Source: NTP (1991)

it appears that saturation of 1,2-dichloroethane metabolism occurs when blood levels reach 5–10 µg/mL blood or after exposure to 150–250 ppm 1,2-dichloroethane. When blood concentrations of 1,2-dichloroethane exceed these levels (i.e., at exposure concentrations >150 ppm), manifestations of toxicity became more apparent. For example, Maltoni et al. (1980) reported that most of the toxicity associated with inhalation exposure to 250 ppm 1,2-dichloroethane in rats and mice was alleviated when exposure levels were reduced to 150 ppm, and no treatment-related effects were noted at 50 ppm. These findings suggest that 1,2-dichloroethane-induced toxicity occurs once a threshold blood level has been exceeded.

Reitz et al. (1982) also studied the metabolism of 1,2-dichloroethane following the administration of single oral doses of 150 mg/kg [^{14}C]-1,2-dichloroethane. Again, the exact metabolic pathways were not determined, but the observation that hepatic nonprotein sulfhydryl groups were depressed indicated that glutathione may also play a major role in the metabolism of 1,2-dichloroethane following oral exposure. Saturation of biotransformation enzymes was apparent at this dose since only 60% of the administered radiolabel was recovered as urinary metabolites, and 29% of the administered radiolabel was associated with unchanged parent compound in the expired air. As with inhalation, it appeared that saturation of 1,2-dichloroethane metabolism occurred when blood levels reached 5–10 µg/mL blood or after administration of ~25 mg/kg 1,2-dichloroethane (D'Souza et al.1988; Reitz et al. 1982; Spreafico et al. 1980). This blood threshold level again correlated with observed toxicity in animal studies (NCI 1978), as discussed above.

Although the saturable pathways appear to be the same for both oral and inhalation exposure, oral administration of 1,2-dichloroethane by gavage results in saturation at lower administered doses than inhalation exposure. Reitz et al. (1982) demonstrated that administration of 150 mg/kg 1,2-dichloro-ethane by gavage resulted in a 1.3-fold higher absolute dose to the animals than 150 ppm via inhalation (which is approximately equal to 502 mg/kg). Gavage administration produced approximately twice as much total metabolite as inhalation, and peak levels of 1,2-dichloroethane in blood were almost 5 times higher following gavage versus inhalation. Gavage administration does not represent typical oral exposure in humans. Gavage administration results in large bolus doses absorbed at one time thereby leading to spikes in blood levels and a more pronounced expression of toxicity. Oral exposure to 1,2-dichloroethane by humans will most likely occur via ingestion of contaminated drinking water in small doses spread out over the course of a day. In such instances, biotransformation processes will probably not become saturated; thus, the risk for adverse effects is not as high as would be predicted from gavage administration of equivalent doses.

In female albino mice given 1,2-dichloroethane intraperitoneally, the metabolism of 1,2-dichloroethane appeared to be initiated by hydrolytic dehalogenation followed by reduction to yield 2-chloroethanol (Yllner 1971). This was then converted to 2-chloroacetic acid by microsomal oxidation. Final metabolites identified in the urine of these animals in percent radioactivity recovered included *S*-carboxymethyl-L-cysteine (44–46% free; 0.5–5% conjugated), thiodiacetic acid (33–34%), *S,S'*-ethylene-*bis*-cysteine (1.0%), which are indicative of glutathione conjugation, in addition to chloroacetic acid (6–23%) and 2-chloroethanol (0–0.8%) (see Figure 3-1).

The pathways of 1,2-dichloroethane metabolism have been elucidated primarily by *in vitro* studies in isolated rat hepatic microsomes.

In one *in vitro* study, 1,2-dichloroethane was metabolized mainly to 2-chloroacetaldehyde by hepatic nuclear CYP (Casciola and Ivanetich 1984). Guengerich et al. (1980) proposed a pathway involving microsomal CYP (in the presence of oxygen and NADPH) and mixed function oxidase (MFO) to explain the production of 2-chloroacetaldehyde. 1,2-Dichloroethane undergoes oxygen insertion to yield an unstable chlorohydrin, which spontaneously dechlorinates to form 2-chloroacetaldehyde that can react with macromolecules. 2-Chloroacetaldehyde can also be reduced to chloroethanol or be further oxidized to chloroacetic acid. Guengerich et al. (1991) demonstrated that CYP2E1 is the primary oxidation catalyst of 1,2-dichloroethane in humans.

Conjugation of 1,2-dichloroethane with glutathione is proposed to be a major metabolic pathway *in vivo* (Yllner 1971); this has been confirmed by the *in vitro* studies of Livesey and Anders (1979), Anders and Livesey (1980), and Jean and Reed (1989). This pathway is outlined on the right side of Figure 3-1. The depletion of hepatic glutathione by 1,2-dichloroethane has been demonstrated *in vitro* (Albano et al. 1984) and *in vivo* in rats exposed by inhalation or gavage (Saghir et al. 2006). Johnson (1967) demonstrated that, *in vitro*, conjugation of 2-chloroacetic acid with glutathione also proceeded by a nonenzymatic process, yielding S-carboxymethylglutathione. This compound subsequently degraded to yield glycine, glutamic acid, and S-carboxymethylcysteine. S-Carboxymethylcysteine may then be further oxidized to thiodiglycolic acid. Both S-carboxymethylcysteine and thiodiglycolic acid were found as urinary metabolites in rats and mice given 1,2-dichloroethane *in vivo* (Spreafico et al. 1980; Yllner 1971). This scheme is also supported by studies with 1,2-dibromoethane (Nachtomi et al. 1966; van Bladeren 1983).

3.1.4   Excretion

Women inhaling approximately 15.6 ppm 1,2-dichloroethane present in the workplace air eliminated the compound unchanged in the expired air. Similar observations were also reported in women exposed via dermal contact to liquid 1,2-dichloroethane. In both cases, the amount of 1,2-dichloroethane in the expired air was greater immediately following exposure and decreased gradually with time (Urusova 1953).

Elimination of 1,2-dichloroethane following inhalation exposure in rats occurred primarily via the excretion of soluble metabolites and unchanged parent compound in the urine and carbon dioxide in the expired air (Reitz et al. 1982; Spreafico et al. 1980). Urinary metabolites accounted for 84% of the absorbed dose, unchanged fecal 1,2-dichloroethane accounted for 2%, and carbon dioxide accounted for 7% of the absorbed dose following the inhalation of 150 ppm by rats (Reitz et al. 1982). The primary urinary metabolites identified in rats following inhalation exposure were thiodiacetic acid (70%) and thiodiacetic acid sulfoxide (26–28%). The rapidity of elimination is demonstrated by the fact that a few hours after exposure, 1,2-dichloroethane was not detected in blood and was detected only to a small extent 48 hours after exposure in various tissues (liver, kidney, lung, spleen, forestomach, stomach, carcass) (Reitz et al. 1982). Rapid elimination was also observed in mice exposed to 25, 87, or 185 ppm 1,2-dichloroethane for 6 hours, with clearance from the blood being near complete within an hour after exposure (Liang et al. 2021; Zhong et al. 2022). A concentration-dependent increase in urinary 2-chloroacetic acid levels was observed in mice following 28 days of exposure to 25, 87, or 185 ppm 1,2-dichloroethane for 6 hours/day, 5 days/week (approximately 300, 1,000, and 1,300 µg/L, respectively) (Liang et al. 2021).

Spreafico et al. (1980) studied the kinetics of 1,2-dichloroethane excretion in rats following inhalation exposure of 50 or 250 ppm 1,2-dichloroethane for 5 hours. They determined that elimination was monophasic with the half-times of 12.7 and 22 minutes at 50 and 250 ppm exposure, respectively. The disappearance of 1,2-dichloroethane was dose-dependent since the percentage of parent compound recovered in the expired air increased exponentially with dose. This was presumably a reflection of the saturable metabolic processes. Spreafico et al. (1980) also determined that elimination of 1,2-dichloro-ethane from adipose tissue was slower than elimination of 1,2-dichloroethane from the blood, liver, and lung.

No studies were located regarding excretion in humans after oral exposure to 1,2-dichloroethane.

Elimination of 1,2-dichloroethane following oral administration in rats was also rapid and occurred primarily via excretion of soluble metabolites in the urine, and unchanged parent compound and carbon dioxide in the expired air (Mitoma et al. 1985; Payan et al. 1993; Reitz et al. 1982; Spreafico et al. 1980). Reitz et al. (1982) conducted a complete ^{14}C-balance study in male Osborne-Mendel rats and found that urinary metabolites accounted for 60% of the radioactivity administered as a single oral dose of 150 mg ^{14}C-1,2-dichloroethane/kg body weight. Unchanged 1,2-dichloroethane in the breath accounted for 29% and carbon dioxide in the breath accounted for 5% of the administered radioactivity. The remaining 6% of the administered radioactivity was recovered in the carcass, feces, and cage washes. The primary urinary metabolites identified were the same as those seen following inhalation exposure: thiodiacetic acid (70%) and thiodiacetic acid sulfoxide (26–28%). Elimination of 1,2-dichloroethane was 96% complete within 48 hours. The results were similar in rats given a single gavage dose of 150 mg/kg following 2 years of intermittent inhalation exposure to 50 ppm of 1,2-dichloroethane (Cheever et al. 1990).

Mitoma et al. (1985) studied the elimination of single gavage doses of ^{14}C-labeled 1,2-dichloroethane from rats and mice (doses of 100 and 150 mg/kg, respectively, in corn oil) after pretreatment with unlabeled compound 5 days/week for 4 weeks. At 48 hours after administration of the radiolabeled compound, expired volatile metabolites, CO_2, excreta (feces and urine), and the carcass accounted for approximately 11.5, 8.2, 69.5, and 7% of administered radioactivity in rats, respectively, and 7.7, 18.2, 81.9, and 2.4% of the administered dose in mice, respectively.

Spreafico et al. (1980) studied the kinetics of 1,2-dichloroethane excretion in rats following the oral administration of 50 mg/kg 1,2-dichloroethane (in corn oil) and found that kinetics were best described by a two-compartment model. Withey et al. (1983) reported that administration in water resulted in a shorter elimination half-time than administration in vegetable oil. Reitz et al. (1982) also reported a two-compartment model of elimination following the gavage administration of 150 mg/kg 1,2-dichloroethane. The initial elimination phase had a half-life of 90 minutes, but elimination became more rapid when blood levels fell to 5–10 µg/mL, characterized by a half-life of approximately 20–30 minutes. This is in contrast, however, to what was observed following inhalation exposure. Spreafico et al. (1980) suggested that the oral profile represented both an absorption-distribution phase and an elimination phase, whereas the inhalation profile reflected only elimination. This elimination of 1,2-dichloroethane was also dose-dependent following oral administration in rats, as the percentage of parent compound recovered in the expired air increased exponentially with dose. Again, this is a

reflection of saturable metabolic processes. The rate of elimination from adipose tissue was similar to that from blood and other tissues, in contrast to the results for inhalation exposure.

These results indicate that 1,2-dichloroethane will most likely not accumulate in nonlipid components of the human body following repeated exposure by any route, as elimination of the compound is rapid and complete. Available data also suggest that 1,2-dichloroethane is not particularly persistent in adipose tissue following oral exposure (Spreafico et al. 1980), but it may accumulate to some extent in adipose tissue after inhalation exposure (Spreafico et al. 1980). In the past, 1,2-dichloroethane was detected in human milk of nursing women (Urusova 1953), but more recent data showing 1,2-dichloroethane in breast milk were not located. Historic data likely reflect exposures from former use patterns that are no longer relevant today.

1,2-Dichloroethane was eliminated unchanged in the expired air following dermal exposure of nursing mothers to liquid 1,2-dichloroethane in the workplace (Urusova 1953). The amount of 1,2-dichloroethane in the expired air was greatest immediately after skin contact and gradually decreased with time.

No studies were located regarding excretion in animals after dermal exposure to 1,2-dichloroethane.

Studies conducted in animals in which 1,2-dichloroethane was administered via other routes (e.g., intraperitoneal or intravenous) support the findings of the studies discussed above; excretion of 1,2-dichloroethane via urine and expired air was rapid and complete, and the route of excretion as well as the form of the chemical excreted were dose-dependent (Spreafico et al. 1980; Yllner 1971).

Estimates of an elimination constant (ke) for 1,2-dichloroethane were similar between two- and three-compartment pharmacokinetic models fitted to a time-series of blood concentration data that were obtained from rats given single intravenous doses (Withey and Collins 1980). The ke values for elimination from blood were roughly inversely related to dose; mean values of 0.143, 0.122, 0.091, 0.096, or 0.097 were obtained at dose levels of 3, 6, 9, 12, or 15 mg/kg, respectively.

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.

Two PBPK models have been developed to describe the amount of 1,2-dichloroethane and its metabolites that reach the blood and target tissues following different exposure routes in rats (D'Souza et al. 1987, 1988; Sweeney et al. 2008). The Sweeney et al. (2008) PBPK model was developed as an extension and refinement of the previously developed D'Souza et al. (1987, 1988) model.

The D'Souza et al. (1987, 1988) model simulates the metabolism and distribution of 1,2-dichloroethane in rats using five compartments: lung, liver, richly perfused tissues (such as kidney and spleen), slowly perfused tissues (such as muscle and skin), and fat. The model assumes that metabolism of 1,2-dichloro-ethane in the body only occurs in the lung and the liver and is designed to account for exposure by the inhalation and ingestion routes. 1,2-dichloroethane is metabolized by both a saturable oxidation pathway and direct conjugation with glutathione. The model predicts that inhalation exposures to 1,2-dichloro-ethane produce less glutathione-conjugate metabolites in the liver and lung of rats than equivalent oral exposures. The model was validated experimentally for both rats and mice.

Sweeney et al. (2008) used the D'Souza et al. (1987, 1988) model as the basis for developing an updated PBPK model that reflected advances in knowledge of 1,2-dichloroethane metabolism since the first model was developed. This updated model had a revised oral absorption rate, a revised constant for the time delay for resynthesis of glutathione following depletion and included a revision to the levels of glutathione in the lungs versus the liver. The updated model also included two new gastrointestinal compartments, as well as a separate compartment for the kidney, which was previously grouped with the richly perfused tissues. The model also added an additional metabolism pathway through unspecified extrahepatic enzymes. The predictions from this updated model were then compared with 1,2-dichloroethane kinetics study results from a multitude of studies with varying routes of exposure: intravenous dosing, closed chamber inhalation, open chamber inhalation, gavage in water, and gavage in oil. The model performed well for single or repeated exposure to the chemical for each of these routes of exposure in four strains of rats. The Sweeney et al. (2008) model was used in Sweeney and Gargas (2016) to extrapolate the oral NOAEL and LOAEL of existing health effect studies in rats to the

inhalation route. However, it is unclear how well the Sweeney et al. (2008) model would perform in extrapolating doses between species, such as between rats and humans.

3.1.6 Animal-to-Human Extrapolations

The metabolism of 1,2-dichloroethane has not been studied in humans. The lack of this information precludes a non-speculative attempt to discuss potential interspecies differences or similarities in the toxicity of 1,2-dichloroethane, as well as a determination of which animal species is the most appropriate model for humans. Extrapolations of 1,2-dichloroethane oral toxicity data from animals to humans should consider the type of exposure because some of the differences in toxic and carcinogenic responses in animal studies can be explained on the basis of saturation of the detoxification/excretion mechanism due to bolus (gavage) administration. Frasch et al. (2007), however, did provide evidence that the use of hairless guinea pig skin was a strong model for 1,2-dichloroethane dermal permeability in humans, as no significant differences were found between human and hairless guinea pig skin in permeability coefficients or lag times.

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

3.2.1 Children's Susceptibility

Data on the health effects of 1,2-dichloroethane exposure in children are limited to a single case report of a 14-year-old boy who swallowed 15 mL of the compound (Yodaiken and Babcock 1973). The most immediate signs of toxicity were headache and staggering gait within 2 hours of exposure, followed soon after by lethargy and vomiting. During the next few days, the boy developed symptoms of toxicity, increasing in variety and severity, that involved several organ systems, including adverse hematological effects, pulmonary edema, cardiac arrest (he was resuscitated), and eventual death on the 5th day after exposure from massive hepatic necrosis and renal tubular necrosis. Data from this case report and from reports of adult humans who died following acute-duration exposure to high levels by inhalation or ingestion are consistent with animal studies indicating that the main targets of acute toxicity include the central nervous system, respiratory tract, stomach, liver, and kidneys. Considering the consistency of effects in acutely exposed humans and animals, and data showing that the liver, kidney, and immune system are sensitive targets of lower-dose and longer-term inhalation and oral exposures in animals, it is reasonable to assume that effects in these tissues would also be seen in similarly exposed adults and children.

No studies that provide reliable information on adverse developmental effects in humans exposed to 1,2-dichloroethane are available. A cross-sectional epidemiologic study that investigated whether elevated levels of routinely sampled organic contaminants in New Jersey public water systems, including 1,2-dichloroethane, were associated with increased prevalence of adverse birth outcomes (Bove 1996; Bove et al. 1995) was located. A number of associations between various chemicals and birth outcomes were found, including a positive association between ingestion of 1,2-dichloroethane in drinking water and major cardiac birth defects; however, the mixed chemical exposures indicate that the results are only suggestive, do not establish a cause-and-effect relationship, and should be interpreted with caution.

Studies in rats, mice, and rabbits indicate that 1,2-dichloroethane is not developmentally toxic following inhalation or oral gestational exposure, although indications of embryo lethality at maternally toxic doses have been reported (Kavlock et al. 1979; Lane et al. 1982; Payan et al. 1995; Rao et al. 1980). While Kavlock et al. 1979 reported no developmental effects, limitations of the study include treatment using municipal water sources and a mixture of volatile components, so the concentration and relative contribution of 1,2-dichloroethane is unclear.

3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

Evidence from mouse studies suggests that the specific nature of oral exposure may play a role in the degree of immunotoxicity expressed in young animals. Bolus doses of 1,2-dichloroethane appear to be more effective in eliciting an immunotoxic response than drinking-water exposures in 5-week-old mice. There was a significant, dose-related reduction in IgM response to sheep erythrocytes, and a significant, but not dose-related, reduction in delayed-type hypersensitivity response to sheep erythrocytes in 5-week-old CD-1 mice exposed for 14 days by gavage to 4.9 and 49 mg/kg/day (Munson et al. 1982). In mice provided 49 mg/kg/day, these effects were accompanied by a 30% decrease in total leukocyte number. In contrast, mice given drinking water containing 189 mg/kg/day of 1,2-dichloroethane for 90 days beginning at 5 weeks of age displayed no treatment-related effects on either the antibody-forming cell response or the delayed-type hypersensitivity response after immunization with sheep erythrocyte antigens (Munson et al. 1982). The fact that the animal evidence for oral immunotoxicity of 1,2-dichloroethane includes decreased immune responses in 5-week-old mice provides a limited indication of the potential susceptibility of children to immunotoxic effects, particularly after bolus ingestion by children, that could occur, for example, with accidental ingestion of older household products that contain 1,2-dichloroethane.

Young mice were also susceptible to reduced immune function after brief inhalation exposure to 1,2-dichloroethane. A single 3-hour exposure to 5–11 ppm of 1,2-dichloroethane induced increased susceptibility to *S. zooepidemicus* (i.e., increased mortality following infection) in 4–5-week-old female mice, suggesting reduced pulmonary immunological defenses in the exposed mice (Sherwood et al. 1987). No immunological effects were observed at 2.3 ppm. Young female mice exposed to 11 ppm also had reduced bactericidal activity in the lungs 3 hours after inhalation challenge with *K. pneumoniae*. In contrast, young male rats (ages ranging from 4 to 5 weeks) that were exposed once to 200 ppm for 5 hours or 100 ppm 5 hours/day for 12 days did not exhibit any increased susceptibility to infection from these microbes, suggesting that rats may be less susceptible to the detrimental immunological effects of 1,2-dichloroethane than mice and/or that male rodents are less susceptible than females (Sherwood et al. 1987). The relevance of the young mouse inhalation data to child susceptibility is unknown, particularly in the light of the observed interspecies differences. However, the data do suggest that it would be prudent to prevent 1,2-dichloroethane inhalation exposures in children.

No studies that evaluated for the distribution of 1,2-dichloroethane or its metabolites across the placenta in humans were located. However, there is some evidence that 1,2-dichloroethane and/or its metabolites cross the placenta after inhalation and oral exposures in animals. 1,2-Dichloroethane was found in maternal blood (83.6±20.2 mg %) [per 100 mL], placental tissue (43.0±9.6 mg % [per 100 mg]), amniotic

3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

fluid (55.5±11.1 mg % [per 100 mL]), and fetal tissue (50.6±11.5 mg % [per 100 mg]) after inhalation exposure of female rats to 247±10 ppm 1,2-dichloroethane during pregnancy (Vozovaya 1977). Additional evidence of transplacental distribution of 1,2-dichloroethane after inhalation exposure is provided by Withey and Karpinski (1985), who found that the geometric mean concentration of 1,2-dichloroethane in the fetuses of rats that inhaled 150–2,000 ppm for 5 hours increased linearly with increasing exposure level. However, the reliability of the Vozovaya (1977) data is unclear, and the methods for evaluating 1,2-dichloroethane tissue concentrations were not reported in Withey and Karpinski (1985).

There is clearer evidence for transplacental distribution of 1,2-dichloroethane and/or its metabolites after maternal oral exposure. Payan et al. (1995) evaluated [^{14}C]-1,2-dichloroethane distribution in maternal rats following a single oral bolus dose of approximately 160 mg/kg on GD 12 or 18. In both cases, transplacental distribution of radiocarbon was demonstrated by the presence of radioactivity in the developing conceptus. A greater accumulation occurred in the developing fetus and placenta 48 hours after the GD 18 administration than after the GD 12 administration. At 48 hours after the GD 18 dosing, the majority of residual radioactivity burden was located in the fetus (0.167% of administered dose) and the liver (0.156% of administered dose).

No studies regarding 1,2-dichloroethane metabolism in children were located. The metabolism of 1,2-dichloroethane is well described (see Figure 3-1), and it is reasonable to assume that the metabolic pathways are, for the most part, the same between adults and children. However, the expression of certain enzymes is known to be developmentally regulated, and one of these enzymes may be involved in 1,2-dichloroethane metabolism. NAT is involved in 1,2-dichloroethane metabolism at a step subsequent to glutathione conjugation (see Figure 3-1). NAT performs the N-acetylation of S-carboxymethyl-L-cysteine to N-acetyl-S-carboxymethyl-L-cysteine, a major urinary metabolite. There are, however, two NATs (NAT1 and NAT2) that are expressed in humans with separate but overlapping substrate specificities (Parkinson 1996). NAT2 is apparently expressed only in the liver and the gut (Parkinson 1996) and is known to be developmentally regulated (Leeder and Kearns 1997). Some NAT2 activity is present in the fetus at 16 weeks, but NAT2 activity is low in virtually 100% of infants, not reaching adult activity levels until 1–3 years of age (Leeder and Kearns 1997). It is not clear in NTP (1991) whether the NAT involved in 1,2-dichloroethane metabolism is NAT1 or NAT2, although both enzymes N-acetylate some xenobiotics equally well (Parkinson 1996). Additionally, CYP2E1 levels in human infants steadily increase from infancy to adulthood, where fetal samples were found to have undetectable levels of CYP2E1, infants 1–3 months of age exhibited mean levels of the enzyme of about 10% of adult values,

infants 3–12 months of age exhibited mean values of about 30% of adult values, and children between
1 and 10 years of age exhibited mean values no different than adults, suggesting an age-dependent
increase in CYP2E1 levels (Hines 2008; Vieira et al. 1998). There is less of a consensus about the
general ontogeny of glutathione in humans (Hines 2008).

In the past, 1,2-dichloroethane has been detected in human milk (EPA 1980; Urusova 1953), indicating
that developing children could possibly be exposed to 1,2-dichloroethane from breast-feeding mothers.
However, historic data likely reflect exposures from former use patterns that are no longer relevant today.
Thus, the importance of this route of developmental exposure is unclear because current data on the
concentration of 1,2-dichloroethane in breast milk are not available. 1,2-Dichloroethane also
accumulated in the adipose tissue of rats after inhalation exposure and was eliminated from fat more
slowly than from blood, liver, and lung (Spreafico et al. 1980), suggesting the possibility that the maternal
body burden of 1,2-dichloroethane in fat could be available for exposure to the fetus or nursing infant for
a somewhat extended period after maternal exposure. Supporting data for relatively slow elimination of
1,2-dichloroethane from fat are provided in an intravenous exposure study in rats (Withey and Collins
1980).

3.2.2   Other Populations that are Unusually Susceptible

Populations that drink alcohol may be likely to experience increased liver toxicity when exposed to
1,2-dichloroethane. 1,2-Dichloroethane is a known substrate for human CYP2E1 (Gonzalez and Gelboin
1994). CYP 2E1 is induced in people who frequently drink alcohol, as well as people with medical
conditions such as diabetes. It is likely that the induction of this enzyme increases the amount of
1,2-dichloroethane that is metabolized via this pathway rather than by glutathione conjugation, allowing
for binding of the increased quantities of oxidative metabolites to the target organ. Mice with increased
CYP2E1 expression exhibited enhanced metabolism of 1,2-dichloroethane to reactive intermediates,
increased oxidative stress, resulting in enhanced susceptibility to hepatotoxic effects (Sun et al.
2016). Cottalasso et al. (2002) found that 1,2-dichloroethane increased liver toxicity in rats following
chronic ethanol consumption. In *in vitro* experiments conducted on isolated hepatocytes from rats
chronically exposed to ethanol (which induces CYP2E1) and 25 µL of 1,2-dichloroethane added to the
closed system, it was found that 1,2-dichloroethane hepatotoxicity was enhanced by further increasing
levels of ALT, AST, and LDH (Cottalasso et al. 2002).

Inactivation of plasma alpha-1-proteinase inhibitor has been proposed to be an important factor in the development of lung emphysema. The occurrence of a synergistic inactivation of plasma alpha-1 proteinase inhibitor by 1,2-dichloroethane and cigarette smoke components (acrolein and pyruvic aldehyde) *in vitro* suggests that smokers as well as those exposed to passive smoke may be more susceptible to lung emphysema following repeated exposure to 1,2-dichloroethane (Ansari et al. 1988). Further, those with genetically reduced plasma alpha-1-proteinase inhibitor, who are predisposed to emphysema, may be at increased risk.

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 1,2-dichloroethane 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-dichloroethane 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-dichloroethane 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

Levels of 1,2-dichloroethane in breath, blood, and urine may be used to indicate exposure to this chemical. However, 1,2-dichloroethane is rapidly eliminated from the body (see Section 3.1.4); thus, if measurements are not made close to the time of exposure, 1,2-dichloroethane may not be detected. Therefore, the rapid elimination would limit the time when sampling may result in detection of 1,2-dichloroethane. A number of studies have investigated the relationship between tissue and environmental levels of 1,2-dichloroethane. In general, small amounts of 1,2-dichloroethane detected in the breath and urine (trace–0.2 ppb and 50–140 ng/L, respectively) were associated with exposure to 1,2-dichloroethane in air and water (trace–100 ng/m^3 and 50 mg/L, respectively) (Barkley et al. 1980; Conkle et al. 1975). In two studies conducted by Wallace et al. (1984, 1986), levels of 1,2-dichloroethane in breath samples from 350 residents of New Jersey were consistently below the detection limit; therefore, no conclusions could be drawn from these studies. 1,2-Dichloroethane was also detected in the breath (14.3 ppm) and breast milk (0.54–0.64 mg %) of nursing women working in factory premises containing 15.6 ppm 1,2-dichloroethane in air (Urusova 1953). These data are insufficient to quantify the relationship between environmental exposure to 1,2-dichloroethane and resultant tissue and fluid levels.

Urinary excretion of thioethers is another biomarker of exposure to 1,2-dichloroethane. Payan et al. (1993) showed in male Sprague-Dawley rats during a 24-hour post-administration period that total excreted urinary thioethers increased linearly with increasing oral dose (for doses between 0.25 and 4.04 mmol/kg [11.9 mg/kg/d and 400 mg/kg/d, respectively]), at a rate of 0.028 mmol thiol group eliminated per millimole of 1,2-dichloroethane administered. This occurred in spite of the fact that the total percentage of orally administered radioactivity excreted in the urine decreased with increasing dose (possibly due to saturation of certain metabolic pathways leading to urinary metabolites). Thioethers are commonly produced by conjugation reactions involving glutathione and comprise the primary urinary metabolites of 1,2-dichloroethane (see Sections 3.1.3 and 3.1.4). Increased urinary excretion of thioethers following exposure to 1,2-dichloroethane has been demonstrated in rats (Igwe et al. 1988; Payan et al. 1993), showing that this endpoint is sensitive to 1,2-dichloroethane exposure. Payan et al. (1993),

however, found that urinary thiodiglycolic acid (measured by gas chromatography), a thioether compound that is not extractable by alkaline hydrolysis, is a more sensitive marker of 1,2-dichloroethane exposure than total thioethers. As discussed above for the parent compound, rapid excretion of 1,2-dichloroethane and metabolites (essentially complete after 48 hours in animal studies) means that measurements would have to be made at a known time since exposure to be of any quantitative value. There is an additional problem with use of increased urinary thioether excretion as a biomarker for 1,2-dichloroethane exposure. Since many xenobiotics form conjugates with glutathione, exposure to any number of compounds may increase urinary excretion of total thioethers (Monster 1986). Therefore, its use as a biomarker of 1,2-dichloroethane exposure is limited unless exposure to other compounds can be ruled out.

Kim and Guengerich (1989) found that urinary mercapturic acid was linearly dose-related to intraperitoneally injected 1,2-dibromoethane in rats, and the urinary excretion of mercapturic acid was correlated with formation of hepatic and renal DNA adducts. It is possible that a similar relationship exists for relevant 1,2-dichloroethane exposures, although the methods proposed by Kim and Guengerich (1989) would not discriminate between other halogenated ethane compounds (e.g., 1,2-dibromoethane).

Erve et al. (1996) investigated whether human hemoglobin, alkylated with the episulfonium ion of S-(2-chloroethyl) glutathione (a 1,2-dichloroethane metabolite via the glutathione-conjugation metabolic pathway), could be a useful biomarker for human exposure to 1,2-dichloroethane. They found that the method was not a very sensitive indicator for exposure, since an approximately 100-fold molar excess of S-(2-chloroethyl)glutathione over the hemoglobin concentration was required before alkylation was detectable *in vitro*.

Jin et al. (2018a) used urinary levels of chloroacetic acid, the final metabolite of 1,2-dichloroethane in mice, as a measure of a particular metabolism pathway of 1,2-dichloroethane that is mediated by CYP2E1. The urinary levels of chloroacetic acid increased significantly in the group of mice exposed to 1,2-dichloroethane through inhalation for up to 3 days, while the intervention group that was also exposed to 1,2-dichloroethane but was fed a substance that inhibits CYP2E1 had no significant changes in their urinary levels of chloroacetic acid.

The National Health and Nutrition Examination Survey (NHANES) also measures levels of 1,2-dichloroethane in the human blood and has done so since the 2003–2004 data collection cycle of the survey to the 2015–2016 cycle. The NHANES used an analytical method that quantifies trace levels of 1,2-dichloroethane in the blood using solid-phase microextraction, capillary gas chromatography, and

quadrupole mass spectrometry together (Blount et al. 2006). Blood levels of 1,2-dichloroethane from recent NHANES data are presented in Chapter 5 and show that most of the values collected are below the limit of detection.

3.3.2   Biomarkers of Effect

The health effects observed in humans exposed to 1,2-dichloroethane are all nonspecific effects and may be produced from any number of causes, including other causes that do not involve environmental exposure to xenobiotics such as 1,2-dichloroethane. Therefore, these effects would not be useful as specific indicators of effect from exposure to 1,2-dichloroethane. Even if other causes could be ruled out, the specific levels that produce the various effects in humans are not known, so it would not be possible to quantify exposure based on the observed effects. The effects discussed below are not unique effects of exposure to 1,2-dichloroethane, but are the most sensitive effects than may occur.

The primary probable targets of 1,2-dichloroethane identified in humans are the central nervous system, liver, and kidney (for a detailed description of the health effects of 1,2-dichloroethane, see Chapter 2). Another likely target is the immune system, for which very limited information was available in humans but was a sensitive target of 1,2-dichloroethane in animals. The effect on the immune system is immunosuppression (Munson et al. 1982; Sherwood et al. 1987). The observed biomarkers for this effect are reduced ability to fight induced bacterial infection, reduced immunoglobulin response to sheep erythrocytes, and reduced delayed-type hypersensitivity response to sheep erythrocytes, all of which show reduced immune system response to a challenge. The neurological effects observed included a variety of symptoms such as headache, irritability, drowsiness, tremors, partial paralysis, and coma (Chen et al. 2015; Dang et al. 2019; Garrison and Leadingham 1954; Liu et al. 2010; Nouchi et al. 1984; Wirtschafter and Schwartz 1939; Zhan et al. 2011). These effects were accompanied by histopathological changes in the brain in both humans and animals (Chen et al. 2015; Dang et al. 2019; Jin et al. 2018a, 2018b; Liu et al. 2010; Wang et al. 2014, 2018; Zhan et al. 2011; Zhang et al. 2011; Zhou et al. 2015, 2016). The symptoms that occur at the lowest levels (such as headache, irritability, drowsiness, and tremors) may be considered biomarkers for the neurological effects of 1,2-dichloroethane. However, these suggested biomarkers of effects are not specific to 1,2-dichloroethane-induced toxicity.

Liver damage is a prominent feature of 1,2-dichloroethane exposure. Biomarkers for hepatotoxicity observed in humans and animals were alkylation of hepatocellular macromolecules, increased liver weight, and elevated levels of serum enzymes (ALT, AST, SDH) (Alumot et al. 1976; Chen et al. 2015;

Cheng et al. 1999; Daniel et al. 1994; Heppel et al. 1946; Morgan et al. 1990; Nouchi et al. 1984; NTP 1991; Spencer et al. 1951; Sun et al. 2016; van Esch et al. 1977; Wang et al. 2017). Kidney damage is another major effect of 1,2-dichloroethane; kidney failure has been reported in humans following high-level exposure (Hueper and Smith 1935; Lochhead and Close 1951; Nouchi et al. 1984; Schönborn et al. 1970; Yodaiken and Babcock 1973). Biomarkers of renal effects in humans and animals included binding of macromolecules in renal cells and increased kidney weight (Daniel at al. 1994; Hubbs and Prusmack 1955; Morgan et al. 1990; NTP 1991; van Esch et al. 1977). Glomerular involvement may be indicated by urinary excretion of the glomerular structural protein fibronectin (Bundschuh et al. 1993). Research also shows that reproductive effects are characteristic of exposure to 1,2-dichloroethane. A study in humans showed increased rates of premature births in female workers and wives of workers exposed to 1,2-dichlorethane (Zhao et al. 1989). Animal studies have shown reproductive toxicity in males, including pathological changes in reproductive organs, and morphological changes in sperm (Zhang et al. 2017). Although embryo lethality, decreased fertility, and stillbirths have been observed in gestational studies of 1,2-dichloroethane exposure (Vozovaya 1974, 1977; Zhao et al. 1989), the literature supporting this evidence is mixed.

3.4   INTERACTIONS WITH OTHER CHEMICALS

No studies regarding interactions of 1,2-dichloroethane with other chemicals in humans were located. Based on metabolic data resulting from animal studies, various interactions can be expected to occur. Inducers and inhibitors of CYP enzymes, glutathione precursors and depleting agents, and dietary/nutritional status can all influence the rate of formation and excretion of the various toxic intermediates resulting from exposure to 1,2-dichloroethane.

Induction of hepatic CYP enzymes by phenobarbital and/or Aroclor 1254 increases the rate metabolism of 1,2-dichloroethane by CYP mixed function oxidases (MFO) *in vitro* (Hayes et al. 1973; Sipes and Gandolfi 1980). Alterations in metabolism could potentially produce profound effects on toxicity. Enhanced enzymatic metabolism of 1,2-dichloroethane also occurs after treatment with ethanol *in vitro* (Sato et al. 1981). Ethanol is an inducer of CYP2E1, the major MFO enzyme involved in 1,2-dichloroethane metabolism (Guengerich et al. 1991). Since ethanol and 1,2-dichloroethane are both CYP2E1 substrates, they act as competitive metabolic inhibitors when administered together. However, the effect of the consumption of ethanol before *in vitro* exposure to 1,2-dichloroethane varies greatly depending on the actual tissue concentration of ethanol reached during the metabolism of 1,2-dichloro-ethane (Sato et al. 1981). At low tissue ethanol concentration, CYP activity is stimulated. At high tissue

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ethanol concentrations, especially just before exposure to 1,2-dichloroethane, suppression of 1,2-dichloroethane metabolism occurs (Sato et al. 1981). Metabolism of 1,2-dichloroethane (50 ppm in air) was unaffected by chronic co-exposure to ethanol (5% in drinking water) in a 2-year study in rats (Cheever et al. 1990). Toxicity was also unaffected in this study.

Concurrent administration of 0.15% disulfiram (also known as tetraethylthiuram disulfide, Antabuse, and DSF; disulfiram is common in the rubber industry and as a treatment for alcohol use disorder) in the diet and inhaled 1,2-dichloroethane (10, 153–304, 455 ppm) in animals markedly increased hepatotoxicity much more than would occur with exposure to 1,2-dichloroethane alone (Igwe et al. 1986a, 1988). Similarly, after chronic co-treatment with 50 ppm of 1,2-dichloroethane by inhalation and 0.05% disulfiram in the diet for 2 years, a series of neoplastic lesions were produced in rats that were not produced by 1,2-dichloroethane (or disulfiram) alone (Cheever et al. 1990). The lesions included intrahepatic bile duct cholangiomas, subcutaneous fibromas, hepatic neoplastic nodules, interstitial cell tumors in the testes, and mammary adenocarcinomas.

Metabolism studies on rats co-exposed to 1,2-dichloroethane and disulfiram for 2 years showed that following a 7-hour exposure, blood levels of 1,2-dichloroethane were elevated 5-fold by co-treatment with disulfiram (Cheever et al. 1990). In addition, the amount of ^{14}C eliminated as unchanged 1,2-dichloroethane in the breath was elevated by disulfiram co-treatment, with a corresponding decrease in the amount of radioactivity excreted as metabolites in the urine. These results support the suggestion that disulfiram reduces the MFO metabolism of 1,2-dichloroethane, leading to accumulation of 1,2-dichloroethane in the blood and toxic effects. Diethyldithiocarbamate, the reduced form of disulfiram, is a relatively selective inhibitor of CYP2E1, the primary MFO enzyme involved in 1,2-dichloroethane metabolism (Guengerich et al. 1991).

Conjugation with glutathione is an important metabolic pathway for 1,2-dichloroethane. However, glutathione conjugation with 1,2-dichloroethane has also been hypothesized to produce reactive sulfur half-mustard metabolites, such as S-(2-chloroethyl) glutathione (D'Souza et al. 1987; Igwe et al. 1986b; Jean and Reed 1989; Lock 1989; Reitz et al. 1982). There is considerable evidence supporting the hypothesis that reactive intermediates formed by glutathione conjugation are responsible for 1,2-dichloro-ethane toxicity. However, studies also show a protective effect of glutathione. The administration of glutathione, precursors of glutathione, or amino acids capable of donating a sulfhydryl group for the biosynthesis of glutathione all decrease the toxic effects and mortality in rats given 1,2-dichloroethane orally (Heppel et al. 1947). This protective action of glutathione and precursors also occurs in young rats

exposed to 1,2-dichloroethane by inhalation (Johnson 1967). It is not clear how the protective effect of glutathione reported in these studies may be reconciled with the hypothesis that reactive intermediates formed by glutathione conjugation are responsible for 1,2-dichloroethane-induced toxicity. By analogy to 1,2-dibromoethane, however, the protective effect of co-administered glutathione in 1,2-dichloroethane exposures might be explained by the reaction of S-(2-chloroethyl)glutathione with glutathione, which is a nonenzymatic reaction occurring at physiological glutathione concentrations (Cmarik et al. 1990), although work with 1,2-dibromoethane indicates that levels of DNA adducts are correlated with glutathione content (Kim and Guengerich 1990). Methionine, p-aminobenzoic acid, aniline, and sulfanilamide have been shown to protect against toxicity of 1,2-dichloroethane (Heppel et al. 1945). A good correlation has been found between the urinary excretion of mercapturic acid and the formation of DNA adducts in liver and kidney DNA of 1,2-dibromoethane-treated rats (Kim and Guengerich 1989). This finding suggests that the extent of formation of adducts may be correlated with the toxic effects of 1,2-dichloroethane.

Nutritional status affects the rate of metabolic formation of toxic intermediates; liver from fasted animals showed an increased rate of 1,2-dichloroethane metabolism in vitro (Nakajima and Sato 1979) because fasting induces the formation of CYP2E1 (Johansson et al. 1988), the primary MFO enzyme involved in oxidation of 1,2-dichloroethane (Guengerich et al. 1991). Fasting also may lower hepatic levels of glutathione. According to the hypothesis that reactive intermediates formed by glutathione conjugation are responsible for 1,2-dichloroethane-induced toxicity, toxicity would be reduced under these conditions. However, the actual effect of fasting on 1,2-dichloroethane toxicity is unknown.

A few studies that investigated the toxic interactions between 1,2-dichloroethane and other xenobiotic toxicants were located. Pretreatment with orally administered 2-hexanone did not potentiate the nephrotoxicity of 1,2-dichloroethane administered by intraperitoneal injection in rats (Raisbeck et al. 1990). Co-treatment with 1,1-dichloroethylene produced only a slightly greater-than-additive effect on lipid droplet changes in rat hepatocytes (EPA 1989). A mixture of 1,2-dichloroethane (80 mg/kg) and carbon tetrachloride (200 mg/kg) administered in a single oral dose to rats produced lower liver triglyceride levels than observed with carbon tetrachloride alone. These levels were still increased above 1,2-dichloroethane-only levels (Aragno et al. 1992). Studies of *in vitro* interactions produced more positive results, though interactions observed *in vitro* do not always generalize to the intact system. tert-Butyl hydroperoxide potentiated lipid peroxidation induced by 1,2-dichloroethane in rat liver slices *in vitro* (Sano and Tappel 1990). The occurrence of lipid peroxidation is associated with physical damage to

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tissues. There was a synergistic inactivation of plasma alpha-1 proteinase inhibitor when 1,2-dichloro-ethane was tested together with the cigarette smoke components acrolein and pyruvic aldehyde *in vitro* (Ansari et al. 1988). Inactivation of plasma alpha-1 proteinase inhibitor has been proposed as an important factor in the development of lung emphysema.

Oral administration of 1,2-dichloroethane in drinking water for 16 weeks together with three other chemical carcinogens commonly found at hazardous waste sites (arsenic, vinyl chloride, and trichloroethylene) resulted in inhibition of the promotion of preneoplastic hepatic lesions and pulmonary hyperplasia and adenomas (Pott et al. 1998). The four chemicals, including 1,2-dichloroethane, have been shown to be individually carcinogenic in laboratory animals, yet they interacted antagonistically to inhibit promotion of precancerous lesions. The study is limited, however, by a short exposure duration, small numbers of test animals, and the use of only male rats; the interactive effect of lifetime exposure to the four chemicals cannot be inferred with confidence from these results. The mechanism for this interactive effect has not been elucidated, but Pott et al. (1998) hypothesized that decreased cell proliferation, increased apoptosis, or enhanced remodeling of preneoplastic lesions may play a role. It is also possible that this effect could have been due to competitive metabolic inhibition, as vinyl chloride, trichloroethylene, and 1,2-dichloroethane are all CYP2E1 substrates (Pohl and Scinicariello 2011).