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

3.1 TOXICOKINETICS

A single volunteer study provides limited quantitative information on absorption and excretion. No additional human studies were located. Chloroethane toxicokinetic studies in rats and mice provide limited qualitative data for absorption and distribution and some quantitative data on metabolism and excretion. In summary:

- Chloroethane is readily absorbed following inhalation exposure in humans, rats and mice and oral exposure in rats. Following a 30-second inhalation exposure in humans, approximately 82% of the inhaled dose was retained in the body. The extent of absorption was not quantified in studies using rats and mice. The dermal absorption potential is low, as indicated by the estimated dermal flux rate of 0.99 mg/cm^2 hour.

- Rat partition coefficients indicate that chloroethane, once absorbed, would have a greater affinity for fat than for muscle or the liver. Distribution was widespread in rats following inhalation and oral exposure, with the highest concentrations found in ovaries, adrenals, fat, and skin.

- In rats and mice, the two major pathways of chloroethane metabolism are the production of acetaldehyde by cytochrome P450 (CYP), and conjugation of chloroethane with GSH to form S-ethyl-glutathione.

- Acetaldehyde is rapidly metabolized to acetic acid. The GSH metabolites are further metabolized to S-ethyl-L-cysteine in mice and S-ethyl-N-acetyl-L-cysteine in both rats and mice. GSH conjugate metabolites of chloroethane (i.e., mercapturic acids) are detected in the urine of rats and mice.

- Following a 30-second inhalation exposure in humans, 30% of the retained dose was excreted in expired air in the first hour. The rate of urinary excretion in the first hour was described as slow (i.e., <0.01% per minute).

- In animals exposed to relatively low concentrations or doses of chloroethane, excretion as exhaled CO_2 predominates, suggesting complete metabolism. At higher concentrations or doses in rats, where metabolism is saturated, exhalation of unchanged chloroethane is the primary excretion pathway. A similar pattern is observed in mice following exposure to high oral doses (i.e., exhalation of chloroethane is predominant); however, a shift towards higher urinary excretion of chloroethane metabolites occurs following inhalation of high concentrations in mice.

3.1.1 Absorption

Chloroethane is readily absorbed following inhalation exposure in humans, rats, and mice and oral exposure in rats (Dobkin and Byles 1971; Dow 1992; Finer 1966; Konietzko 1984; Lawson 1965;

Lehmann and Flury 1943; Morgan et al. 1970; Torkelson and Rowe 1981). Inhalation absorption of chloroethane is rapid in humans, which is demonstrated by the rapidity of anesthesia in humans following inhalation exposure (Dobkin and Byles 1971; Finer 1966; Lawson 1965). Human subjects were exposed to about 5 mg ^{38}Cl-labeled chloroethane for 30 seconds by taking one breath through the mouth and then holding it for 30 seconds (Morgan et al. 1970). Approximately 18% of the radioactivity was exhaled in the first two breaths, indicating that about 82% was retained.

Chloroethane is readily absorbed through the lungs and gastrointestinal tract in laboratory animals; however, the extent of absorption has not been quantified (Dow 1992; Konietzko 1984; Lehmann and Flury 1943; Torkelson and Rowe 1981). A dermal flux rate of 0.99 mg/cm^2/hour was estimated based on the physical-chemical properties of chloroethane (Fiserova-Bergerova et al. 1990). Based on the estimated dermal flux rate, the study authors considered chloroethane to have no significant dermal absorption potential. No quantitative studies were located regarding absorption in humans or animals following dermal exposure to chloroethane.

3.1.2 Distribution

Representative partition coefficients for chloroethane in humans, rats, and mice are provided in Table 3-1. These partition coefficients were measured *in vitro* using a vial equilibration method. The data for rats indicate that chloroethane has a higher affinity for fat than for blood, liver, or muscle (Gargas et al. 1989).

Table 3-1. Chloroethane Partition Coefficients

			Partition coefficient			
Species	Strain	Sex	Blood/air	Liver/air	Muscle/air	Fat/air
Human[a]	NA	NR	1.9	–	–	–
Human[b]	NA	NR	2.69±0.20	–	–	–
Rat[b]	Fischer 344	M	4.08±0.39[c]	3.61±0.32	3.22±0.68	38.6±0.7
Mouse[d]	B6C3F1	F	5.1±1.8	–	–	–

[a]Morgan et al. 1970.
[b]Gargas et al. 1989.
[c]The rat blood/air partition coefficient measured for rats by Gargas et al. (1989) was adjusted to 5.5 for the Gargas et al. (1990) PBPK model based on fit to closed-chamber gas data.
[d]Gargas et al. 2008.

– = no data; F = female; M = male; NA = not applicable; NR = not reported; PBPK = physiologically based pharmacokinetic

Distribution was widespread in rats following inhalation and oral exposure, with the highest concentrations found in ovaries, adrenals, fat, and skin (Dow 1992). In female Fischer 344 rats and B6C3F1 mice exposed to ^{14}C-chloroethane by inhalation (15,000 ppm for 6 hours) or gavage (single dose of 37 or 1750 mg/kg in corn oil), detectable levels of radioactivity were found in all organs examined (adrenal, blood, brain, fat, heart, liver, lung, muscle, kidney, ovary, uterus, skin, and remaining carcass) (Dow 1992). The highest levels of radioactivity were found in the ovaries, adrenals, fat (oral exposure only), and skin (inhalation exposure only). No accumulation was found in the uterus of rats or mice following inhalation or oral exposure.

Review articles provided some additional information about the distribution of chloroethane; however, the species in which the information was obtained was not stated (Konietzko 1984; Lehmann and Flury 1943). In the blood, approximately 75% of the chloroethane is bound to red blood cells and 25% is in the plasma (Konietzko 1984). The highest concentration of chloroethane in the animal body was found in fatty tissue around the kidney and the lowest was found in the cerebrospinal fluid (Konietzko 1984). The brain was said to accumulate a concentration 2 times that of the blood. Lehmann and Flury (1943) reported that chloroethane content in the brain and medulla oblongata was especially high.

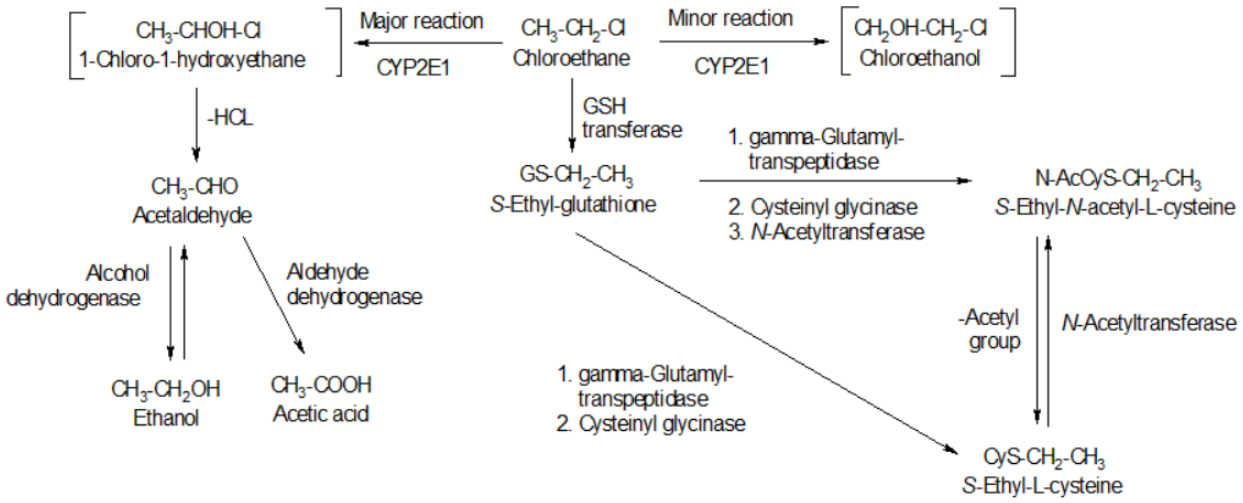
One study determined that chloroethane can be detected in the breast milk of nursing mothers (Pellizzari et al. 1982). The study was not quantitative and did not offer data concerning the percentage of nursing mothers that might excrete the compound in milk after exposure. It did not provide a range of concentrations of the compound in this medium. No studies were identified that determined if chloroethane was stored in maternal tissues. However, the rapid clearance of chloroethane, as well as its volatility, suggest that it would not be stored within the body for an extended period of time, so preconception maternal exposure is not likely to result in exposure to children during gestation or lactation.

3.1.3 Metabolism

Although no studies were located regarding metabolism of chloroethane by humans, the proposed metabolic pathways for chloroethane in rats and mice (Fedtke et al. 1994b; Figure 3-1) are relevant for humans. The two major pathways are the production of acetaldehyde by CYP and the conjugation of chloroethane with GSH to form S-ethyl-glutathione.

The metabolism of chloroethane to acetaldehyde was studied *in vitro* using livers from rats and mice exposed to chloroethane at 0 or approximately 15,000 ppm 6 hours/day for 5 days (Fedtke et al. 1994a). The amounts of acetaldehyde detected ranged from 26.9 to 49.3% of the chloroethane metabolized, depending on pre-exposure to chloroethane, for the individual microsome preparations from rats and mice. The investigators found that exposure to chloroethane induced its own metabolism by approximately 100% in mice and female rats, with no effect in male rats. Based on studies using specific CYP enzyme inducers and inhibitors, the investigators concluded that CYP2El was responsible for chloroethane metabolism. CYP2E1 also metabolizes alcohols, aldehydes, and ketones, and plays a role in gluconeogenesis within the body (Vieira et al. 1996). Most acetaldehyde is rapidly metabolized to acetic acid by aldehyde dehydrogenase; a small portion may be reduced by alcohol dehydrogenase to ethanol. Therefore, increased acetaldehyde relative to normal levels was not detected in the serum of chloroethane-exposed rats or mice (15,000 ppm) or in the urine of exposed rats (Fedtke et al. 1994a). Small increases in acetaldehyde were detected in the urine of chloroethane-exposed mice (Section 3.1.4, Excretion). Except for the approximately 3-fold greater metabolism of chloroethane in mice compared to rats, there was little difference between the species.

Figure 3-1. Metabolic Pathways for Chloroethane Biotransformation



Source: Fedtke et al. (1994b)

[ ] = known metabolites that were not detected in the referenced study; GSH = glutathione

GSH levels were studied in rats and mice exposed to chloroethane at 0 or 15,000 ppm 6 hours/day for 5 days (Dow 1992; Fedtke et al. 1994b). The animals were sacrificed immediately after the last exposure.

Compared to controls, GSH concentrations were significantly decreased in exposed animals. The significant GSH decreases occurred in the livers of male rats, in the kidneys of female rats, in the lungs of both sexes of rats and mice, and in the uterus of both rats and mice that were exposed to 15,000 ppm. The decreases in GSH levels were greatest in chloroethane exposed animals, particularly in the uterus of both species and in the lungs of mice, in which levels were approximately two-thirds lower than in controls (Fedtke et al. 1994b). Exposure to 15,000 ppm for 6 hours depleted tissue GSH levels to a greater extent in female mice than in female rats (Table 3-2); however, the species differences were less pronounced in the uterus and ovary compared to the liver, kidney, brain, and lung (Dow 1992). The time course of hepatic GSH depletion from chloroethane exposure demonstrated rapid depletion and recovery in female mice. Hepatic GSH depletion occurred more slowly in female rats; however, the rate of recovery was similar to mice, showing complete recovery by 18 hours after chloroethane exposure. Chloroethane exposure did not exhibit species differences for the rates of kidney and uterine GSH depletion and recovery. GSH depletion was not observed in rats and mice exposed to 150 or 3,000 ppm chloroethane for 6 hours (Dow 1992).

Table 3-2. Glutathione Depletion in Tissues of Female Fischer 344 Rats and B6C3F1 Mice Exposed to 15,000 ppm Chloroethane for 6 Hours

	Percent decrease in glutathione levels	
Tissue	Rats	Mice
Liver	35	79
Kidney	22	41
Brain	10	20
Lung	21	68
Ovary	43	44
Adrenal	68	32
Uterus	32	45

Source: Dow 1992

In vitro studies of chloroethane conjugation to GSH, using liver cytosolic fractions from control and chloroethane-exposed rats and mice, indicated that the conjugation was catalyzed by glutathione-S-transferase enzymes (Fedtke et al. 1994b). GSH conjugation rates, in nmol chloroethane conjugated/minute mg protein, were greater in mice (0.71±0.19 in males; 1.01±0.19 in females) than in rats (0.17±0.19 in males; 0.16±0.03 in females). Chloroethane exposure had no effect on these rates in rats and slightly decreased the rates in mice. When urine was analyzed for GSH metabolites, S-ethyl-N-acetyl-L-cysteine was detected in both rats and mice. However, only S-ethyl-L-cysteine was detected

in the urine of mice. The total amount of GSH metabolites excreted during the 5-day exposure period was about 5-fold higher in mice than in rats. The study authors concluded that rats completely metabolized S-ethyl-L-cysteine to more hydrophilic metabolites before urinary excretion, while these metabolic pathways were not available to the same extent in mice under the conditions of this study.

After GSH conjugation of chloroethane, three other enzymes convert the conjugate to a more hydrophilic form to be excreted by the body. These enzymes are γ-glutamyltranspeptidase, cysteinyl glycinase, and N-acetyltransferase (NAT) (Sipes and Gandolfi 1991). These three enzymes convert relatively hydrophobic GSH conjugates to their respective mercapturic acids, which can be excreted more readily.

The metabolic rates for chloroethane were estimated for male Fischer 344 rats using a gas uptake method (Gargas et al. 1990) (Table 3-3). The rats were exposed to an initial concentration of 100, 535, 1,200, or 2,350 ppm, and the disappearance of the gas was studied for about 5 hours. A physiologically based pharmacokinetic (PBPK) model that assumed metabolism occurred exclusively in the liver was used to analyze the data. The metabolism of chloroethane was best described by a combination of a saturable pathway and a first-order pathway.

Table 3-3. Estimates of Metabolic Parameters Obtained from Gas Uptake Experiments in Male Fischer 344 Rats

V_{maxc}, mg/hour*kg	4.0
V_{maxc}, µmol/hour	62.0
K_m, mg/L	0.1
K_m, µM	1.55
k_{tc}, $hour^{-1}*kg^{-1}$	1.0

V_{maxc} = maximum reaction velocity (scaled to 1 kg animal); K_m = concentration at ½ V_{max} (Michaelis constant); k_{tc} = first-order rate constant (scaled to 1 kg animal)

Source: Gargas et al. 1990

3.1.4 Excretion

Excretion of chloroethane by the lungs is rapid in humans and animals (Konietzko 1984; Lehmann and Flury 1943; Torkelson and Rowe 1981). In animals exposed to relatively low concentrations or doses of chloroethane, excretion as exhaled CO_2 predominates, suggesting complete metabolism (Dow 1992). At higher concentrations or doses in rats, where metabolism is saturated, exhalation of unchanged chloroethane is the primary excretion pathway (Dow 1992). A similar pattern is observed in mice

following exposure to high oral doses (i.e., exhalation of chloroethane is predominant); however, a shift towards higher urinary excretion of chloroethane metabolites occurs following inhalation of high concentrations in mice (Dow 1992).

In humans exposed briefly by inhalation to chloroethane, 30% of the retained dose was excreted in the breath within 1 hour (Morgan et al. 1970). Excretion over a longer period of time could not be measured because of the short half-life of the ^{38}Cl radioisotope used in this study. Morgan et al. (1970) found that the rate of excretion of radioactivity in the urine of humans was very slow (i.e., <0.01% per minute) 1 hour after inhalation.

The excretion pattern was similar in rats and mice exposed to 150 ppm for 6 hours, with the highest percentage of radioactivity recovered as expired CO_2, followed by tissues and carcass, urine, and feces (Table 3-4). Less than 2% was found as unchanged chloroethane in expired air in animals exposed to 150 ppm, suggesting that most of the radioactivity was eliminated as metabolites at this concentration. Exposure to 15,000 ppm chloroethane caused a shift in the excretion pattern which differed in rats and mice. In rats, the highest percentage of radioactivity was recovered as unchanged chloroethane; decreases in recovered radioactivity were observed in expired CO_2, urine, feces, and tissue/carcass compared to the 150-ppm group. In mice, expired chloroethane was also increased, but to a much lesser extent than seen in rats (i.e., 60-fold in rats versus 4-fold in mice). The time course of excretion was characterized by rapid exhalation of unchanged chloroethane (i.e., within the first hour). Exhalation as ^{14}C-CO_2 occurred primarily during the first 12 hours, while urinary excretion occurred over the first 24 hours.

Table 3-4. Excretion of Chloroethane and Metabolites Following Inhalation Exposure in Female Fischer 344 Rats and B6C3F1 Mice

	Percent of recovered radioactivity 48 hours after a 6-hour inhalation exposure	
Media	150 ppm	15,000 ppm
Rats		
Expired chloroethane	1.12	62.81
Expired CO_2	53.57	19.17
Urine	9.66	8.68
Feces	3.15	1.60
Tissues and carcass	32.03	7.64
Total	99.53	99.90

Table 3-4. Excretion of Chloroethane and Metabolites Following Inhalation Exposure in Female Fischer 344 Rats and B6C3F1 Mice

	Percent of recovered radioactivity 48 hours after a 6-hour inhalation exposure	
Media	150 ppm	15,000 ppm
Mice		
Expired chloroethane	1.72	6.96
Expired CO_2	41.76	31.80
Urine	15.86	38.37
Feces	6.02	7.05
Tissues and carcass	34.65	16.02
Total	100.01	100.20

Source: Dow 1992

Following single gavage exposures of 57 mg/kg in Fisher 344 rats or 37 mg/kg in B6C3F1 mice, the excretion pattern suggested more excretion as unchanged chloroethane in rats compared to mice and more excretion as expired ^{14}C-CO_2 in mice compared to rats (Table 3-5). Repeated administration of 37 mg/kg for eight daily doses in mice showed a similar excretion pattern as seen after a single gavage dose. Administration of a higher oral dose (1,999 mg/kg in rats, 1,970 mg/kg in mice) enhanced the excretion of unchanged chloroethane in expired air in both species, suggesting that metabolic pathways may be saturated at high doses. The time course of excretion was similar for oral and inhalation exposure with unchanged chloroethane excreted within the first hour, exhalation as ^{14}C-CO_2 occurring primarily during the first 12 hours and urinary excretion occurring over the first 24 hours.

Table 3-5. Excretion of Chloroethane and Metabolites Following Oral Exposure in Female Fischer 344 Rats and B6C3F1 Mice

Media	Percent of recovered radioactivity 48h after oral gavage dosing	
Rats (single dose)	57 mg/kg	1,998 mg/kg
Expired chloroethane	42.78	84.28
Expired CO_2	40.34	4.53
Urine	2.09	0.81
Feces	0.77	0.19
Tissues and carcass	7.04	0.76
Total	93.02	90.57

Table 3-5. Excretion of Chloroethane and Metabolites Following Oral Exposure in Female Fischer 344 Rats and B6C3F1 Mice

Media	Percent of recovered radioactivity 48h after oral gavage dosing		
Mice	34 mg/kg (single dose)	37 mg/kg (eight daily doses)	1,970 mg/kg (single dose)
Expired chloroethane	16.75	13.82	73.60
Expired CO_2	60.82	64.29	9.62
Urine	2.88	2.93	1.68
Feces	1.44	1.19	0.37
Tissues and carcass	7.02	5.80	1.14
Total	88.91	88.03	86.41

Source: Dow 1992

Small increases in acetaldehyde were detected in the urine of chloroethane-exposed mice but not rats (Fedtke et al. 1994a). Acetaldehyde concentrations in the urine were 7.9–20.3 and 0–18.1 µmol/L, respectively, in control male and female mice and 15.4–70.1 and 11.6–17 µmol/L, respectively, in mice exposed to 15,000 ppm chloroethane for 6 hours. Acetaldehyde is rapidly metabolized to acetic acid; therefore, it would be difficult to detect in whole animal studies. GSH conjugates have also been detected in the urine of rats and mice exposed to chloroethane (Fedtke et al. 1994b). Rats excreted the more hydrophilic S-ethyl-N-acetyl-L-cysteine, while mice excreted both S-ethyl-N-acetyl-L-cysteine and S-ethyl-L-cysteine. During the 5 days that rats and mice were exposed to chloroethane at 15,000 ppm for 6 hours/day, the total amount of GSH metabolites excreted in the urine was about 5-fold higher in mice than in rats.

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).

Gargas et al. 1990

Gargas et al. (1990) used a rat dosimetry model to analyze gas uptake curves from closed-chamber exposures to chloroethane. Three rats placed in a closed chamber were exposed to target concentrations of 100, 535, 1,200, or 2,350 ppm chloroethane for 3–6 hours. The chamber atmosphere was sampled every 10 minutes and analyzed by gas chromatography to monitor the time course of gas uptake. The series of uptake curves was analyzed using the rat dosimetry model which included physiological parameters (body and organ weights, alveolar ventilation blood flow) and blood and tissue solubility as reported by Gargas et al. (1989). The dosimetry model included compartments for fat, liver, other rapidly perfused tissues (i.e., adrenals, kidney, brain, uterus, ovaries, and testes) and slowly perfused tissues. Metabolism was assumed to occur solely in the liver and the mass balance equation accounted for saturable metabolism, defined by V_{maxc} and $K_{m,}$ and a first order rate constant (k_{tc}) (Table 3-3). Chamber uptake curves were simulated and compared to the experimental data. Kinetic constants were adjusted, and simulations were repeated to obtain an adequate visual fit and computer optimization was performed by varying V_{maxc} and k_{tc} values until the best least-square fit was achieved.

Chloroethane metabolism was described as a combination of saturable and first-order processes. In a second experiment, rats were exposed to a chamber concentration of 600 ppm chloroethane after pretreatment with pyrazole; the uptake kinetics were significantly altered by pretreatment with pyrazole. Analysis using the PBPK model showed near complete inhibition of oxidative CYP metabolism by pyrazole pretreatment.

Gargas et al. 2008

Gargas et al. (2008) expanded the existing PBPK model for rats (Gargas et al. 1990) and developed chloroethane PBPK models for mice and humans (women) to facilitate species comparisons. Tissue compartments represented in the model included gas exchange in the lung, fat, adrenals, kidneys, brain, uterus, ovaries/testes, liver, other richly perfused tissues, and slowly perfused tissues. The tissue:blood partition coefficients for mice and humans were calculated by dividing the rat tissue:air partition

coefficients by the mouse or human blood air partition coefficients. Blood:air partition coefficients for rats, mice, and humans are shown in Table 3-1 (Gargas et al. 1989, 1990, 2008).

Oxidative metabolism by CYP was saturable with respect to chloroethane concentration and GSH conjugation was considered saturable with respect to both chloroethane concentration and GSH level. Tissue GSH concentrations were evaluated as a balance between zero-order synthesis or delivery and first order loss due to use (i.e., GSH conjugation), degradation, or export. Reported tissue levels of GSH (liver, kidney, brain, ovary, adrenal gland, and uterus) and GSH depletion data (Dow 1992) were used to calculate GSH-conjugation rates for rats and mice. GSH levels and conjugation rates in humans were estimated using a parallelogram method. Parameter fitting for oxidative (mouse only) and GSH metabolism (rats and mice) was accomplished using closed chamber uptake (Gargas et al. 1990) and GSH depletion data (Dow 1992). Validation of the rat and mouse models was accomplished by comparing measured (Fedtke et al. 1994b; Landry et al. 1982) and model-predicted GSH values in the liver, kidney, and uterus. Predicted GSH values were reasonably accurate following a single chloroethane exposure but were somewhat less accurate following repeated exposure. Limited data are available for validating the human model. The volunteer study of chloroethane uptake and retention (Morgan et al. 1970) was used for this purpose and modeled estimates of retention were similar to the measured values.

Species comparisons using the rat, mouse, and human models predict CYP saturation to occur at 200–500 ppm chloroethane in rats, >1,000 ppm in mice, and between 1,000 and 3,000 ppm in humans. Saturation of GSH metabolism is predicted to occur between 6,000 and 9,000 ppm in all three species. Mice were predicted to produce more GSH metabolites of chloroethane, compared to rats and humans. The PBPK modeling results are consistent with the hypothesis that a GSH-derived metabolite of chloroethane, formed via oxidation by CYP (likely producing acetaldehyde), and conjugation with GSH may be involved with the mode of action for uterine tumors in mice. Model limitations include the small number of animals used for the closed chamber uptake experiments and the inability of the GSH submodel to account for an increase in GSH levels that exceed initial concentrations. In addition, validation of the human model is limited by the availability of human data.

3.1.6 Animal-to-Human Extrapolations

Inhalation absorption of chloroethane and excretion in expired air and urine occurs in humans, rats, and mice. Data are not available to compare the extent of absorption or excretion in humans and animals. The distribution and metabolism of chloroethane have not been studied in humans. A PBPK model was

developed to compare the internal dosimetry of chloroethane following inhalation in rats, mice, and humans (Gargas et al. 2008); however, limited data were available to validate the human model.

Animals and humans appear to have similar respiratory and neurological toxicity. Stimulation of the respiratory rate was seen in exposed humans and guinea pigs (Cole 1967; USBM 1929). Stimulation of the vagus nerve was observed in humans and dogs at anesthetic levels (Bush et al. 1952). Dogs also showed cardiac depression, but no attempt to evaluate cardiac depression was made in humans (anesthetic level maintained at stage 3 for subject safety). The symptoms of intoxication and the anesthetic properties of chloroethane were similar in humans, guinea pigs, and dogs (Bush et al. 1952; Davidson 1925; Demarest et al. 2011; Morris et al. 1953; USBM 1929). Animal data are extensive for respiratory, cardiovascular, and neurological effects; however, data in humans are limited to case reports of poisoning following use as a recreational inhalant and reports of chloroethane used as a local or general anesthetic.

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

There are limited reports concerning children being exposed to chloroethane. Effects observed in humans exposed to chloroethane have resulted primarily from inhalation exposure. Respiratory paralysis was reported to be the cause of death of a 14-year-old child who died during anesthesia with chloroethane (Kuschinsky 1970); however, the concentration of chloroethane administered was not known. Another

study reported vagal stimulation in children briefly exposed to reportedly high concentrations of chloroethane; the specific levels were not indicated (Bush et al. 1952).

Chloroethane has also been used and sometimes misused as a topical anesthetic in both children and adults (Nibhanipudi 2015; Noble 1979; Ramsook et al. 2001; Soueid and Richard 2007; van Ketel 1976). Misuse occurs when excessive amounts of chloroethane are sprayed on the skin for long periods of time. Three children suffered frostbite on the exposed skin of their ears and necks after having their earlobes sprayed with chloroethane for several minutes (Noble 1979).

Effects seen in adults exposed to chloroethane are also expected in children. In particular, the nervous system is likely to be a sensitive target of chloroethane, as it is in adults. Since infants and young children have a larger proportion of their bodies as brain mass with a greater cerebral blood flow than adults (Swenberg et al. 1992), the pharmacokinetics indicate a higher potential for chloroethane to reach the brain of a child. Infants and young children (ages not specified) would therefore be more susceptible to the anesthetic effects of chloroethane than adults.

No studies were identified that reported effects in adults from chloroethane exposure that occurred during childhood. There is no information on the health effects of exposures in young animals after birth. There are no data concerning the effects of chloroethane exposure on human development and there are only two developmental studies in animals (Dow 1985; Scortichini et al. 1986). Inhalation of chloroethane doses (≤4,946 ppm) during GDs 6–15 were not maternally toxic, although a trend for an increased incidence of mouse fetuses with increased skull foramina was observed (Scortichini et al. 1986). Dow (1985) reported that the fetuses exposed *in utero* (GDs 6–15) to 5,000 ppm appeared normal; however, fetuses were not examined for skeletal or visceral alterations.

There are no data available concerning the pharmacokinetics of chloroethane in children. There are no human or animal studies available concerning the ability of chloroethane or its metabolites to reach and cross the placenta.

One study determined that chloroethane is present in the breast milk of nursing mothers (Pellizzari et al. 1982). The study was not quantitative and did not offer data concerning the percentage of nursing mothers who might excrete the compound in milk after exposure. Further, the study did not provide a range of concentrations of the compound in this medium.

No studies were identified that determined if chloroethane was stored in maternal tissues. However, the rapid clearance of chloroethane, as well as its volatility, suggest that it would not be stored within the body for an extended period of time, so preconception maternal exposure is not likely to result in exposure to children during gestation or lactation.

No data are available to indicate that distribution of chloroethane is different in children. However, chloroethane distribution may be very different in children relative to adults due to the difference in fat and water content and lean body mass in children. Physical-chemical properties of chloroethane indicate that it would be readily soluble in fat. In the newborn and young infant, fat tissue is relatively scarce (15% of body weight) (Morselli et al. 1980) as compared to an adult, indicating that distribution of lipophilic chloroethane will differ in infants and young children relative to adults. In addition, infants and younger children have much more water (total body and extracellular) relative to body weight than adults. Given this, the distribution of water-soluble compounds, such as chloroethane metabolites, will differ in children as compared with adults (Morselli et al. 1980).

No data are currently available to indicate that the metabolism of chloroethane is different in children when compared to adults. However, the chloroethane metabolism scheme has enzyme families that are developmentally regulated. Chloroethane is metabolized by both CYP and by glutathione S-transferase. Studies have shown that liver glutathione S-transferase activities are low in prepubertal male and female rats, but as the rats reach sexual maturity (at around 30–50 days of age), GSH-conjugating activity is 2–3-fold higher in males than females (Lamartiniere and Lucier 1983). The difference in glutathione S-transferase activity was dependent on pituitary secretions. Further research on hypophysectomized male and female rats revealed that growth hormones may contribute to the establishment of glutathione S-transferase activities (Lamartiniere 1981). No data are available to indicate that glutathione S-transferase activity is also developmentally or sexually expressed in humans.

During the process of metabolism, NAT enzymes may convert the chloroethane conjugate to a less hydrophilic form, allowing it to be excreted (Sipes and Gandolfi 1991). There are two NAT enzyme families, NAT 1 and NAT2. Of these enzymes, only NAT2 is developmentally regulated. It is unknown which NAT enzyme metabolizes chloroethane; therefore, it is unknown whether chloroethane is developmentally regulated by this pathway.

Studies have shown that CYP2E1 is developmentally expressed in humans (Vieira et al. 1996). This enzyme is not detectable from livers of fetuses at 14–40 gestational weeks. However, the level of the

protein rises sharply in the first day after birth (1 unit/mg protein) and continues to increase until it reaches adult values of approximately 5 units/mg protein, in children from 1 to 10 years of age (Vieira et al. 1996).

It is unknown whether children differ from adults in their susceptibility to chloroethane, despite the theoretical reasons for which they might potentially differ, as discussed above.

In humans, there are no data concerning parental exposure affecting children, including preconception exposure. There are no data concerning preconception exposure of either parent to germ line mutations, developmental defects, childhood cancer, or other health effects in humans. Chloroethane is mutagenic in bacterial and mammalian cells incubated *in vitro*. However, chloroethane is negative for mutagenicity of mammalian cells *in vivo*. These inconclusive results do not allow the prediction of chloroethane genotoxicity in humans.

No population has been identified that is unusually susceptible to toxic effects resulting from chloroethane exposure. Since chloroethane is metabolized by CYP2E1 (Fedtke et al. 1994b), it is possible that individuals with polymorphisms in CYP2E1 may be more susceptible to toxic effects of chloroethane. Also, CYP2E1 is induced in people who frequently drink alcohol, as well as people with medical conditions such as diabetes. Therefore, populations that frequently drink alcohol or have diabetes, may be more susceptible to effects of chloroethane.

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 chloroethane 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 chloroethane 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

Chloroethane levels can be measured in the blood with a limit of detection of 0.045 µg/L. These measurements were added to the National Health and Nutrition Examination Survey (NHANES) study in 2013 to evaluate exposure in the U.S. population (CDC 2017, 2018, 2020). Because a portion of the chloroethane inhaled is exhaled, measurement of chloroethane in breath may also serve as a useful biomarker of exposure.

In rats and mice, chloroethane is metabolized to acetaldehyde and the GSH conjugates, S-ethyl-N-acetyl-L-cysteine and S-ethyl-L-cysteine (Fedtke et al. 1994a, 1994b). The GSH conjugates, S-ethyl-N-acetyl-E-cysteine and S-ethyl-L-cysteine, would not be biomarkers unique to chloroethane exposure. Acetaldehyde forms adducts with plasma proteins. Ethanol is also metabolized to acetaldehyde; thus, measurement of adducts, or of antibodies produced in response to these adducts, would also indicate ethanol exposure (Worrall et al. 1994); these therefore would not be a specific biomarker for chloroethane.

3.3.2 Biomarkers of Effect

Clinical signs of toxicity in volunteers exposed to 13,000–20,000 ppm chloroethane for short exposure durations include neurological (e.g., intoxication) and gastrointestinal effects (e.g., abdominal cramps and vomiting) (Davidson 1925; USBM 1929). Anesthesia occurs in humans by inhalation of chloroethane at a concentration of approximately 40,000 ppm (Dobkin and Byles 1971). Other effects reported at anesthetic concentrations include cardiac irregularities, respiratory paralysis, and nausea. Since these effects occur following exposure to many chemicals, they would not serve as useful biomarkers for chloroethane exposure.

3.4 INTERACTIONS WITH OTHER CHEMICALS

In the past, chloroethane, combined with nitrous oxide and oxygen, was used to maintain anesthesia in patients previously made unconscious by administration of either thiopentone (thiopental), nitrous oxide, or a mixture of nitrous oxide, chloroethane, and oxygen (Cole 1956). A concentration of 20,000 ppm chloroethane was initially required to maintain anesthesia, but this could slowly be reduced to as low as 5,000 ppm in some cases. Anesthesia could be maintained up to an hour using chloroethane in this manner. In a similar study using 36,000 ppm chloroethane, the length of time required to recover from anesthesia varied from 3 to 15 minutes in 33 subjects (Cole 1967). Vomiting occurred in 10 of 23 patients who were anesthetized with 36,000 ppm chloroethane combined with nitrous oxide and oxygen (Cole 1967).

A study in cats demonstrated that the extent of methemoglobinemia induced by intravenous administration of aniline was significantly reduced in cats anesthetized with chloroethane compared to unanesthetized cats (McLean et al. 1967). The rate at which the methemoglobin disappeared, however, was also significantly reduced in the anesthetized cats compared with unanesthetized cats. The results suggest that concurrent exposure to aniline and chloroethane may induce less methemoglobin than exposure to aniline alone, but the methemoglobin induced by the combined exposure would persist longer than that induced by exposure to aniline alone. A similar effect was not observed when cats were anesthetized with chloralose and treated with phenylhydroxylamine, the aniline metabolite that results in methemoglobin formation. Therefore, the study authors concluded that chloralose acts by inhibiting the metabolism of aniline. It is not known if chloroethane acts in the same manner.

No studies were available investigating the interactions of chloroethane with other chemicals in children or in adults.