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

3.1 TOXICOKINETICS

Human studies of vinyl chloride provide limited quantitative information on absorption, metabolism, and excretion. Vinyl chloride toxicokinetics have been studied in nonhuman primates (e.g., rhesus monkeys) and rodents, with most of the quantitative information derived from studies conducted in rats. An overview of these data is summarized below.

- Studies in humans and animals indicate that vinyl chloride is readily absorbed through the lungs following inhalation. Animal studies demonstrate that vinyl chloride is rapidly and almost completely absorbed from the gastrointestinal tract after oral exposure. A single study in monkeys suggests that dermal absorption of vinyl chloride gas is not likely to be significant.
- No human studies were identified that provided reliable information about the distribution of vinyl chloride in tissues other than blood.
- Animal studies indicate that the distribution of vinyl chloride is rapid and widespread; however, storage in the body is limited because of rapid metabolism and excretion. Metabolites of vinyl chloride can be found in the liver, kidney, spleen, skin, and brain, but tissue concentrations do not increase following repeated exposure.
- Vinyl chloride can cross the placenta after inhalation exposure in rat dams.
- Metabolism in humans and experimental animals occurs via the oxidation of vinyl chloride by CYP to form an epoxide intermediate, 2-chloroethylene oxide, which spontaneously rearranges to form 2-chloroacetaldehyde. Intermediates are detoxified primarily via glutathione conjugation and conjugates are excreted in urine as substituted cysteine derivatives.
- Metabolism follows Michaelis-Menten kinetics in rats, with enzyme saturation near 100 ppm in air or between 1 and 100 mg/kg/day for a single gavage dose.
- Vinyl chloride metabolites are excreted primarily in the urine following oral or inhalation exposure to low doses. At higher doses where metabolic saturation has been exceeded, vinyl chloride is exhaled as the parent compound.

3.1.1 Absorption

Inhalation absorption of vinyl chloride is rapid in humans. Five young adult male volunteers were exposed to vinyl chloride concentrations of 2.9, 5.1, 11.7, or 23.5 ppm by way of a gas mask for 6 hours (Krajewski et al. 1980). Retention was estimated by measuring the difference between inhaled and exhaled concentrations. An average retention of 42% was estimated. Although the results varied among

the individuals tested, the percentage retained was independent of the concentration inhaled. Since retention did not change with increasing vinyl chloride concentrations, it appears that saturation of the major pathway of overall metabolism did not occur in this exposure regimen.

Animal data demonstrate that the inhalation absorption of vinyl chloride occurs readily and rapidly. Physiologically based pharmacokinetic (PBPK) models developed to provide quantitative estimates of uptake are discussed in Section 3.1.5. Peak blood levels occurred at 30 minutes in rats exposed (head only) to 7,000 ppm (Withey 1976). On removal from the vinyl chloride-containing atmosphere, blood levels fell rapidly. After 2 hours, concentrations were barely detectable.

Several studies in rats indicate that vinyl chloride is rapidly and virtually completely absorbed from the gastrointestinal tract following oral exposure. Peak blood levels of vinyl chloride were observed within 10–20 minutes after gavage dosing of rats with vinyl chloride in an aqueous solution (single doses of 44–92 mg/kg) (Withey 1976). Peak blood levels varied from 6 to >40 µg/mL. Data from another study in which rats were administered single gavage doses of 0.05, 1, and 100 mg/kg vinyl chloride labelled with radioactive carbon (^{14}C-vinyl chloride) (in corn oil) suggested that absorption of vinyl chloride was nearly complete (Watanabe et al. 1976a).

The fraction of the administered dose recovered in the feces, roughly indicative of the proportion unabsorbed, ranged from 0.47 to 2.39%; total recovery ranged from 82.3 to 91.3%. Loss of radioactivity might be attributed either to experimental error or to incomplete sampling of the carcass. Fecal excretion was measured in rats fed 0, 1.8, 5.6, and 17.0 mg/kg/day of vinyl chloride monomer (from powdered PVC containing a high level of the monomer) (Feron et al. 1981). Fecal excretion accounted for 8, 10, and 17% of the vinyl chloride present in the low-, middle-, and high-dose groups, respectively. The investigators hypothesized that the vinyl chloride recovered from the feces was encapsulated by PVC, thereby not available to the rats for absorption, and that absorption of bioavailable vinyl chloride was virtually complete.

No studies were located regarding absorption in humans after dermal exposure to vinyl chloride. Animal data suggest that dermal absorption of vinyl chloride gas is not likely to be significant. Dermal absorption was measured in two rhesus monkeys that received full body (except head) exposure to vinyl chloride gas. It was estimated that 0.031 and 0.023% of the total available vinyl chloride was absorbed at 800 and 7,000 ppm, respectively, after a 2–2.5-hour exposure (Hefner et al. 1975a). The investigators concluded that, after short-term exposure to high concentrations, dermal absorption was far less

significant than inhalation absorption. No information is available regarding dermal absorption of vinyl chloride from liquid or solid media.

3.1.2 Distribution

Representative vinyl chloride partition coefficients for humans, rats, mice, and hamsters are provided in Table 3-1. These partition coefficients were obtained for use in PBPK models. They were estimated using a vial equilibration technique (U.S. Air Force 1990). Further details about how the values were obtained, including the number of experiments completed and whether the errors shown are standard deviations or standard errors, were not provided. In general, concentrations of vinyl chloride found in fat are higher than would be found in other tissues. Partition coefficients for vinyl chloride range from 10 to 20 (fat/air) and from 1 to 3 (muscle/air, blood/air, and liver/air). In animal studies, females have shown greater partitioning from air to fat than males.

Table 3-1. Vinyl Chloride Partition Coefficients

			Partition coefficient			
Species	Strain	Sex	Blood/air	Liver/air	Muscle/air	Fat/air
Rat	CDBR[a]	M	1.79±0.216	3.0±0.407	2.18±0.470	14.6±0.917
		F	2.12±0.437	1.66±0.429	1.28±0.245	19.2±0.96
	F-344[a]	M	1.60±0.328	1.99±1.96	2.06±0.703	11.8±0.811
		F	1.55±0.11	2.05±0.17	2.39±0.46	21.1±1.3
	Wistar[a]	M	2.10±0.313	2.69±0.555	2.72±0.575	10.2±1.61
		F	1.62±0.0664	1.48±0.28	1.06±0.221	22.3±0.542
	Sprague-Dawley[b]	M	2.4±0.5	–	–	–
Mouse	B6C3F1[a]	M	2.83±0.22	–	–	–
		F	2.56±0.14	–	–	–
	CD-1[a]	M	2.27±0.0725	–	–	–
		F	2.37±0.16	–	–	–
Hamster	Golden Syrian[a]	M	2.74±0.151	3.38±0.362	2.56±0.457	14.3±5.32
		F	2.21±0.47	1.31±0.28	1.96±0.28	21.10±2.01
Human[c]	NA	NR	1.16	–	–	–

[a]U.S. Air Force 1990; values determined using vial equilibration method.
[b]Barton et al. 1995.
[c]EPA 1987.

– = no data; F = female; M = male; NA = not applicable; NR = not reported

Tissue/blood partition coefficients in male Sprague-Dawley rats measured using a vial equilibration method were reported as 10±3 for fat/blood, 0.4±0.2 for muscle/blood, 0.7±0.3 for liver/blood, and 0.7±0.4 for kidney/blood (Barton et al. 1995).

Data from rat studies suggest that the distribution of inhaled vinyl chloride is rapid and widespread, but storage of vinyl chloride in the body is limited by rapid metabolism and excretion. In rats exposed to ^{14}C-vinyl chloride and pretreated with 6-nitro-1,2,3-benzothiadiazole to block metabolism of vinyl chloride by microsomal CYP oxidation pathways, the highest levels of radiolabel were located in the fat, with lesser amounts in the blood, liver, kidney, muscle, and spleen. When metabolism was not blocked, the highest levels of radiolabeled metabolites were located in the liver and kidney (Buchter et al. 1977).

Immediately after a 5-hour exposure to ^{14}C-vinyl chloride at 50 ppm, tissue levels of ^{14}C-activity, expressed as the percentage incorporated per gram of tissue, were highest in the kidney (2.13%) and liver (1.86%), with lower levels in the spleen (0.73%) and brain (0.17%) (Bolt et al. 1976a). Radioactivity in tissue was measured in rats 72 hours after exposure to 10 or 1,000 ppm ^{14}C-vinyl chloride for 6 hours. In order of decreasing concentration for rats exposed to 10 ppm, ^{14}C-labeled compounds (expressed as percentage present as nonvolatile metabolites), were measured in the liver (0.14), kidney (0.08), skin (0.07), lung (0.07), muscle (0.05), carcass (0.05), plasma (0.05), and fat (0.03). For rats exposed to 1,000 ppm, the tissue radiolabel percentages were: liver (0.15), skin (0.12), kidney (0.06), carcass (0.05), lung (0.05), muscle (0.04), fat (not detected), and plasma (not detected) (Watanabe et al. 1976b).

There was no difference in the routes or rate of excretion between repeated-dose versus single-dose exposure of rats to 5,000 ppm of ^{14}C-vinyl chloride (Watanabe et al. 1978a). The concentration of radiolabel detected in tissues 72 hours after exposure revealed no statistically significant difference between rats exposed once or repeatedly to vinyl chloride. Percentages of radioactivity after 72 hours measured in tissues are as follows (for single and repeated doses, respectively): liver (0.12 and 0.16), kidney (0.06 and 0.07), skin (0.05 and 0.08), carcass (0.03 and 0.04), and fat (not detected and not detected).

Placental transfer of vinyl chloride can occur rapidly in rats. Female rats exposed to approximately 0, 2,000, 7,000, or 13,000 ppm vinyl chloride for 2.5 hours on GD 18 showed high concentrations of vinyl chloride in maternal and fetal blood and amniotic fluid (Ungvary et al. 1978). Vinyl chloride concentrations in maternal blood were 19.02, 32.40, and 48.43 μg/mL, respectively, while fetal blood concentrations were 12.80, 22.67, and 30.52 μg/mL, respectively. Vinyl chloride concentrations in

amniotic fluid were 0, 4.27, 4.93, and 13.50 μg/mL at 0, 2,000, 7,000, and 13,000 ppm vinyl chloride, respectively (Ungvary et al. 1978).

The level of ^{14}C-nonvolatile metabolites was measured in tissues of rats 72 hours after single gavage doses (0.05–100 mg/kg) of ^{14}C-vinyl chloride in corn oil (Watanabe et al. 1976a). The highest levels of radioactivity for each dose level occurred in the liver. These levels were 2–5 times higher than in the other tissues examined (skin, plasma, muscle, lung, fat, and carcass).

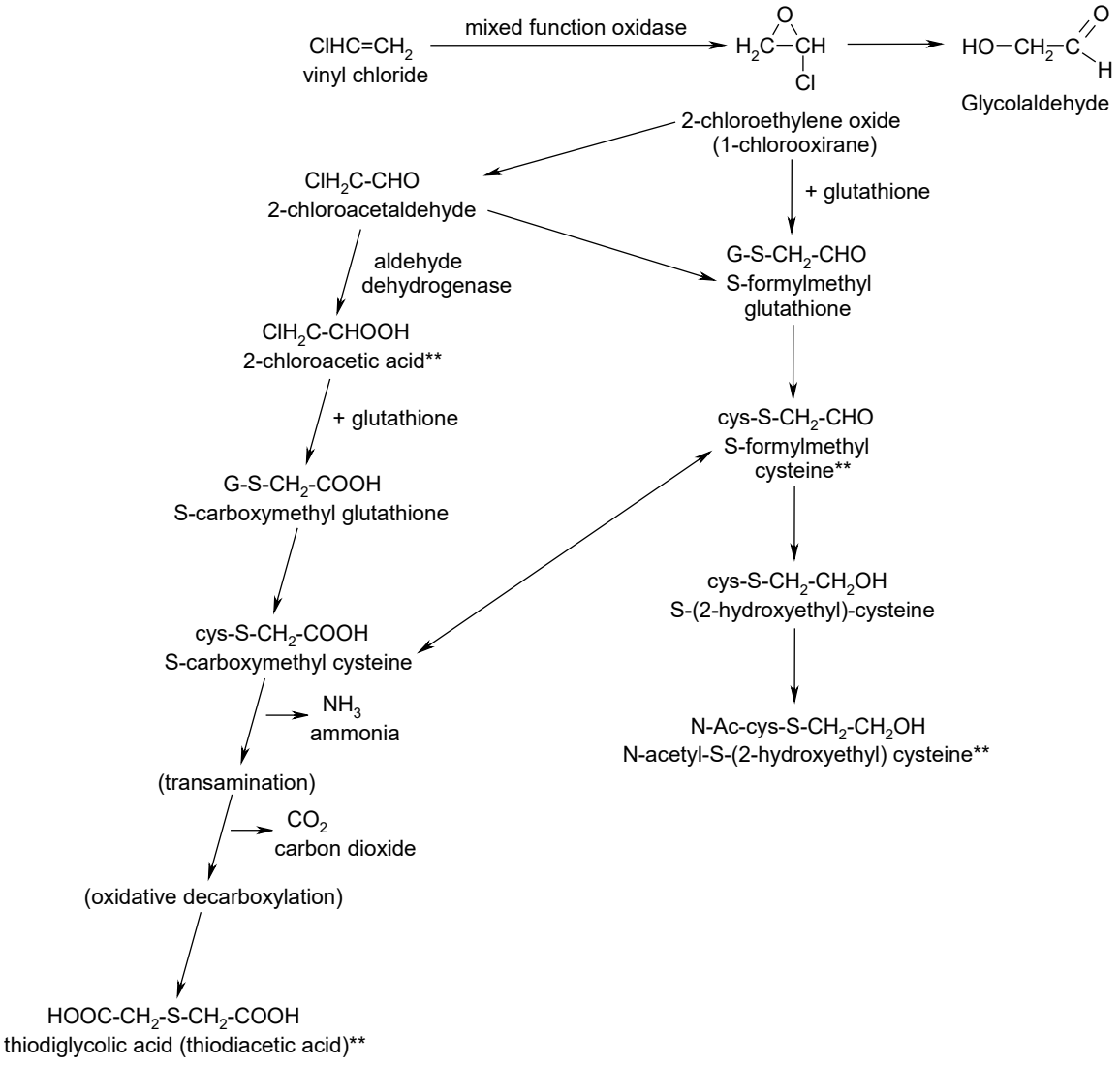
3.1.3 Metabolism

Vinyl chloride metabolism in humans is attributed to the CYP monooxygenases in the liver (Ivanetich et al. 1977; Sabadie et al. 1980; Salmon 1976). The proposed metabolic pathways for vinyl chloride are shown in Figure 3-1. Data obtained in rats indicate that metabolic pathways are consistent for both inhalation and oral exposure (Bartsch et al. 1976, 1979; Green and Hathway 1975, 1977; Hathway 1977; Watanabe and Gehring 1976; Watanabe et al. 1976a). Metabolism occurs via the oxidation of vinyl chloride by CYP to form an epoxide intermediate, 2-chloroethylene oxide, which spontaneously rearranges to form 2-chloroacetaldehyde (Guengerich et al. 1979, 1981; Gwinner et al. 1983; Laib 1982). 2-Chloroethylene oxide can also be detoxified by epoxide hydrolase to yield glycolaldehyde (IARC 2012). These intermediates are detoxified mainly through conjugation with glutathione catalyzed by glutathione *S*-transferase. The conjugated products are excreted in urine as substituted cysteine derivatives and include thiodiglycolic acid, *S*-formylmethylcysteine, and *N*-acetyl-*S*-(2-hydroxyethyl) cysteine (Bolt et al. 1980; Hefner et al. 1975b). Urinary metabolites identified in rats exposed by inhalation include polar compounds at low exposure concentrations (Hefner et al. 1975b; Watanabe et al. 1976b) and 2-chloroacetic acid at high exposure concentrations (Hefner et al. 1975b). Mitochondrial aldehyde dehydrogenase 2 (ALDH2) may also play a role in detoxifying 2-chloroacetaldehyde (Chen et al. 2019). Activation of ALDH2 with an agonist (Alda-1) was shown to attenuate liver injury and reduce oxidative stress in mice exposed to vinyl chloride (Chen et al. 2019).

Metabolic saturation was not demonstrated in volunteers exposed to vinyl chloride at concentrations of 2.9, 5.1, 11.7, and 23.5 ppm for 6 hours (Krajewski et al. 1980). In rats, metabolism follows Michaelis-Menten kinetics, with enzyme saturation near 100 ppm in air or between 1 and 100 mg/kg/day for a single gavage dose (Hefner et al. 1975b; Watanabe et al. 1976a).

Isolated rat liver cells converted ^{14}C-vinyl chloride into nonvolatile metabolites (Hultmark et al. 1979), indicating that the *in vitro* liver cell microsomal metabolism was NADPH-dependent and probably involved CYP. Pretreatment with 6-nitro-1,2,3-benzothiadiazole, an inhibitor of some microsomal CYP oxidation pathways, was sufficient to totally block the metabolism of vinyl chloride in rats exposed to 0.45 ppm in a closed system for 5 hours (Bolt et al. 1977). This observation suggests that metabolism of vinyl chloride proceeds primarily through a CYP pathway with likely production of an epoxide intermediate.

Figure 3-1. Proposed Metabolic Pathways for Vinyl Chloride*



**Excreted in urine.

Sources: Bolt et al. (1980); Hefner et al. (1975b); IARC (2012); Park et al. (1993); Plugge and Safe (1977)

Inhalation exposure of rats to high concentrations of vinyl chloride was associated with a reduction in the liver nonprotein sulfhydryl functional group concentration (Barton et al. 1995). A reduction in these functional groups is expected since there are limited amounts of liver glutathione and/or cysteine to conjugate the metabolites of vinyl chloride. (Bolt et al. 1976b; Hefner et al. 1975b; Jedrychowski et al. 1984; Watanabe et al. 1978b).

Saturation of metabolic pathways was observed in rats and monkeys that were exposed in a closed system to ^{14}C-vinyl chloride (Bolt et al. 1977; Buchter et al. 1980; Filser and Bolt 1979). In Wistar rats, metabolic saturation was determined to occur at approximately 250 ppm, and a metabolic rate (V_{max}) of 110 µmol/hour/kg was estimated (Bolt et al. 1977; Filser and Bolt 1979). Kinetic constants of 58 µmol/hour/kg for V_{max} and 1 µM for the K_m in male Sprague-Dawley rats were also reported (Barton et al. 1995). In an experiment using rhesus monkeys, metabolic saturation occurred at 200 ppm, with a V_{max} of 50 µmol/hour/kg (Buchter et al. 1980). The V_{max} of 50 µmol/hour/kg estimated using rhesus monkeys was suggested as a closer approximation of metabolism in humans than the value of 110 µmol/hour/kg estimated for rats by Filser and Bolt (1979).

Kinetic constants for vinyl chloride metabolism were derived from *in vitro* studies in rat liver microsomes (el Ghissassi et al. 1998). Metabolism followed Michaelis-Menton kinetics with a K_m of 7.42 µM and a V_{max} of 4,674 pmol/mg protein/minute. Inhibitor studies using chemical and immunological inhibitors demonstrate that vinyl chloride is metabolized primarily by CYP2E1.

Urinary metabolites identified from rats ingesting ^{14}C-vinyl chloride are consistent with the metabolic pathways postulated for inhalation exposure, in particular with the formation of 2-chloroethylene oxide and 2-chloroacetaldehyde. Metabolites identified include *N*-acetyl-*S*-(2-hydroxyethyl) cysteine, 2-chloroacetic acid, and thiodiglycolic acid (Green and Hathway 1975, 1977; Watanabe and Gehring 1976; Watanabe et al. 1976a). Metabolic saturation appears to occur with a single gavage dose of between 1 and 100 mg/kg/day (Watanabe et al. 1976a).

Several investigators observed the binding of nonvolatile metabolites of ^{14}C-vinyl chloride to liver macromolecules both *in vitro* and in rats exposed by inhalation (Guengerich and Watanabe 1979; Guengerich et al. 1979, 1981; Kappus et al. 1976; Watanabe et al. 1978a, 1978b). In single-exposure experiments at different concentrations, the extent of macromolecular binding increased proportionately to the amount of vinyl chloride metabolized and disproportionately to the exposure concentration (Watanabe et al. 1978b). The extent of macromolecular binding was increased by repeated exposure to

vinyl chloride (Watanabe et al. 1978a) and by pretreatment with phenobarbital (Guengerich and Watanabe 1979). Macromolecular binding was attributed to the reactive intermediate 2-chloroethylene oxide, which binds to DNA and RNA, and to its rearrangement product, 2-chloroacetaldehyde that can form an adduct with some amino acid side-chains, altering the protein conformation (Guengerich and Watanabe 1979; Guengerich et al. 1979, 1981; Kappus et al. 1976; Watanabe et al. 1978a, 1978b).

3.1.4 Excretion

Studies demonstrated that the primary route of vinyl chloride excretion is dose-dependent (Krajewski et al. 1980; Watanabe and Gehring 1976; Watanabe et al. 1976b, 1978a). Vinyl chloride metabolites are excreted primarily in the urine following oral and inhalation exposure at low doses or concentrations. In humans exposed by inhalation, exhalation of vinyl chloride was a minor pathway of elimination even at low concentrations (Krajewski et al. 1980). Animal studies have shown that at higher doses where metabolic saturation has been exceeded, vinyl chloride is exhaled as the parent compound (Watanabe and Gehring 1976; Watanabe et al. 1976b, 1978a).

Human data suggest that exhalation of unmetabolized vinyl chloride is not an important pathway of elimination at low exposure concentrations. The mean concentration in expired air for humans exposed for 6 hours to air containing 2.9–23.5 ppm ranged from 0.21 to 1.11 ppm, representing from 7.23 to 4.73% of the inhaled amounts, respectively (Krajewski et al. 1980). After dermal exposure in monkeys, most of the minimal vinyl chloride absorbed was excreted in exhaled air (Hefner et al. 1975a).

The mode of excretion of vinyl chloride and its metabolites following inhalation exposure of animals to different concentrations reflects the saturation of metabolic pathways. The cumulative excretion of radioactivity over a 72-hour postexposure period was measured in rats exposed to 10–1,000 ppm (Watanabe and Gehring 1976; Watanabe et al. 1976b) or 5,000 ppm (Watanabe et al. 1978a) ^{14}C-vinyl chloride for 6 hours. Radioactivity expired as carbon dioxide or vinyl chloride, excreted in the urine and feces, and retained in the carcass was expressed as a percentage of the total radioactivity recovered. The results suggest that metabolism was nearly complete at 10 ppm because <2% of the recovered radioactivity occurred as unchanged parent compound. The predominant route for excretion of radioactive metabolites was through the urine, accounting for about 70% of the recovered radioactivity. At 1,000 ppm, the fraction of unchanged vinyl chloride increased to 12.3% and urinary radioactivity decreased to 56.3%, indicating that metabolism was saturated at this concentration.

Increasing vinyl chloride concentrations may have different effects for animals and humans. In humans exposed to low concentrations, a higher percentage of unmetabolized vinyl chloride was found in expired breath (Krajewski et al. 1980). This is the opposite of what is observed in animals, wherein there is a trend for a greater percentage of vinyl chloride being exhaled at higher concentrations. In rats exposed to 5,000 ppm for 6 hours, more than half of the recovered radioactivity appeared as unchanged vinyl chloride in expired air, and urinary excretion accounted for about 27% of the recovered activity (Watanabe et al. 1978a). Generally, there was little change in the proportion of recovered radioactivity excreted in the feces or exhaled as carbon dioxide. The percentage of the radioactivity retained in the carcass and tissues of rats appeared to be somewhat decreased at 5,000 ppm compared with 10 and 1,000 ppm, suggesting preferential retention of metabolites rather than unchanged vinyl chloride (Watanabe and Gehring 1976; Watanabe et al. 1978a, 1976b). However, it is possible that a reversal of this trend would occur in humans if concentrations were increased to those used in the animal studies or to concentrations closer to the K_m for human metabolism.

Pulmonary excretion of unaltered vinyl chloride in rats followed first-order kinetics regardless of exposure concentrations, with half-lives of 20.4, 22.4, and 30 minutes following 6-hour exposures at 10, 1,000, and 5,000 ppm, respectively (Watanabe et al. 1976b). After oral exposure, pulmonary excretion of vinyl chloride appeared to be monophasic at <1.0 mg/kg, with a half-life of about 55–58 minutes (Watanabe et al. 1976a). At 100 mg/kg, pulmonary excretion of vinyl chloride was biphasic, with half-lives of 14.4 and 40.8 minutes for the rapid and slower phases, respectively. Exhalation of unchanged vinyl chloride was generally complete within 3–4 hours; however, excretion of metabolites in urine continued for days (Green and Hathway 1975).

The urinary excretion of radioactivity was biphasic, with the second or slow phase accounting for <3% of the total urinary excretion (Cheng et al. 2001; Watanabe et al. 1976a). Estimated half-lives for the rapid (first-order) phase were 4.6, 4.1, and 4.5 hours at 10, 1,000, and 5,000 ppm, respectively (Cheng et al. 2001) and 4.5–4.6 hours for oral doses of 0.05–100 mg/kg (Watanabe et al. 1976a). Single oral doses of ^{14}C-vinyl chloride (0.05, 0.25, 1.0, 20, 100, and 450 mg/kg) were administered to rats, and the excretion of radioactivity was monitored over a 72-hour period (Green and Hathway 1975; Watanabe and Gehring 1976; Watanabe et al. 1976a). A striking increase in exhalation of unchanged vinyl chloride and compensatory decreases in urinary and fecal excretion of radioactivity and exhalation of carbon dioxide were observed at >20 mg/kg, suggesting that metabolic saturation had occurred at that dosage. At <1.0 mg/kg, the predominant route of elimination was urinary excretion of polar metabolites.

Urinary metabolites included *N*-acetyl-*S*-(2-hydroxyethyl) cysteine, thiodiglycolic acid, and possibly *S*-(2-hydroxyethyl) cysteine (Watanabe et al. 1976b). Identification of these metabolites of vinyl chloride in the urine indicates that vinyl chloride is transformed in the body to a reactive metabolite, which is then detoxified by reaction with glutathione (GSH, gamma-glutamylcysteinylglycine). Subsequently, the glutamic acid and glycine moieties of the tripeptide are cleaved, and the cysteine conjugate of the reactive metabolite of vinyl chloride is either acetylated or further oxidized and excreted. Thiodiglycolic acid is the major metabolite of vinyl chloride detected in the urine of exposed workers (Cheng et al. 2001). Urinary thiodiglycolic acid levels were correlated with vinyl chloride levels in air at concentrations >5 ppm; however, this correlation appears to be more variable at lower vinyl chloride concentrations in air (Chen et al. 2019).

Metabolites identified in the urine of orally treated rats were consistent with the formation of 2-chloroethylene oxide and 2-chloroacetaldehyde (Green and Hathway 1977; Watanabe et al. 1976a), as postulated for metabolism following inhalation exposure. The major metabolites were identified as thiodiglycolic acid, *N*-acetyl-*S*-(2-hydroxyethyl) cysteine, *N*-acetyl-*S*-(2-chloroethyl)cysteine, and *S*-(2-chloroethyl)cysteine (Green and Hathway 1977; Watanabe et al. 1976a). Minor metabolites included urea, glutamic acid, and 2-chloroacetic acid (Green and Hathway 1975).

Dermal exposure of high concentrations of vinyl chloride gas resulted in most excreted in expired air for the small fraction that was absorbed. Hefner et al. (1975a) reported that two rhesus monkeys received whole-body (except head) exposure to vinyl chloride gas (800 and 7,000 ppm) for 2–2.5 hours and most was excreted in expired air (Hefner et al. 1975a). The percentages of absorbed vinyl chloride that were exhaled were 0.028% at 700 ppm and 0.014% at 8,000 ppm (Hefner et al. 1975a).

The elimination of radioactivity following intraperitoneal administration of ^{14}C-vinyl chloride to rats resembles the pattern observed following inhalation or oral administration. Following an intraperitoneal dose of 0.25 mg/kg, exhalation of unchanged vinyl chloride, exhalation of carbon dioxide, and urinary and fecal excretion of radioactivity accounted for 43.2, 11.0, 43.1, and 1.8% of the administered dose, respectively (Green and Hathway 1975). At 450 mg/kg, exhaled vinyl chloride increased to 96.2% of the administered dose, carbon dioxide decreased to 0.7%, urinary radioactivity decreased to 2.6%, and fecal radioactivity decreased to 0.1%.

Doses administered intravenously were eliminated very rapidly and almost entirely by exhalation of unchanged vinyl chloride. Green and Hathway (1975) administered a 0.25-mg/kg intravenous dose of

^{14}C-vinyl chloride to rats and recovered 80% of the dose within 2 minutes and 99% within 1 hour as unchanged compound in expired air.

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

Models are simplified representations of a system with the intent of reproducing or simulating its structure, function, and behavior. PBPK models are more firmly grounded in principles of biology and biochemistry. They use mathematical descriptions of the processes determining uptake and disposition of chemical substances as a function of their physicochemical, biochemical, and physiological characteristics (Andersen and Krishnan 1994; Clewell 1995; Mumtaz et al. 2012a; Sweeney and Gearhart 2020). PBPK models have been developed for both organic and inorganic pollutants (Ruiz et al. 2011) and 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 (Mumtaz et al. 2012b; Ruiz et al. 2011; Sweeney and Gearhart 2020; Tan et al. 2020). PBPK models can also be used to more accurately extrapolate from animal to human, high dose to low dose, route to route, and various exposure scenarios and to study pollutant mixtures (El-Masri et al. 2004). 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 (Clewell 1995).

PBPK models are available for vinyl chloride. These models predict the metabolism and distribution of vinyl chloride. The overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

3.1.5.1 EPA (1987) Animal Models

EPA (1987) developed a PBPK model to estimate the metabolized dose of vinyl chloride when coupled to a multistage model to estimate cancer risk in animals. This PBPK model consists of four compartments: the liver, fat, highly perfused tissue, and poorly perfused tissue. All metabolism is assumed to occur in the liver by one saturable (reflecting Michaelis-Menten kinetics) first-order metabolism pathway.

The dose delivery provided by the vinyl chloride model developed by EPA (1987) was validated by the U.S. Air Force (1990) study and by additional vinyl chloride metabolism studies conducted in rats. At

low concentrations, this model fit *in vivo* data in rats by Gehring et al. (1978) well, but at concentrations above 25 ppm, the model predicted a greater level of vinyl chloride metabolism than was observed.

3.1.5.2 U.S. Air Force (1990) Rat, Mouse, and Hamster Models

The U.S. Air Force (1990) modified the EPA (1987) model to improve the fit with actual data, particularly as it relates to glutathione depletion and doses above 25 ppm. In the first modification, both vinyl chloride and the epoxide metabolite were assumed to react with glutathione. This model had difficulty predicting glutathione depletion at high doses; for example, it predicted glutathione depletions higher than observed at 4,600–5,800 ppm vinyl chloride concentrations. The second alternative model assumed that only the product of the first-order metabolism reacted with glutathione. It also predicted glutathione depletions higher than observed at high exposure concentrations. To improve the model, the investigators suggested the addition of a low-affinity glutathione pathway.

Using vinyl chloride concentration data obtained from Wright-Patterson Air Force Base (AFB), the U.S. Air Force (1990) extended the first glutathione conjugation model, developed in rats, to different strains of rats, mice, and hamsters. Vinyl chloride gas uptake experiments were completed in which animals were exposed to various concentrations of vinyl chloride in closed chambers for up to 6 hours, and the disappearance of vinyl chloride was monitored. The glutathione content of the animals was also measured immediately after exposure. Using data from these studies with the physiologic parameters shown in Table 3-2, the investigators estimated metabolic parameters for vinyl chloride and the rate constant for the conjugation of vinyl chloride with glutathione (Table 3-3). Using the metabolic parameters determined from the gas uptake experiments, the model predictions showed good agreement with the actual data for all of the animal strains tested.

Table 3-2. Physiological Parameters Used to Estimate Parameters from Vinyl Chloride Gas Uptake Experiments[a]

Parameter	Rats	Mice	Hamsters
Ventilation rate (L/hour/body weight$^{0.74}$)	14	23–35[b]	13
Total cardiac output (L/hour/body weight$^{0.74}$)	14	23–35[b]	13
Blood flow to the liver (fraction of total cardiac output)	0.25	0.24	0.24
Blood flow to highly perfused tissue (fraction of total cardiac output)	0.51	0.52	0.52
Blood flow to fat (fraction of total cardiac output)	0.09[c]	0.05	0.09
Blood flow to poorly perfused tissue (fraction of total cardiac output)	0.15[c]	0.20	0.15
Volume of tissue (L/body weight)	0.04	0.04	0.04

Table 3-2. Physiological Parameters Used to Estimate Parameters from Vinyl Chloride Gas Uptake Experiments[a]

Parameter	Rats	Mice	Hamsters
Volume of highly perfused tissue (L/body weight)	0.05	0.05	0.05
Volume of fat tissue (L/body weight)	0.07–0.1[d]	0.04	0.07
Volume of poorly perfused tissue (L/body weight)	0.72–0.75[d]	0.78	0.75

[a]U.S. Air Force (1990); units of body weight were not provided.
[b]Ventilation rates and total cardiac outputs were 23 for male B6C3F1 mice, 25 for female B6C3F1 mice, 28 for female CD-1 mice, and 35 for male CD-1 mice.
[c]Male Wistar rats blood flow to fat = 0.08 and blood flow to slowly perfused tissue = 0.16.
[d]Female F-344 and female Wistar rats had volume of fat tissue = 0.07 and volume of slowly perfused tissue = 0.75; male F-344 and female Wistar rats had volume of fat tissue = 0.08 and volume of slowly perfused tissue = 0.74; male Wistar rats and male CDBR rats had volume of fat tissue = 0.1 and volume of slowly perfused tissue = 0.72.

Table 3-3. Estimates of Metabolic Parameters Obtained from Gas Uptake Experiments

Species	Strain	Sex	V_{max}/body weight$^{0.7}$ (mg/hour/body weight$^{0.7}$)	Kfc (body weight$^{0.3}$/hour)	Kgsc (body weight$^{0.3}$/hour/µmol/L GSH)
Rat	CDBR	M	2.50	0.63	ND
		F	2.47	1.00	0.000241
	F-344	M	3.17	1.08	0.000249
		F	2.95	1.03	0.000227
	Wistar	M	3.11	0.45	0.000093
		F	2.97	1.55	0.00040
Mouse	B6C3F1	M	5.89	5.5	0.000827
		F	5.53	8.93	0.001670
	CD-1	M	6.99	5.1	0.000563
		F	5.54	6.62	0.000809
Hamster	Golden Syrian	M	4.94	1.67	0.000330
		F	4.76	2.06	ND

Source: U.S. Air Force 1990

F = female; GSH = glutathione; Kfc = first order of epoxide formation; Kgsc = rate constant for conjugation of vinyl chloride with glutathione; M = male; ND = not determined; V_{max} = maximum velocity of reaction

It does not appear that the investigators further validated the Wright-Patterson AFB model with data from studies other than those used to determine the metabolic parameters. This model was not used to estimate metabolized doses for humans because the investigators indicated that human data to estimate all of the required parameters were not available. They suggested that allometry may have to be used to estimate some of the parameters for humans.

3.1.5.3 Clewell et al. (1995) Human Models

Clewell et al. (1995) used PBPK modeling coupled with a linearized multistage model to predict human cancer risk. The model again had four compartments as described for the EPA (1987) study, and the same EPA physiologic parameters were used. Partition coefficients were from *in vitro* experiments and are shown in Table 3-1. Metabolism was modeled by two saturable pathways: one high affinity, low capacity (P4502E1), and one low affinity, high capacity (2C11/6 and 1A1/2). The metabolic parameters used were not provided, but they were estimated from the U.S. Air Force (1990) model. This model assumed that the metabolites (chloroethylene oxide and chloroacetaldehyde) were further degraded to carbon dioxide, reacted with glutathione, or reacted with DNA. The parameters (not stated) for the degradation reactions of chloroethylene oxide and chloroacetaldehyde were estimated from vinylidene chloride data (D'Souza and Andersen 1988) using appropriate allometric scaling.

Based on the Clewell et al. (1995) PBPK model and a linearized multistage model using liver angiosarcoma data from animal studies, the human risk estimates for lifetime exposure to 1 ppb vinyl chloride ranged from 1.1 to 15.7/million persons. Based on the incidence of liver angiosarcoma in human epidemiological studies, the risk estimates for lifetime exposure to 1 ppb vinyl chloride were 0.4–4.22/million persons. Clewell et al. (1995) indicated that the risk estimates in the occupational exposure range using PBPK modeling are about 30–50 times lower than estimates using external dose calculations based on the linearized multistage model.

Clewell et al. (2001) further refined the PBPK model for vinyl chloride. This model was applied by the EPA to develop quantitative toxicity values for vinyl chloride (i.e., reference dose [RfD], reference concentration [RfC], inhalation unit risk, oral slope factor) (EPA 2000). The model had four compartments and metabolism was modeled by two saturable pathways: one high affinity, low capacity (P4502E1), and one low affinity, high capacity (2C11/6 and 1A1/2). A description of glutathione kinetics was also included in the model. Cancer risk estimates in the occupational exposure range calculated using the PBPK model were consistent with risk estimates from epidemiological studies and were approximately 80-fold lower than cancer risk estimates from animal studies without PBPK modeling. The inhalation portion of the PBPK model is well documented with experimental inhalation data sufficient to ensure a high degree of confidence in the derived dose metrics. Less confidence is associated with the oral dose metrics due to the limited experimental data available (EPA 2000).

The Clewell et al. (2001) model was also applied to evaluate the potential impact of age- and sex-specific pharmacokinetic differences on the dosimetry of vinyl chloride (Clewell et al. 2004). The rate of metabolite production per volume of liver was estimated to rise rapidly from birth until about age 16 years, after which it remains relatively constant before rising again late in life. Other factors that may affect vinyl chloride toxicity at early life stages include the presence of fetal P450s and the level of glutathione transferase.

The PBPK model described in Clewell et al. (2001) and EPA (2000) was used to derive the chronic-duration oral MRL. For more information on ATSDR's use of the Clewell model, refer to Appendix A.

3.1.5.4 Reitz et al. (1996) Rat, Mouse, and Human Models

Reitz et al. (1996) also developed a PBPK model that coupled measures of delivered dose in rats to a linearized multistage model to predict the incidence of hepatic angiosarcoma in mice and humans. The model incorporated four compartments: fat, muscle, rapidly perfused tissues, and liver. Physiological parameters in the model were based on similar ones used in an earlier multispecies PBPK model developed for methylene chloride. Partition coefficients were estimated by vial equilibration techniques similar to those described in the U.S. Air Force (1990) study. Metabolic rate constants were obtained from *in vivo* gas uptake experiments performed at Wright-Patterson AFB.

Based on the PBPK-based procedure utilized by Reitz et al. (1996), the predicted human risk estimates ranged from about 200 cases of angiosarcoma per 100,000 (for workers employed 10 years at a plant where the time-weighted average [TWA] was 50 ppm) to almost 4,000 cases/100,000 in workers employed for 20 years in a plant where the TWA was 2,000 ppm. The predictions of human risk were compared with the data reported by Simonato et al. (1991). The predictions of angiosarcoma incidence in humans were almost an order of magnitude higher than actually observed in exposed human populations and were more than two orders of magnitude lower than risk estimations that did not utilize pharmacokinetic data.

3.1.5.5 Other Models

Yoon et al. (2007) evaluated the impact of assuming extrahepatic metabolism by CYP2E1 in PBPK models for vinyl chloride inhalation. The study concluded that predictions for the rat and human models were not significantly affected by the inclusion of extrahepatic metabolism by CYP2E1 in the kidney and lung. Chiu and White (2006) described the development of a simplified steady-state solution of a generic

PBPK model for volatile organic compounds. This steady-state analysis was shown to produce similar results to the full PBPK model reported in the EPA (2000) risk assessment for vinyl chloride. Mumtaz et al. (2012a) developed a generic seven-compartment PBPK model, which added compartments for blood, kidney, and skin. A comparison of the results of this model to the Clewell et al. (2001) model showed that both models adequately predicted blood concentrations during, and immediately following, exposure.

3.1.6 Animal-to-Human Extrapolations

Limited information is available regarding the toxicokinetic differences between species. Toxicokinetic data in humans are limited (Krajewski et al. 1980; Sabadie et al. 1980). A primate study suggested that metabolism may saturate at lower concentrations in primates (>300–400 ppm) than in rats (Buchter et al. 1980).

PBPK models were also developed to predict the metabolism and distribution of vinyl chloride in laboratory animals and humans (Section 3.1.5). The most recent PBPK model for vinyl chloride (Clewell et al. 2001) was applied by EPA to develop quantitative toxicity values for vinyl chloride (RfD, RfC, inhalation unit risk, oral slope factor) (EPA 2000). The model has four compartments and metabolism was modeled by two saturable pathways: one high affinity, low capacity (P4502E1), and one low affinity, high capacity (2C11/6 and 1A1/2). A description of glutathione kinetics was also included in the model. Cancer risk estimates calculated using the PBPK model were consistent with risk estimates from epidemiological studies.

There appears to be a correlation of vinyl chloride toxicity between humans and animals with regard to respiratory, cardiovascular, hematological, hepatic, dermal, immunological, neurological, reproductive and cancer effects. Renal effects of vinyl chloride, including increased relative kidney weight and an increase in severity of tubular nephrosis, are reported in several rat studies (Bi et al. 1985; Feron and Kroes 1979; Feron et al. 1979a). However, kidney toxicity was only observed in a single human study of exposure to multiple chlorinated solvents in drinking water (Chen and Wu 2017). Evidence for developmental effects of vinyl chloride has not been reliably demonstrated in epidemiology studies (Bao et al. 1988; Edmonds et al. 1975, 1978; Rosenman et al. 1989; Ruckart et al. 2013; Swartz et al. 2015; Talbott et al. 2015; Theriault et al. 1983) but did occur in studies of several animal species (John et al. 1977, 1981).

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 vinyl chloride are discussed in Section 5.7, Populations with Potentially High Exposures.

Data suggest that fetuses, infants, and young children are susceptible to the toxic effects of vinyl chloride. Some epidemiologic studies (Infante et al. 1976a, 1976b; NIOSH 1977) suggested an association between fetal death and birth defects and parental vinyl chloride exposure. However, the design and analysis of these studies has been criticized (Hatch et al. 1981; Stallones 1987). Some inhalation studies with animals have suggested that vinyl chloride is a developmental toxicant (e.g., produces delayed ossification) at doses that also produce maternal toxicity (John et al. 1977, 1981; Mirkova et al. 1978; Sal'nikova and Kotsovskaya 1980; Ungvary et al. 1978). However, no adverse effects on embryo-fetal development were noted in a rat inhalation study (Thornton et al. 2002).

Vinyl chloride can cross the placenta and enter the blood of the fetus (Ungvary et al. 1978). Studies by Drew et al. (1983) and Maltoni et al. (1981) have shown that animals exposed by inhalation prior to adolescence or during pregnancy may have a greater death rate and increased likelihood of developing cancer than adult animals exposed for similar periods. This may relate to the length of the induction period of hepatic angiosarcoma rather than to an increased susceptibility of the young, *per se*. Lifetime cancer risk was also dependent on the age of the animals at the time of exposure to vinyl chloride. Refer to Section 2.19 for more details on studies addressing cancer and age of vinyl chloride exposure.

It is also possible that there are explanations for these findings. Cogliano and Parker (1992) suggested that in the multistage model of carcinogenesis, carcinogens that induce an initial transition early in the life of an animal would be more effective since there would be a longer period of time remaining in the lifespan for completion of the remaining transitions. Their empirical model of the effect of age at exposure on the development of cancer suggests that there is an age-sensitive period of exposure to vinyl chloride.

An age-related increase in DNA adduct formation was noted in an inhalation study of lactating rats and their 10-day-old pups exposed to 600 ppm of vinyl chloride, 4 hours/day for 5 days (Fedtke et al. 1990). Concentrations of two adducts found in livers of pups were 4-fold higher than those found in livers of dams; however, pups were exposed to contaminated breast milk in addition to air concentrations of vinyl chloride. In another study, immature rats exposed to vinyl chloride formed 6 times more etheno-nucleosides compared with adults (Ciroussel et al. 1990). The concentration of ethenoguanine adducts was 2–3-fold greater in weanling rats as compared to adult rats exposed at the same dose for the time period (0, 10, 100, or 1,100 ppm, 6 hours/day for 5 days) (Morinello et al. 2002a).

Taken together, the studies cited above suggest an early life stage sensitivity to vinyl chloride carcinogenicity (Cogliano et al. 1996). EPA has recommended an adjustment of the cancer risk estimates to account for early life-stage sensitivity to vinyl chloride (EPA 2000; Ginsberg 2003).

The toxicokinetic behavior of vinyl chloride in children is expected to be similar to that in adults (Clewell et al. 2004; EPA 2000; Gentry et al. 2003). Urinary metabolites of vinyl chloride and other volatile compounds have been measured in preterm infants in a neonatal intensive care unit (El-Metwally et al. 2018). An evaluation of pharmacokinetic differences across life stages suggests that the largest difference in pharmacokinetics occurs during the perinatal period (Gentry et al. 2003). The most important factor appears to be the potential for decreased clearance due to immature metabolic enzymes systems. For instance, clearance is hampered in the embryonic liver because CYP2E1 is not expressed but rapidly increases during the first 24 hours after birth. Between the developmental ages of 1 and 10 years, children's CYP2E1 protein levels and enzyme activity are comparable to adults (EPA 2000).

Young children appear to have the capacity of metabolizing vinyl chloride to reactive intermediates that form DNA adducts that lead to cancer. A PBPK model was applied to evaluate the potential impact of age- and sex-specific pharmacokinetic differences on the dosimetry of vinyl chloride (Clewell et al. 2004). The rate of metabolite production per volume of liver was estimated to rise rapidly from birth

until about age 16, after which it remains relatively constant before rising again late in life. The data on CYP2E1 levels in the developing organism suggests that early life stage sensitivity to vinyl chloride-induced cancer is not solely due to an increase in the production of reactive intermediates via this isozyme. Fetal CYP isoforms may play a role in metabolism of vinyl chloride to reactive intermediates in the fetus and neonate. Glutathione conjugation may also differ in the developing organism. DNA repair capacity and other pharmacodynamic factors may also be associated with an early life stage susceptibility to cancer.

Individuals with comorbidities (e.g., obesity and liver disease) and genetic polymorphisms of HLA-DR5, HLA-DR3, and B8 alleles are unusually susceptible to the effects of vinyl chloride. Lifestyle factors such as exposure to organochlorine pesticides, consuming high-calorie diets, ethanol, or barbiturates, or taking Antabuse for alcoholism may make people have increased susceptibility to vinyl chloride effects. Irregular heart rhythms, impaired peripheral circulation, and systemic sclerosis (Section 3.3.2) may also increase susceptibility.

Mice fed a high-fat diet are more susceptible to liver injury induced by low concentrations of vinyl chloride. High-fat diet mice exposed to 0.85 ppm vinyl chloride for 12 weeks showed liver damage, neutrophil infiltration, non-parenchymal cell apoptosis, mitochondrial dysfunction, and oxidative and endoplasmic reticulum stress compared to mice fed a normal or low-fat diet (Chen et al. 2019; Fujiwara 2018; Lang et al. 2018, 2020; Liang et al. 2018; Liu et al. 2023; Wahlang et al. 2020). High-fat diet mice exposed to ≥63 ppm of vinyl chloride (2 hours/day, 5 days/week for 13 weeks) also showed steatosis, oxidative and endoplasmic reticulum stress in the liver, and upregulated expression of *de novo* lipogenesis-related proteins (Jia et al. 2022). Liu et al. (2023) reported that high-fat diet mice exposed to 0.85 ppm vinyl chloride for 6 hours/day, 5 days/week for 12 weeks had an increase in the number of hepatic tumors observed 9 months after exposure had ended, compared to mice fed a low-fat diet.

Mice injected with lipopolysaccharide or fed diets high in fat and exposed orally to 2-chloroethanol also experienced enhanced liver injury when compared to mice fed a normal or low-fat diet (Anders et al. 2016a, 2016b; Kaelin et al. 2020; Lang et al. 2019). This effect was attenuated by rapamycin, which protects against mitochondrial damage and subsequent oxidative stress (Lang et al. 2019). Mitochondrial ALDH2 may also play a role in detoxifying 2-chloroacetaldehyde (Chen et al. 2019). Activation of ALDH2 with an agonist (Alda-1) was shown to attenuate liver injury and reduce oxidative stress in high-fat diet mice exposed to vinyl chloride (Chen et al. 2019).

Vinyl chloride is metabolized in the liver in a multistep process. The prevalence of liver ultrasound abnormalities (not further defined) was associated with polymorphism of the CYP2E1 gene (c1c2/c2c2 genotype) (Zhu et al. 2005a). A genetic polymorphism of CYP2E1 (increase in CYP2E1 c2c2 genotype) was also associated with liver fibrosis, diagnosed by ultrasonography in 13 of 320 workers employed in five PVC manufacturing plants (Hsieh et al. 2007). No association was found between liver effects and genetic polymorphisms of glutathione transferase or aldehyde dehydrogenase in these studies. Polymorphisms of genes involved in metabolism (CYP2E1, GSTP1, ALDH2), DNA repair (hOGG1, MGMT, XRCC1, XPA, XPC, XPD, XPF, TDG, APE1), apoptosis (MDM2, BCL2) and cell cycle control (p53, p21) have been associated with increased micronuclei, sister chromatid exchange frequency, DNA damage and retention of DNA adducts in vinyl chloride workers (Feng et al. 2017; Ji et al. 2010; Li et al. 2006, 2009a, 2013; Qiu et al. 2008, 2011a; Wang et al. 2010a, 2010b, 2013b; Wen-Bin et al. 2009; Wong et al. 2003b; Zhu et al. 2005b, 2008). The occurrence of the mutation biomarkers in serum was correlated with polymorphisms of the DNA repair genes XRCC1 (mutant p53), excision repair cross complementation group 2 (ERCC2)/XPD (mutant p53 and ras-p21) and ALDH2 and CYP2E1 in vinyl chloride workers (Li et al. 2003b, 2006, 2009b). The presence of a polymorphism for CYP2E1 (variant c2 allele) was also associated with the occurrence of mutant p53 and ras-p21 serum biomarkers (Schindler et al. 2007). The risk of developing liver cancer also appeared to be elevated in those with a history of Hepatitis B viral infection (Du and Wang 1998; Wong et al. 2003b).

Vinyl chloride workers with genetic polymorphisms of genes related to metabolism, DNA repair, and cell cycle control may be more susceptible to liver toxicity and liver cancer. A polymorphism of the CYP2E1 gene was associated with an increase in liver abnormalities evaluated by ultrasound (Hsieh et al. 2007; Zhu et al. 2005a). Genetic polymorphisms of several genes were associated with increased micronuclei frequency, DNA damage, retention of DNA adducts, and an increase in tumor biomarkers in serum (Ji et al. 2010; Li et al. 2006, 2009a; Qiu et al. 2008, 2011a; Schindler et al. 2007; Wang et al. 2010a, 2010b, 2013b; Wen-Bin et al. 2009; Zhu et al. 2005b, 2008). The risk of developing liver cancer also appears elevated in those with a history of Hepatitis B viral infection (Du and Wang 1998; Wong et al. 2003b). Work by Black et al. (1983, 1986) has shown that persons with the HLA allele, HLA-DR5, may have an increased likelihood of developing vinyl chloride disease, and those with the alleles, HLA-DR3 and B8, may have an increased severity of the disease.

Phenobarbital and Aroclor 1254 increase mixed function oxidase (MFO) activity and have been shown to greatly increase the hepatotoxicity of vinyl chloride (Conolly and Jaeger 1979; Conolly et al. 1978; Jaeger et al. 1974, 1977; Jedrychowski et al. 1985; Reynolds et al. 1975a, 1975b). The intermediary metabolites

of vinyl chloride, 2-chloroethylene oxide and 2-chloroacetaldehyde, have been suggested to be responsible for some of the adverse effects produced by vinyl chloride. Thus, individuals taking barbiturates or who might be exposed to organochlorine pesticides that are known to induce microsomal enzymes (such as Aroclor 1254) would be expected to be at increased risk for developing vinyl chloride-induced hepatotoxicity.

Radike et al. (1981) demonstrated that ethanol-consuming rats exposed to vinyl chloride had an increased incidence of cancer and an earlier death rate than animals exposed to vinyl chloride in the absence of ethanol. Some persons consume the agent, Antabuse, to curb the desire for alcohol. In its role as a therapeutic agent, Antabuse blocks aldehyde dehydrogenase and causes a build-up of acetaldehyde, which is emetic, in the body when alcohol is consumed. If persons taking Antabuse are exposed to vinyl chloride, the alternative metabolic pathway for vinyl chloride metabolism will be blocked, causing more vinyl chloride to be metabolized to the toxic metabolite, 2-chloroethylene oxide. Thus, these persons may be at increased risk for hepatotoxicity, cancer, and death at an early age.

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 2006).

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 2006). 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 vinyl chloride are discussed in Section 3.3.1.

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 2006). 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 vinyl chloride 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

The only exposure biomarker specific to vinyl chloride is the measurement of this compound in expired air. Other exposure biomarkers are not specific to vinyl chloride exposure only. As such, there is limited utility in urine tests for thiodiglycolic acid and N-acetyl-S-(2-hydroxyethyl)-cysteine.

Vinyl chloride may be quantified in expired air following acute moderate-to-high exposures (Azari et al. 2016). The expiration of vinyl chloride follows first-order kinetics; therefore, this parameter can be directly correlated with exposure levels (Baretta et al. 1969). This measure provides the most direct evidence for vinyl chloride exposure. However, measurement of exposure by this technique is limited by the rapidity with which vinyl chloride is expired during breathing. The half-life of vinyl chloride in expired air is between 20 and 30 minutes following an inhalation exposure and is approximately 60 minutes following oral dosing (Watanabe and Gehring 1976; Watanabe et al. 1976b, 1978a, 1978b). Thus, testing must be initiated as soon as possible following termination of exposure. Measurement of vinyl chloride in expired air has limited utility for low-level exposures (<50 ppm) because of competition between absorptive uptake and rapid metabolism (Baretta et al. 1969). In addition, it provides no information on the duration of exposure.

Thiodiglycolic acid is a major urinary metabolite of vinyl chloride. Measurement of thiodiglycolic acid in urine can be used to monitor occupationally exposed workers (Cheng et al. 2001; Lee et al. 2020; Müller et al. 1979) and children living in the vicinity of industrial vinyl chloride-using facilities (Huang et al. 2016; Wang et al. 2019b). The validity of this biomarker for community health studies has been questioned in cases where exposure concentrations in air are generally low (<5 ppm) (Chen et al. 2018). The amount of thiodiglycolic acid in the urine varies according to individual metabolic idiosyncrasies because metabolism of vinyl chloride to thiodiglycolic acid is a saturable process. Therefore, when

exposure exceeds a certain level, the excretion of vinyl chloride as thiodiglycolic acid will plateau (Watanabe and Gehring 1976). In addition, the rate of metabolism of vinyl chloride to thiodiglycolic acid can be influenced by the presence of liver disease and by ethanol consumption as well as intakes of other substances such as barbiturates (Hefner et al. 1975b).

Similar to the measurement of vinyl chloride in expired air, the measurement of thiodiglycolic acid must take place shortly after exposure because of its rapid excretion. The half-life for excretion of thiodiglycolic acid following an acute-duration exposure is between 4 and 5 hours (Watanabe and Gehring 1976; Watanabe et al. 1978a, 1978b). Cheng et al. (2001) suggested that urinary thiodiglycolic acid levels should not be measured at the end of a work shift but are best detected at the beginning of the following workday. Excretion of thiodiglycolic acid is not unique to vinyl chloride exposure. For example, thiodiglycolic acid can be excreted in the urine as the result of exposure to vinylidene chloride, ethylene oxide, or 2,2-dichloroethylether (Norpoth et al. 1986; Pettit 1986). Infants delivered prematurely can have high levels of urinary thiodiglycolic acid. A correlation was observed between the thiodiglycolic acid levels and the number of weeks that the infant was born prematurely. The origin of this thiodiglycolic acid in neonates is unknown but is likely not associated with vinyl chloride exposure (Pettit 1986).

Boyle et al. (2016) suggest that urinary levels of N-acetyl-S-(2-hydroxyethyl)-cysteine may be a useful biomarker for combined exposure to vinyl chloride, ethylene oxide, and acrylonitrile. This compound is measured as a urinary biomarker for the listed volatile compounds in the National Health and Nutrition Examination Survey (NHANES) (Konkle et al. 2020).

3.3.2 Biomarkers of Effect

Biomarkers of effect for vinyl chloride include altered liver function, DNA adducts, and measures of genotoxicity including chromosomal aberrations, micronuclei, and DNA damage (i.e., strand breaks).

Liver function tests are sensitive indicators of the hepatic damage resulting from vinyl chloride exposure. These assays include the indocyanine clearance test, measurement of serum bile acids, and measurement of serum hyaluronic acid concentration (Berk et al. 1975; Liss et al. 1985; McClain et al. 2002; Vihko et al. 1984). In general, serum enzymes were found to be of limited value in monitoring the progression of vinyl chloride-induced hepatic changes (Berk et al. 1975; Liss et al. 1985; Vihko et al. 1984). This is likely due to the extent of hepatic damage produced by vinyl chloride and the late development of

necrotic areas in the disease process (Popper et al. 1981). A study of hepatic ultrasound abnormalities suggests that functional and imaging tests may be useful biomarkers of liver toxicity in workers exposed to vinyl chloride (Wang et al. 2008). Cave et al. (2010) suggested that an elevation of total cytokeratin 18 levels in serum may be indicative of liver cell necrosis (a known vinyl chloride effect).

The intermediary metabolites, 2-chloroethylene oxide and 2-chloroacetaldehyde, bind to macromolecules in the body. 2-Chloroethylene oxide is hypothesized to bind primarily to DNA and RNA, whereas 2-chloroacetaldehyde binds primarily to proteins (Bolt 1986; Guengerich and Watanabe 1979; Guengerich et al. 1979, 1981; Kappus et al. 1976; Watanabe et al. 1978a, 1978b). Several DNA adducts have been reported following vinyl chloride exposure (Mutlu et al. 2010, 2012; Pottenger et al. 2014; Swenberg et al. 2011; Yun et al. 2020). 7-(2-Oxoethyl) guanine (7-OEG) is the primary DNA adduct; however, it is not mutagenic (i.e., does not cause mispairing during replication) and would not be a biomarker of effect (Mutlu et al. 2010). N^2,3-Ethenoguanine is a mutagenic adduct and may be an important effect biomarker of vinyl chloride (Mutlu et al. 2010). Liquid chromatography-mass spectrometry (LCMS) and stable isotope methods have been used to detect DNA adducts in several tissues, including white blood cells and oral cells in humans (Yun et al. 2020) and liver, lung, and kidney in animals (Mutlu et al. 2010, 2012; Pottenger et al. 2014; Swenberg et al. 2011).

Ethenoguanine adducts may be quantified from urine following base excision repair and excretion where they can be measured using an LCMS method (Gonzalez-Reche et al. 2002). This method would also include the measurement of endogenously formed etheno-adducts; thus, it is critical to determine the background level of urinary adducts in a control population.

Chromosomal aberrations found in lymphocytes can be indicative of the genotoxic effects of vinyl chloride (Anderson 2000; Anderson et al. 1980; Ducatman et al. 1975; Fucic et al. 1990a, 1990b, 1992; Funes-Cravioto et al. 1975; Garaj-Vrhovac et al. 1990; Hansteen et al. 1978; Hrivnak et al. 1990; Kucerova et al. 1979; Purchase et al. 1978; Sinués et al. 1991). However, any of a number of genotoxic substances can produce chromosomal aberrations. de Jong et al. (1988) found that variability in the control population may obscure the observation of chromosomal aberrations in persons exposed to low levels of vinyl chloride. G-banding analysis appeared to provide a more sensitive indication of chromosomal alteration than sister chromatid exchanges (Zhao et al. 1996). DNA damage in lymphocytes can be directly assessed using a single-cell gel electrophoresis technique. The severity of the damage may correlate with the duration of exposure (Awara et al. 1998). The DNA adducts produced by the reactive intermediary metabolites of vinyl chloride, including 1,N^6-ethenoadenosine and

3,N^4-ethenocytidine, may be more specific indicators of vinyl chloride's genotoxic potential (Bolt 1986; Guengerich and Watanabe 1979; Guengerich et al. 1979, 1981; Kappus et al. 1976; Watanabe et al. 1978a, 1978b).

The micronucleus assay, performed using peripheral lymphocytes of 32 vinyl chloride workers, was used to indicate the time elapsed since the last vinyl chloride exposure occurred (Fucic et al. 1994, 1997). The study showed a decrease in the frequency of micronuclei and mitotic activity in proportion to the length of the interval after the last vinyl chloride exposure. For the group with 10 years of employment, the percentage of micronuclei decreased from 12.82 when exposure occurred on the day of blood sampling to 3.16 when the last exposure occurred 90 days before blood sampling (Fucic et al. 1994). Similar changes were noted when the mean duration of employment was 5 years. However, this use of the micronucleus assay must consider the total duration of exposure. Micronucleus frequency was shown to be several times higher in binucleated lymphocytes as compared to mononuclear lymphocytes in 25 workers exposed to vinyl chloride for an average of 10 years (Fučić et al. 2004). Zheng et al. (2019) suggested that reduced relative telomere length and gene expression of telomere associated proteins (i.e., shelterin complex) were associated with increased micronuclei and could be considered as potential biomarkers; however, these effects may be caused by many genotoxic compounds and are not specific to vinyl chloride.

3.4 INTERACTIONS WITH OTHER CHEMICALS

ATSDR (2007) prepared an interaction profile for chloroform, 1,1-dichloroethylene, trichloroethylene, and vinyl chloride. This report indicated that no direct data are available to characterize health hazards (and dose-response relationships) from mixtures containing all four components. In addition, PBPK/PD models have not yet been developed that would predict pertinent target doses of the components under mixture exposure scenarios. Toxicological data for the individual compounds suggest that sites of joint toxic action may include hepatic, renal, immunological, neurological, developmental effects, and cancer; however, no experimental data are available for mixtures (ATSDR 2007).

Studies have been performed to examine the effect of agents intended to alter the metabolism of vinyl chloride on its toxicity. For example, the effects of phenobarbital pretreatment on vinyl chloride-induced hepatotoxicity were examined by Jaeger et al. (1974, 1977), Jedrychowski et al. (1985), and Reynolds et al. (1975a, 1975b). Pretreatment of rats with phenobarbital for 7 days prior to a 4-hour vinyl chloride exposure produced an increase in microsomal CYP activity (Reynolds et al. 1975b) and enhanced

hepatotoxicity (Jaeger et al. 1974, 1977; Jedrychowski et al. 1985; Reynolds et al. 1975a, 1975b). In the absence of the phenobarbital pretreatment, a single exposure to approximately 50,000 ppm had no detectable adverse effect on the livers of exposed rats. However, following phenobarbital pretreatment, 50,000 ppm of vinyl chloride produced increased serum activity of hepatic enzymes (Jaeger et al. 1977; Jedrychowski et al. 1985), areas of hepatic necrosis (Reynolds et al. 1975a), or both (Jaeger et al. 1974; Reynolds et al. 1975b). Activation of ALDH2 with an agonist (Alda-1) was shown to attenuate liver injury and reduce oxidative stress in high-fat diet mice exposed to vinyl chloride (Chen et al. 2019).

Another agent known to increase CYP activity, Aroclor 1254, was also tested for its ability to enhance vinyl chloride-induced hepatotoxicity (Conolly and Jaeger 1979; Conolly et al. 1978; Jaeger et al. 1977; Reynolds et al. 1975b). Pretreatment of rats with Aroclor 1254 for several days prior to exposure to vinyl chloride resulted in an increase in serum activity of hepatic enzymes (Conolly and Jaeger 1979; Conolly et al. 1978; Jaeger et al. 1977; Reynolds et al. 1975b) and areas of hepatic necrosis (Conolly et al. 1978; Reynolds et al. 1975b). Additional support for a role for CYP in the enhanced toxicity of vinyl chloride was obtained using SKF525A, a CYP inhibitor. If SKF525A was administered following phenobarbital pretreatment and before vinyl chloride exposure, it blocked the ability of phenobarbital pretreatment to enhance vinyl chloride-induced hepatotoxicity (Jaeger et al. 1977).

The role of glutathione conjugation in vinyl chloride-induced toxicity was also examined (Conolly and Jaeger 1979; Jaeger et al. 1977). The investigators hypothesized that depletion of glutathione might enhance the toxicity of vinyl chloride by preventing the excretion of toxic intermediary metabolites. However, diethylmaleate, an agent known to deplete hepatic glutathione levels, had no effect on the toxicity produced by vinyl chloride following pretreatment with either phenobarbital (Jaeger et al. 1977) or Aroclor 1254 (Conolly and Jaeger 1979). Trichloropropene oxide (TCPO), another agent known to deplete hepatic glutathione, produced enhancement of the hepatic toxicity produced by Aroclor 1254 pretreatment and vinyl chloride exposure but only when the animals had been fasted prior to the vinyl chloride exposure (Conolly and Jaeger 1979). In this study, the authors hypothesized that the enhancement of vinyl chloride toxicity was a result of the ability of TCPO to inhibit epoxide hydrolase rather than its ability to deplete glutathione levels.

Although the depletion of cellular glutathione levels did not appear to enhance vinyl chloride toxicity, treatment with cysteine, the rate-limiting precursor in hepatic glutathione synthesis, increased hepatic glutathione levels and provided partial protection against the toxic effects produced by Aroclor 1254 and vinyl chloride (Conolly and Jaeger 1979).

Mastrangelo et al. (2004) showed that alcohol increased the risk of hepatocellular carcinoma and liver fibrosis in vinyl chloride workers. Possible mechanisms for this synergistic effect include alcohol induction of CYP2E1 and decreased liver glutathione levels resulting in increased formation of mutagenic metabolites (Voigt 2005). CYP2E1 induction may also increase hepatocellular proliferation and formation of ROS. In the experiment by Radike et al. (1981), ethanol-consuming rats exposed to vinyl chloride for a year had an enhanced incidence of hepatic angiosarcomas, hepatomas, and lymphosarcoma, earlier onset of the tumors, and an enhanced death rate. The incidence of vinyl chloride-induced angiosarcomas was potentiated by ethanol, whereas the increased incidences of hepatoma and lymphosarcoma by ethanol were additive in nature.

The effects of smoking on chromosomal aberrations in vinyl chloride-exposed workers was examined by Hrivnak et al. (1990), who found no effect of smoking in 43 workers exposed for an average of 11.2 years to levels of vinyl chloride ranging from 0.8 to 16 ppm. Most cytogenetic studies of the effects of smoking in humans have reported no effect on chromosomal aberrations, although the sister chromatid exchange frequency is usually elevated (Wong et al. 1998).

A study that examined the interaction between vinyl chloride and trichloroethylene using both inhalation exposures of rats and pharmacokinetic modeling found that trichloroethylene exposure inhibited vinyl chloride in a competitive manner (Barton et al. 1995). This interaction was observed only at high concentrations (both chemicals >10 ppm), and the study authors concluded that the interaction is not likely to be important for environmental exposures.