

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

3.1 TOXICOKINETICS

Information on the toxicokinetics of chloromethane are available from limited human studies and several animal studies.

- Chloromethane is readily absorbed from the lungs and rapidly approaches equilibrium with the blood (Putz-Anderson et al. 1981b; Putz-Anderson et al. 1981a).
- Animal studies demonstrate that chloromethane absorbed from the lungs is extensively distributed throughout the body with relatively little variation in the pattern of distribution with respect to dose (Kornburst et al. 1982, Chellman et al. 1986a, von Oettingen et al. 1949, 1950).
- Rapid and biphasic blood clearance was found in humans, rats, and dogs (Landry et al. 1983a, Nolan et al. 1985, Putz-Anderson et al. 1981a).
- Conjugation of chloromethane via glutathione transferase is the main form of metabolism in humans and animals. Cytochrome P-450 may dehalogenate chloromethane to formaldehyde, but oxidation of GSH–chloromethane conjugation intermediates by cytochrome P-450 may also be involved in the formation of formaldehyde (Heck et al. 1982; Kornburst and Bus 1983).
- Very little chloromethane is excreted unchanged. The majority of the metabolites are excreted in the urine or expired as carbon dioxide (Morgan et al. 1970; Putz-Anderson et al. 1981a).

3.1.1 Absorption

Chloromethane is absorbed readily from the lungs of humans following inhalation exposure. Alveolar breath levels of chloromethane approached equilibrium within 1 hour during a 3- or 3.5 hour exposure of men and women (Putz-Anderson et al. 1981b; Putz-Anderson et al. 1981a). Mean \pm SD alveolar expired breath levels were 63 ± 23.6 ppm in 24 men and women exposed to 200 ppm, and 36 ± 12 ppm in 8 men and women exposed to 100 ppm for 3 hours. Mean \pm SD blood levels were 11.5 ± 12.3 ppm for the 200 ppm exposed group, and 7.7 ± 6.3 ppm for the 100 ppm exposed group. The results indicate that uptake was roughly proportional to exposure concentration, but individual levels were quite variable. A high correlation between alveolar air and blood levels ($r=0.85$, $p<0.01$) was found.

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Blood and expired air levels of chloromethane also approached equilibrium during the first hour of exposure in 6 men exposed to 10 or 50 ppm for 6 hours (Nolan et al. 1985). The levels in blood and expired air were proportional to the exposure concentrations. Based on elimination data, the subjects were divided into two groups, fast and slow metabolizers. The difference between inspired and expired chloromethane concentrations indicated that the fast metabolizers absorbed chloromethane at the rate of 3.7 $\mu\text{g}/\text{min}/\text{kg}$, and the slow metabolizers absorbed it at 1.4 $\mu\text{g}/\text{min}/\text{kg}$.

In experiments in rats, uptake of chloromethane approached equilibrium within 1 hour and was proportional or nearly proportional to exposure concentrations of 50-1,000 ppm for 3-6 hours (Landry et al. 1983a; Landry et al. 1983b). Absorbed doses (and absorption rates) for 6-hour exposures were calculated as 67 mg/kg (0.167 mg/min/kg) for rats exposed to 1,000 ppm, and 3.8 mg/kg (0.01 mg/min/kg) for rats exposed to 50 ppm (i.e., a ratio of 17.6). The ratio is nearly proportional to the actual exposure concentration ratio of 20. The difference was assumed to be a slightly lower uptake at the higher dose (perhaps due to a decrease in minute volume such as is observed when animals inhale formaldehyde or another irritant), or to lower metabolism at the higher concentration. Blood chloromethane concentrations reached approximately 90% of equilibrium within 1 hour for dogs exposed to 50 or 1,000 ppm (Landry et al. 1983a), or 15,000 or 40,000 ppm (von Oettingen et al. 1949, 1950) for 6 hours, and the concentration was proportional to the exposure concentration (Landry et al. 1983a; von Oettingen et al. 1949). This proportionality was confirmed at 15,000 and 40,000 ppm chloromethane for which the respective blood concentrations in dogs peaked at 0.12 mmol/100 cc at the lower dose, with proportional extrapolation to approximately 0.32 mmol/100 cc at the higher dose (von Oettingen et al. 1949).

Gaskin et al. (2018) evaluated in vitro skin permeability of gaseous chloromethane using human epidermis. Chloromethane gas was diluted to 20,000 ppm and 2,000 ppm to reflect the lowest reported lethal concentration (LC) and an immediately dangerous to health (IDLH) concentration, respectively. Short-term exposures of less than one hour were used to reflect possible exposures in the workplace or HAZMAT situations. Skin penetration by chloromethane was reported after 15 minutes and increased by a factor of 10 after one hour of exposure at 20,000 ppm. As a result of this analysis a skin notation was assigned by ACGIH (2018).

No studies were located regarding absorption in humans or animals after oral exposure to chloromethane.

3.1.2 Distribution

No studies were located regarding distribution in humans or animals after oral or dermal exposure to chloromethane. One study was located regarding distribution in humans after inhalation exposure to chloromethane.

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Putz-Anderson et al. (1981a) exposed volunteers to 100 ppm (n=8) or 200 ppm (n=24) chloromethane for 3 hours and collected blood and periodic breath samples. Breath concentrations approached equilibrium within one hour and averaged 36 ± 12 ppm and 63 ± 23.6 ppm for the respective doses. The respective blood concentrations were 7.7 ± 6.3 ppm and 11.5 ± 12.3 ppm. There was a high degree of correlation between blood and breath concentrations ($r = 0.85$, $N=29$, $p < 0.01$).

After absorption of chloromethane, distribution of chloromethane and/or its metabolites is extensive in animals. Total uptake of radioactivity (as $\mu\text{mol } ^{14}\text{C}$ -chloromethane equivalents/g wet weight) in whole tissue homogenates following exposure of rats to 500 ppm for 6 hours was 1.21 for lungs, 4.13 for liver, 3.43 for kidneys, 2.29 for testes, 0.71 for muscles, 0.57 for brain, and 2.42 for intestines (Kornbrust et al. 1982). In rats exposed to 5000 ppm for 2 hours and sacrificed 4 hours later, the comparable values were 1.46 for liver, 0.98 for kidneys, 1.02 for testes, 0.69 for epididymides, and 0.36 for brain (Chellman et al. 1986a). Little difference in the pattern of distribution was found at an exposure concentration of 1,500 ppm as compared with 500 ppm. Upon acid precipitation of protein, 80% of the radioactivity present in liver and testes was found in the acid soluble (unbound) fraction. The remainder was found to have been metabolically incorporated into lipid, ribonucleic acid (RNA), DNA, and protein, rather than bound to the macromolecules as a result of direct alkylation. Tissue levels of chloromethane (in mg%) in dogs exposed to chloromethane for 6 hours were 13 in liver, 15 in heart, and 16 in brain at 15,000 ppm and 9.3 in liver, 8.1 in heart, and 9.9 in brain at 40,000 ppm (von Oettingen et al. 1949, 1950).

3.1.3 Metabolism

Information regarding metabolism of chloromethane in humans is limited. Nolan et al. (1985) exposed human volunteers to either 10 or 50 ppm chloromethane and determined that 15% and 61% of the chloromethane was metabolized within 6 hours after exposure, respectively, by those who metabolized chloromethane slowly or more rapidly (termed slow and fast metabolizers). Unlike previously reported assessments, they found that the amounts of urinary S-methylcysteine excreted by each group was comparable to that during the preexposure period. Another finding was that blood levels were 10-fold higher than previously reported, purportedly due to a rapid loss of chloromethane from samples stored at room temperature. Overall, they concluded that measurement of urinary S-methylcysteine is inappropriate for assessing chloromethane exposure and that previously reported blood levels were likely inaccurate. This helped clarify previously reported assessments described below.

In a group of 6 workers exposed to TWA 8-hour workroom concentrations of 30-90 ppm, the urinary excretion of S-methylcysteine showed wide variations, with little correlation to exposure levels (van Doorn et al. 1980). S-methylcysteine is formed from conjugation of chloromethane with glutathione

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(Kornbrust and Bus 1983). In four of the workers, all values were higher than in controls, and appeared to build up during the course of the week. Two of the workers had only minor amounts of S-methylcysteine in the urine, but these workers experienced the highest exposure concentrations. The author concluded that there are two distinct populations of individuals: fast metabolizers with lower body burdens and higher excretion, and slow metabolizers with higher body burdens and lower excretion (van Doorn et al. 1980). The author speculated that the difference may be due to a deficiency of the enzyme glutathione-S-transferase that catalyzes the conjugation of chloromethane with glutathione. Other possible reasons for the differences in chloromethane elimination among subjects include differences in tissue glutathione levels and differences in biliary excretion and fecal elimination of thiolated conjugates. As a working hypothesis, however, the two distinct populations are referred to as fast and slow eliminators.

Two distinct subpopulations were also found based on venous blood and expired concentrations of chloromethane in volunteers (Nolan et al. 1985). In addition, Nolan et al. (1985) observed a five-fold difference in the first order rate constant for elimination with slow metabolizers demonstrating a K_m of 0.039 to 0.069/ min and fast metabolizers demonstrated a K_m of 0.284 to 0.342/min. The urinary excretion of S-methylcysteine in the volunteers exposed to chloromethane was variable, and was not significantly different in pre- and post-exposure levels. No change was detected in the S-methylcysteine concentration or in the total sulfhydryl concentration in the urine of 4 workers before and after a 7-hour shift in a styrene production plant by DeKok and Antheunius (1981), who concluded that S-methylcysteine is not a human metabolite of chloromethane. It is possible, however, that the small number of workers examined by DeKok and Antheunius (1981) were slow eliminators.

Stewart et al. (1980) exposed male and female volunteers to 0-150 ppm chloromethane for periods up to 7.5 hours/day for 2 or 5 consecutive days, and then evaluated blood carboxyhemoglobin saturation before, just following, and 15 and 30 minutes post exposure, and urinary methyl alcohol from 24-hour composites collected twice weekly post exposure. Results indicated that chloromethane was not metabolically converted to either carbon dioxide or methyl alcohol.

Peter et al. (Peter et al. 1989b; Peter et al. 1989a) assayed erythrocyte cytoplasm of humans with chloromethane and monitored the decline of chloromethane and the production of S-methylglutathione. About 60% of the human blood samples showed a significant metabolic elimination of the substance (conjugators), whereas 40% did not (non-conjugators). The results suggested that a minor form of human erythrocyte glutathione S-transferase is responsible for the unique metabolism of chloromethane in human erythrocytes. Hallier et al. (1990) demonstrated that other monohalogenated methanes (methyl iodide and methyl bromide) could undergo enzymatic conjugation with glutathione, but that in contrast to

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chloromethane, methyl iodide and methyl bromide also showed significant non-enzymatic conjugation with glutathione.

Warholm et al. (1994) studied the polymorphic distribution of the erythrocyte glutathione transferases in a Swedish population and found three distinct sub-groups: 11.1% lacked activity, 46.2% had intermediate activity, and 42.8% had high activity. The authors calculated two allelic frequencies, one for a functional allele with a gene frequency of 0.659 and one for a defect allele with a frequency of 0.341. This two allele hypothesis is compatible with the observed distribution of the three phenotypes. A follow-up study on genotype indicated that approximately 10% of the Swedish population lacked the glutathione transferase isoenzyme (Warholm et al. 1995). This 10% number is considerably smaller than a previously proposed proportion of non-conjugators of 30-40% for a German population (Peter et al. 1989a). A different study by Kempkes et al. (1996) found a frequency of 15% for non-conjugators in a German cohort of 40 people. Whether this lack of activity poses an increased risk of developing disease such as cancer is not known. Warholm et al. (1995) suggest that additional ethnic groups be evaluated for percentage of non-conjugators.

Because of this unique polymorphism, these populations have been further studied in the development of physiologically-based pharmacokinetic (PBPK) models to assess the reliability of such models in general (Johanson et al. 1999; Jonsson et al. 2001), and to investigate how the genetic polymorphism affects the metabolism and disposition of chloromethane specifically in vivo (Lof et al. 2000).

Lof et al. (2000) exposed 24 volunteers, (eight with high, eight with medium, and eight with no GSTT1 activity) to 10 ppm chloromethane for 2 hours. The concentration of chloromethane was measured in inhaled air, exhaled air, and blood. The experimental data were used in a 2-compartment model with pathways for exhalation and metabolism. Respiratory uptake averages were 243, 148, and 44 μmol in high, medium, and no GSTT1 activity groups, respectively. During the first 15 minutes of exposure, the concentration of chloromethane in blood rose rapidly and then plateaued. The blood concentrations of chloromethane were similar in all three groups during the 2-hour exposure. At the end of exposure, the blood concentrations declined rapidly in the high and medium metabolizing groups, but declined more slowly in the group lacking GSTT1 activity. The half-times were 1.7, 2.8, and 3.8 minutes, respectively for the first phase and 44, 48, and 60 minutes, respectively, for the second phase. Metabolic clearance was 4.6 and 2.4 L/min in the high and medium GSTT1 groups, but nearly absent in the non-metabolizing group. The rate of exhalation clearance was similar among the three groups, but the non-metabolism group had much higher concentrations of chloromethane in exhaled air after exposure.

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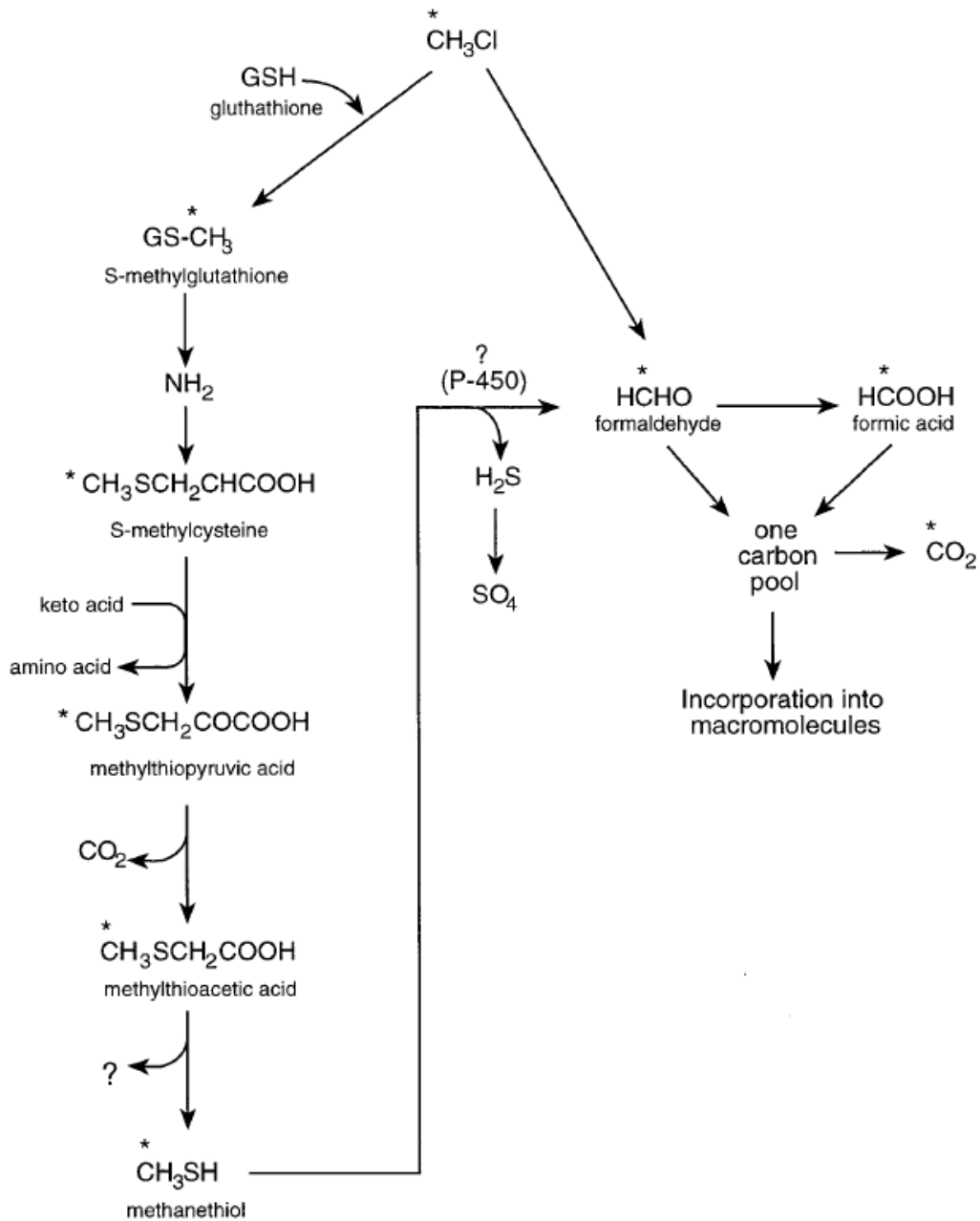
The metabolism of chloromethane has been studied in rats, mice, and dogs *in vivo* after inhalation exposure, and *in vitro*. Based on these studies, the metabolic pathway shown in Figure 3-1 was proposed (Kornbrust and Bus 1983). According to the proposed pathways, chloromethane metabolism involves conjugation with glutathione to yield S-methylglutathione, S-methylcysteine, and other sulfur-containing compounds

(Kornbrust and Bus 1984; Landry et al. 1983a; Landry et al. 1983b; Redford-Ellis and Gowenlock 1971a, 1971b). These compounds can be excreted in the urine (Landry et al. 1983a), or S-methylglutathione may be further metabolized to methanethiol. Cytochrome P-450 dependent metabolism of methanethiol may yield formaldehyde and formic acid, whose carbon atoms are then available to the one-carbon pool for incorporation into macromolecules or for formation of CO₂ (Kornbrust and Bus 1983; Kornbrust et al. 1982). Formaldehyde may also be a direct product of chloromethane metabolism via oxidative dechlorination. Production of methanethiol and formaldehyde, and lipid peroxidation due to glutathione depletion have been suggested as possible mechanisms for the toxicity of chloromethane, but the precise mechanisms are not known (Kornbrust and Bus 1983, 1984). Dekant et al. (1995) demonstrated oxidation of chloromethane to formaldehyde by cytochrome P-450 (2E1) in male mouse kidney microsomes, and that the amount of formaldehyde formed was dependent upon the hormonal status of the animal. Female mouse kidney microsomes produced considerably less formaldehyde than male kidney microsomes. Liver microsomal activity from both sexes was 2-fold higher than in kidney microsomes from the male. In contrast, rat kidney microsomes did not catalyze formaldehyde formation from chloromethane. In addition, Heck et al. (1982) observed a doubling of formaldehyde in the liver and testes of male F344 rats after 4 days of 6-hour exposure to 3000 ppm of chloromethane compared to the control rats. In this same study there was a sevenfold increase in formaldehyde in the brain of exposed rats compared to controls.

Peter et al. (1989a) assayed erythrocyte cytoplasm of a variety of test animals with chloromethane and monitored the decline of chloromethane and the production of S-methylglutathione. Rats, mice, bovine, pigs, sheep, and rhesus monkeys showed no conversion of chloromethane in erythrocyte cytoplasm.

Species differences in the GSTT1 activity for chloromethane in liver and kidney tissues from mice, rats, hamsters and all three phenotypes of humans were studied *in vitro* (Thier et al. 1998). No GSTT1 activity was found in either tissue of the non-metabolizing phenotypic human subjects. The GSTT1 activity in the liver and kidney tissue from the high GSTT1 humans were twice as high as in the low metabolizing group, and two to seven times higher in the liver tissues than in the kidney tissues of either group. The GSTT1 activities in decreasing order were mice >high GSTT1 humans >rat >low GSTT1 humans >hamster >GSTT1-deficient humans. A proposed scheme of metabolism is illustrated in Figure 3-1.

Figure 3-1. Proposed Scheme for Metabolism of Chloromethane



* indicates the position of the radioactive label

Source: Kornburst and Bus 1983

3.1.4 Excretion

Very little unchanged chloromethane is excreted in the urine. In volunteers exposed to chloromethane urinary excretion was <0.01 %/min (Morgan A et al. 1970). Putz-Anderson et al. (1981a) exposed volunteers to 100 or 200 ppm chloromethane for 3 hours, and breath concentrations approached equilibrium within one hour at 36 ppm (SD 12 ppm) and 63 ppm (SD 23.6 ppm), respectively. The excretion patterns of chloromethane following prolonged exposure may be similar to those observed in short term (>1 hr) experiments due to rapid air-blood equilibrium. Therefore, any sampling of blood or serum for occupational exposure assessment should occur during or promptly after exposure ends. Volunteers exposed to 10 or 50 ppm eliminated chloromethane from blood and the expired air in a biphasic manner when exposure ceased (Nolan et al. 1985). Based upon data presented in the report, the half-life for the β -phase was estimated at 50 minutes for fast metabolizers and 90 minutes for slow metabolizers. These fast elimination rates suggest that chloromethane is unlikely to accumulate in tissues, even if exposure is prolonged or repeated.

In rats exposed to [¹⁴C] chloromethane for 6 hours and dogs exposed for 3 hours at concentrations of 50 or 1,000 ppm, blood levels rose rapidly and approached equilibrium proportionate, or nearly proportionate to exposure levels (Landry et al. 1983a). Blood concentrations declined rapidly in a biphasic, non- concentration-dependent manner when exposure was stopped. The disappearance from blood was consistent with a linear 2-compartment open model. Half-lives for the α -phase were 4-5 minutes in rats, and 6-10 minutes in dogs; half-lives for the β -phase were 15 minutes in rats, and 35-50 minutes in dogs. The disappearance of chloromethane from blood probably represents excretion of metabolites rather than the parent compound. As discussed above in Section 3.1.3 on metabolism, chloromethane is conjugated with glutathione and cysteine, leading to urinary excretion of sulfur-containing compounds. Further metabolism of the cysteine conjugate by one-carbon metabolic pathways leads to incorporation of the carbon atom into macromolecules, and the production of carbon dioxide.

No studies were located regarding excretion in humans or animals following oral or dermal exposure to chloromethane.

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

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

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species (Clewell 3rd and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

Jonsson et al. (2001) used the data from the GSTT1 deficient group from the Lof et al. (2000) study (See Section 3.1.3) to develop a standard PBPK model for chloromethane with six tissue compartments: lung, working muscle, resting muscle, well-perfused tissues, liver, and fat. The model also included uptake of chloromethane via ventilation, and all elimination was accounted for by exhalation because these individuals lacked the ability to metabolize chloromethane. The model was fit to the experimental data using a Bayesian approach and assumptions regarding parameters related to metabolism. Although the model provided a good general model, the concentrations in exhaled air and blood were slightly over predicted. The authors noted that the use of non-metabolizing subjects allowed them to assess the kinetics of a volatile chemical without interference from metabolism and to obtain greater knowledge on physiological parameters, but using chloromethane as a model compound had limitations, such as, low solubility of chloromethane in blood, low blood: air partition coefficient, and rapid decay during the first minutes after exposure.

3.1.6 Animal-to-Human Extrapolations

Acute and chronic inhalation studies indicate that mice are more sensitive than rats to the lethal effects of chloromethane (Chellman et al. 1986b; CIIT 1981; Morgan et al. 1982). Smith and von Oettingen (1949a) provided acute mortality data indicating that species susceptibility follows the general order of mice >guinea pig >dog >goat >monkey >rat >rabbit, with a fourfold difference between mice and rabbits. The greater susceptibility of mice may be due to different metabolic rates involving glutathione or different oxidative rates for the production of formaldehyde. Chloromethane conjugates with glutathione to a much greater extent in mouse liver, kidney, and brain compared with rats (Kornburst and Bus 1984).

Pretreatment (*i.p.*) of mice with L-buthionine-S,R-sulfoximine (BSO), a glutathione depleter, protected mice from the chloromethane-induced lethal effects (Chellman et al. 1986b). Thus, the reaction of chloromethane with glutathione to produce S-methylglutathione appears to be a toxifying rather than a detoxifying reaction (Chellman et al. 1986b).

Alternatively, chloromethane can elicit lipid peroxidation as a consequence of depletion of glutathione (Kornburst and Bus 1984). In humans, S-methylcysteine appears as a metabolite of chloromethane, so conjugation with glutathione probably also occurs in humans.

Different P-450 activities between species, sexes, and tissues within the body (i.e., liver versus kidney) affect the dehalogenation of chloromethane to formaldehyde and can thus influence the level of

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formaldehyde-induced DNA or tissue damage (Dekant et al. 1995; Jager et al. 1988; Ristau et al. 1989, 1990).

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

There have been no human studies to determine the health effects of exposure to chloromethane in children, or whether children are more or less susceptible to the potential health effects of chloromethane at a given exposure level and duration of exposure. There is no information on whether the effects in children would be similar to those in adults for either accidental short-term exposures or longer-term lower level exposures. There is a lack of human data on whether chloromethane affects the developing fetus or the development of young children.

There are limited data on the toxicity of chloromethane in children and it is assumed that the toxicity of chloromethane in children is similar to adults. However in guinea pigs, Smith and von Oettingen (1947b) reported that older guinea pigs developed symptoms more rapidly compared to a younger guinea pig, although both young and older animals lost the ability to turn over from a supine position. Also, the older animals were more likely to develop severe effects or die from high exposure (Smith and von Oettingen 1947a, 1947b); young mice, rats, guinea pigs, and dogs were found to have less severe effects compared to older animals exposed to the same amount of chloromethane, and in some cases survived exposure to high levels of chloromethane, while older animals died.

In adults, there appear to be two distinct populations with regard to metabolism and elimination of chloromethane. One population has higher amounts of the metabolizing enzyme, glutathione-S-

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transferase, and thus a higher rate of elimination of chloromethane from the body. The toxicity of chloromethane, however, is thought to result from toxic metabolites formed following the conjugation with glutathione or from the depletion of GSH (Chellman et al. 1986b; Kornbrust and Bus 1983, 1984; Landry et al. 1985). If a polymorphism is present in children, then some children with the same polymorphism as adults (i.e., those with higher levels of glutathione-S-transferase) would be more susceptible to the toxic effects of chloromethane. However, if the mechanism is a result of decreasing GSH (which may protect against peroxidation), these individuals may actually be protected against the impact of chloromethane.

Certain characteristics of the developing human may increase exposure or susceptibility while others may decrease susceptibility to the same chemical.

It is not known whether chloromethane or one of its metabolites (e.g., methanethiol or an altered macromolecule) can cross the placenta and enter into the developing young, or if either compound can enter into breast milk. However, Wolkowski-Tyl et al. (1983a) noted from unpublished observations that mouse dams exposed to 100, 500, or 1,500 ppm chloromethane for 6 hours on gestation day 17 had significant NPSH concentration reductions in both dams (livers and kidneys) and fetuses (livers and carcasses), indicative of potential transplacental passage of chloromethane or its metabolites during late gestation, though no chloromethane was observed in the placenta. Chloromethane is broken down and eliminated from the body very quickly in adult humans (Nolan et al. 1985) and animals (Landry et al. 1983a; von Oettingen et al. 1949, 1950).

Although the breakdown and elimination of chloromethane is expected to be the same in children as in adults, two distinct groups of humans with different metabolic rates have been identified, so more studies are needed to answer this and other questions concerning the movement of chloromethane into the fetus or breast milk, and what levels might result in harmful effects. There is only one PBPK model for chloromethane exposure based on data for GSTT1 deficient individuals. There are otherwise no PBPK models for children, adults, or test animals. There are no good biomarkers of exposure for children (or adults), although clinical symptoms of drunkenness or food poisoning, a smell of acetone around the individual, and a musty and sweet odor of the breath may alert a physician. Attempts to use urinary levels of S-methylcysteine as an indicator of chloromethane exposure have not been successful, so the approach is considered to be invalid (Nolan et al. 1985).

Only limited information is available from animal studies on potential effects in the developing young. In one animal study, pregnant rats were exposed to 1,500 ppm chloromethane by inhalation during gestation. Maternal toxicity, evidenced by decreased body weight gain and retarded development of

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fetuses, was observed in rats exposed to 1,500 ppm chloromethane for 6 hours per day during gestational days (GD) 7-19 (Wolkowski-Tyl et al. 1983a). The fetal effects consisted of reduced fetal body weight and crown-rump length, and reduced ossification in the metatarsals and phalanges, the centra of the thoracic vertebrae, the pubis of the pelvic girdle, and the metatarsals of the hind limbs.

In a mouse study, dams were exposed by inhalation to chloromethane during gestation days 6-17 (Wolkowski-Tyl et al. 1983a). The investigators found increased incidences of heart malformations in the fetuses of mouse dams exposed to 500 ppm chloromethane during gestation days 6-17. The heart malformations consisted of absence or reduction of atrioventricular valves, chordae tendineae, and papillary muscles. Heart malformations, however, were not found in fetuses of mouse dams exposed to higher concentrations of chloromethane during gestation days 11-12.5, which they considered to be the critical period for development of the embryonal heart (John-Greene et al. 1985). John-Greene et al. (1985) suggested that the heart anomaly reported by Wolkowski-Tyl et al. (Wolkowski-Tyl et al. 1983b; Wolkowski-Tyl et al. 1983a) may have been an artifact of the sectioning technique, due to the examination of the fixed as opposed to unfixed fetal tissue, or a misdiagnosis. They also found much inter-animal variability in the appearance of the papillary muscles in control mice. However, Wolkowski-Tyl (1985) countered that the inability of John-Greene et al. (1985) to detect the abnormality was due to the different exposure protocol, and that the critical period is more appropriately gestational day 14. The developmental toxicity of chloromethane in mice is, therefore, controversial; it is not known whether chloromethane could produce developmental effects in humans.

Acute-, intermediate-, and chronic-duration inhalation exposures of male rats to chloromethane have resulted in such reproductive effects as inflammation of the epididymides, sperm granuloma formation in epididymides, disruption of spermatogenesis, decreased fertility at about 500 ppm, and sterility at higher concentrations of 1,000 or 3,000 ppm (Burek et al. 1981; Chapin et al. 1984; Chellman et al. 1986a, Chellman et al. 1986b, Chellman et al. 1987; CIIT 1981; Hamm et al. 1985; Morgan KT et al. 1982; Working and Bus 1986; Working et al. 1985a, Working et al. 1985b). Testicular effects of chloromethane have been manifested as preimplantation loss in unexposed female rats mated with males exposed to chloromethane (Working et al. 1985a). Testicular lesions were also observed in mice after 18 months of exposure to chloromethane (CIIT 1981). Studies on the mechanism of chloromethane-induced testicular effects suggested that preimplantation loss was potentially due to cytotoxicity of chloromethane to sperm in the testes at the time of exposure (Chellman et al. 1986c, Chellman et al. 1987; Working and Bus 1986; Working et al. 1985a, Working et al. 1985b). However, these findings do not negate the possibility of a dominant lethal mutation leading to post-implantation loss. Both mechanism are plausible.

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Chloromethane exposure consistently produced dominant lethal mutations in the sperm of rats, as measured by post implantation loss in females mated to exposed males (Chellman et al. 1986c; Rushbrook 1984; Working et al. 1985a). Because of the known transit times for sperm in the epididymis and the resulting observed times of the post implantation losses, Working et al. (1985a) observed that the timing of the genetic damage to the sperm coincided with their location in the chloromethane-induced inflammation of the epididymis. Since concurrent exposure of male rats to chloromethane and BW755C, an anti-inflammatory agent, greatly reduced the amount of post implantation loss, it is possible both dominant lethal mutations and an epididymal inflammatory response (Chellman et al. 1986c; Working and Chellman 1989) can lead to post implantation loss. The activation of phagocytic cells during the inflammatory process may result in the production of potentially genotoxic chemical species including the superoxide anion radical, hydrogen peroxide, and lipid peroxide decomposition products (Fridovich 1978; Goldstein et al. 1981; Goldstein et al. 1979; Working et al. 1985a).

Chloromethane has been tested for genotoxicity in a number of *in vitro* and *in vivo* studies. Chloromethane gave positive results for gene mutation, sister chromatid exchange, and transformation in cultured mammalian cells, including human lymphoblast cells (Asakura et al. 2008; Fostel et al. 1985; Hatch et al. 1982; Hatch et al. 1983; Working et al. 1986); and appears to be a direct-acting genotoxicant *in vitro*. The ability of inflammatory cells (human phagocytes) to produce superoxides capable of genetic damage has been demonstrated (Weitzman and Stossel 1981). Although chloromethane produced genotoxic effects in human lymphocytes in culture, it is not known whether chloromethane could produce dominant lethal mutations or other genotoxic effects in humans exposed by any route. No information was available on the distribution of chloromethane or metabolites to parental reproductive organs or germ cells in humans that could lead to genetic or epigenetic damage to germ cells. It is also not known whether chloromethane produces a sublethal level of genetic or epigenetic damage to sperm that would, in turn, be sufficiently viable to form an embryo and subsequently be detrimental (at clinical or subclinical levels) to the developing young. Further, chloromethane was found to be a potent mutagen in *Drosophila melanogaster* (University of Wisconsin 1986).

3.3 BIOMARKERS OF EXPOSURE, EFFECT, AND SUSCEPTIBILITY

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

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A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to chloromethane are discussed in Section 3.3.1.

A biomarker of effect is defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by chloromethane are discussed in Section 3.3.2.

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

3.3.1 Biomarkers of Exposure

There are no reliable biomarkers of exposure for children or adults, although clinical symptoms of drunkenness or food poisoning, a smell of acetone around the individual, and a musty and sweet odor of the breath may alert a physician to potential chloromethane exposure. Previous studies have unsuccessfully attempted to correlate exposure levels of chloromethane in air with urinary excretion of S-methylcysteine. In a group of 6 workers exposed to TWA 8-hour workroom concentrations of 30-90 ppm, the excretion of S-methylcysteine in urine showed wide variations, with little correlation with exposure levels (van Doorn et al. 1980). On the basis of variable excretion of S-methyl-cysteine in 6 male volunteers exposed to 10 or 50 ppm chloromethane for 6 hours, Nolan et al. (1985) found no relationship between inhalation exposure and urinary S-methyl-cysteine; blood levels of NPSH assessed in previous research was low due to failure to recognize chloromethane loss from the sample during equilibration at room temperature. They concluded that measurement of S-methylcysteine in urine is not a valid method for monitoring exposure to chloromethane.

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In an evaluation of the use of blood and breath analysis of chloromethane to monitor acute exposure in volunteers, it was concluded that breath sampling is not useful for quantitatively assessing chloromethane exposure. However, breath analysis can identify elevated exposures if promptly sampled and determine which individuals retain higher than normal body burdens such that they are potentially more sensitive. Stewart et al. (1980) exposed male and female volunteers to 0-150 ppm chloromethane for periods up to 7.5 hours/day for 2 or 5 consecutive days. Breath samples were collected starting immediately after to 3 hr after exposure, and early samples for 20 or 100 ppm correlated well with exposure; however, they decreased 5-fold or more in 15 min, and by 2 hours, samples were difficult to interpret. Exposure to 100 ppm could not be distinguished from exposure to 150 ppm after 1 minute postexposure (Stewart et al. 1980).

Xu et al. (1990) evaluated whether covalent binding of chloromethane to hemoglobin would be a viable measure for monitoring exposure to chloromethane in air. In comparison to the other monohalomethanes tested (i.e., methyl bromide and methyl iodide), chloromethane had the lowest reactivity with hemoglobin, limiting its usefulness. The authors supported further assay development for methyl bromide but made no mention of the usefulness of a covalent binding assay for chloromethane, presumably because its reactivity was too low.

3.3.2 Biomarkers of Effect

Biomarkers of effect from chloromethane over-exposure can be difficult to evaluate in borderline and even higher exposure cases. One reason is that symptoms from acute and intermediate duration exposures are not completely consistent; they are similar to those from common viral and bacterial diseases, e.g., headache, dizziness, nausea, and vomiting; and none are specific to chloromethane (Macdonald et al. 1964; Scharnweber et al. 1974). Another reason is large interindividual variability based on neurobehavioral testing (Putz-Anderson et al. 1981b). Attempts to correlate blood levels and expired air concentrations of chloromethane with health effects of occupational and experimental inhalation exposure have been unsuccessful. In a study of 73 behavioral measures of task performance, 4 indices of exposure, and 8 indicators of neurological function in workers exposed to a mean concentration of 34 ppm chloromethane, effects on cognitive time-sharing and finger tremor were found, but correlation coefficients indicated that chloromethane in breath was not a sensitive indicator of performance (Repko et al. 1976). Although volunteers exposed to 200 ppm chloromethane for 3 hours had a 4% decrement in their performance on behavioral tests, individual blood and alveolar air levels of chloromethane were too variable to be of practical use, but group average blood and breath samples were highly correlated (Putz-Anderson et al. 1981a). The decrement in performance was also small and not statistically significant.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Chloromethane may interact with other solvents and its metabolism [genetic polymorphisms of xenobiotic enzymes (Phase I and II)] could be altered by exposure to other chemicals such as the use of alcohol, smoking, etc.

Inhalation exposure of volunteers to 200 ppm chloromethane along with oral dosing with 10 mg diazepam produced an additive impairment in performance on behavioral tests (Putz-Anderson et al. 1981a).

Diazepam alone produced a significant 10% decrease in task performance, whereas exposure to chloromethane produced a non-significant average decrease of 4%, and diazepam and chloromethane together produced a combined 13.5% decrease. The authors suggest that there is no interaction between diazepam and chloromethane exposure, but instead that effects are additive. Group average blood and breathing air concentrations were highly correlated, but there were large interindividual differences.

Minami et al. (1992) report a patient in Japan exposed simultaneously to chloromethane and chloramine gas. The exposure resulted from the patient first cleaning a porcelain toilet with sodium hypochlorite (NaOCl) in an alkaline solution then, without first rinsing off the hypochlorite, spraying a hydrochloric acid (HCl) solution to remove hard salt adhesions. The toilet was connected directly to a sewage storage tank. The resulting fumes produced a toxic response in the patient 30 minutes after cleaning. The patient recovered from the acidosis after bicarbonate transfusion, plasmapheresis, and plasma exchange; but permanent blindness ensued 3 days postexposure. In a follow-up study, Minami et al. (1993) demonstrated an increase in formate excretion in mice dosed via intraperitoneal injection with chloramine after exposure to chloromethane. The authors ascribe this increase to an inhibitory effect of chloramine on formyl tetrahydrofolate dehydrogenase and formaldehyde dehydrogenase. More recently, Wang and Minami (1996) extended their proposed mechanism to include a potentiation of formaldehyde on chloramine inhibition of acetylcholinesterase activity. In their study they state that formaldehyde may potentiate the inhibitory action of chloramine on acetylcholinesterase activity. If formaldehyde is a metabolite of chloromethane, as proposed by Kornbrust and Bus (1983), there may be reason to conclude these two chemicals may have an interactive neurological effect. However, as demonstrated by Jager et al. (1988), but disputed by Heck et al. (1982), there is some debate regarding whether formaldehyde is a metabolite of chloromethane metabolism *in vivo*. Additionally, consideration of how exposure occurs and how each chemical is distributed throughout the body may contribute to hypotheses for potential interactions.

The only other studies that show an effect of other compounds on the toxicity of chloromethane are those in which the effects of BW755C, an anti-inflammatory agent, and BSO, a depleter of glutathione, were

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administered i.p. to rats or mice exposed to chloromethane by inhalation to study the mechanism of chloromethane-induced toxicity (Chellman et al. 1986a, Chellman et al. 1986b). BW755C co-exposure with chloromethane provided protection to several organs (brain, kidneys, liver, and testes). However, it is unlikely that these compounds would be found with chloromethane at hazardous waste sites.