2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of chloromethane. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

This chapter contains descriptions and evaluations of studies and interpretation of data on the health effects associated with exposure to chloromethane. Its purpose is to present levels of significant exposure for chloromethane based on toxicological studies, epidemiological investigations, and environmental exposure data. This information is presented to provide public health officials, physicians, toxicologists, and other interested individuals and groups with (1) an overall perspective of the toxicology of chloromethane and (2) a depiction of significant exposure levels associated with various adverse health effects.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure- inhalation, oral, and dermal; and then by health effect-death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (l-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in Table 2-l and illustrated in Figure 2-1. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or

those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of chloromethane are indicated in Table 2-1 and Figure 2-1. Cancer effects could occur at lower exposure levels, but a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), has not been developed by EPA.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for chloromethane. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

2.2.1 Inhalation Exposure

2.2.1.1 Death

Thirty or more years ago, chloromethane was used as a refrigerant, and many human deaths resulted from exposure to chloromethane vapors from leaks in home refrigerators and industrial cooling and refrigeration systems (Baird 1954; Borovska et al. 1976; Kegel et al. 1929; McNally 1946; Thordarson et al. 1965). In some cases, the individuals were found comatose or dead in their homes. In other cases, patients were admitted to hospitals with typical neurological signs and symptoms of chloromethane poisoning (confusion, staggering, slurred speech). These patients eventually became comatose, developed convulsions, and died. The concentrations and durations of these exposures were not known.

Exposure to high concentrations of chloromethane can result in moderate to severe neurological effects (see Section 2.2.1.4) but death does not always result if exposure ceases and medical attention is received in time. For example, refrigerator repairmen developed neurological symptoms after exposures to chloromethane from leaks at concentrations as high as 600,000 ppm, but no deaths resulted (Jones 1942). In other cases death did occur. Seventeen crew members (male) were exposed for 2 days in 1963 to chloromethane that leaked from a refrigerator on board an Icelandic fishing trawler (no estimates of exposure levels were reported). The refrigerator was located under the sleeping quarters of the crew. In the acute phase of the illness, nine patients exhibited abnormal neurological signs. Four died, one within 24 hours of the exposure. Two patients developed severe depression and committed suicide 11 and 18 months later. The fourth patient was" assessed as 75% disabled due to severe neurological and

psychiatric disturbances, and died 10 years postexposure at the age of 34. Autopsy revealed recent coronary occlusion which was not necessarily connected with the primary illness (Gudmundsson 1977). In a follow-up study, Rafnsson and Gudmundsson (1997) reported an excess mortality from cardiovascular diseases in this exposed population compared to a reference group. The excess mortality was more prominent for the deckhands who received the higher exposures to chloromethane. The results and conclusions from this study, however, are based upon the assumption that the reference group had similar lifestyle factors including smoking habits and diet (which may not have been the case). There was also a relatively low number of individuals with significant exposure.

Animals exposed to sufficiently high levels of chloromethane die after developing severe signs of neurotoxicity. In an extensive investigation, a variety of species including rats, mice, guinea pigs, rabbits, dogs, cats, and monkeys were exposed to lethal concentrations of chloromethane (Dunn and Smith 1947; Smith 1947; Smith and von Oettingen 1947a, 1947b). Severe neurological effects, such as paralysis, convulsions, and opisthotonos, developed before death. Precise determination of concentration-duration-response relationships was not possible from these studies because of limitations including unknown purity of chloromethane, unconventional reporting of lethality data, and generally poor reporting of details. Nonetheless, these earlier studies demonstrated the universal response of animals to the neurotoxic and lethal effects of chloromethane.

More recent studies provide better dose-response information. Sprague-Dawley rats were exposed to 99.5% chloromethane at 0, 200, 500, 1,000, or 2,000 ppm for 48 or 72 hours. One-half of the animals were sacrificed immediately after exposure, and the remaining half were observed for 12 days postexposure prior to sacrifice. At 2,000 ppm for 48 hours, rats were either lethargic, moribund or dead. At 52 hours, rats exposed to 1,000 ppm remained lethargic; rats exposed to 2,000 ppm were all dead or moribund. At 72 hours of exposure, all rats receiving 2,000 ppm were dead. No male and 1 of 10 female rats died by 12 days postexposure to 1,000 ppm for 48 hours. Six of 10 male and 8 of 10 female rats died by 12 days postexposure to 1,000 ppm for 72 hours. No deaths occurred at 200 or 500 ppm for up to 72 hours of exposure. Cause of death was thought to be kidney failure (Burek et al. 1981).

Chellman et al. (1986a) studied the effects of 3-amino-l-[m-(trifluoromethyl)phenyl]-Zpyrazoline (BW755C), a potent anti-inflammatory agent, on chloromethane-induced lethality and reproductive toxicity in male Fischer 344 rats. Rats were exposed to 5,000 ppm chloromethane for 5 days or 7,500 ppm chloromethane for 2 days, 6 hours/day, with or without treatment with BW755C (10 mg/kg,

intraperitoneally 1 hour pre- and postexposure). Exposure to 7,500 ppm chloromethane for 2 days, 6 hours/day was fatal to 8 of 12 rats. No deaths occurred in 6 rats treated with both chloromethane and BW755C. One of 5 rats exposed to 5,000 ppm chloromethane died. No deaths occurred in 5 rats treated with both chloromethane and BW755C. The authors concluded that protection from chloromethane-induced injury by BW755C was not simply the result of altered metabolism because BW755C had no effect on tissue distribution or excretion of ^{14}C-chloromethane and administration of BW755C did not decrease hepatic glutathione content. The protection of BW755C may have been related to an inhibition of leukotriene and prostaglandin synthesis.

Morgan et al. (1982) investigated the lesions induced by inhalation exposure to chloromethane in C3H, C57BL/6, and B6C3F$_1$ mice and in Fischer 344 rats. Ten rats/sex were exposed to chloromethane for 5 days, 6 hours/day with a break in exposure for 2 days, and then a further 4 days of exposure. Rats were exposed to 0, 2,000, 3,500, or 5,000 ppm. Animals were sacrificed 18 hours after the last exposure or immediately after exposure if found to be moribund. After 5 days, 6 males and 5 females exposed to 5,000 ppm, and 2 females exposed to 3,500 ppm, were killed in extremis. Five mice/sex were exposed to chloromethane for 12 days, 6 hours/day. Mice were exposed to 0, 500, 1,000, or 2,000 ppm. In mice exposed to 2,000 ppm, all male B6C3F$_1$ mice were moribund or died by day 2, one C57BL/6 male died on day 2, and others were moribund by day 5. All other mice survived except one male C3H mouse exposed to 1,000 ppm, which died by day 11. This study confirmed the existence of species, sex, and strain differences in susceptibility to chloromethane-induced toxicity. The authors further speculated that, although the mechanism of death is unknown, it may be associated with liver and kidney pathology.

Chellman et al. (1986b) investigated the role of glutathione in the mediation of chloromethane-induced toxicity in the liver, kidney, and brain of male B6C3F$_1$ mice. In one experiment, groups of 5 mice were exposed to chloromethane at concentrations from 500 ppm to 2,500 ppm in increments of 500 ppm with or without pretreatment with buthionine-S,R,-sulfoximine (BSO), a depleter of glutathione (GSH), and were observed for death up to 18 hours after exposure. The resulting mortality data was used to estimate an approximate LC_{50} value. The LC_{50} in the non-pretreated rats was 2,200 ppm, while the LC_{50} for the pretreated rats was 3,200 ppm. The authors concluded that pretreatment with BSO, and hence GSH depletion, protected mice from the lethal effects of chloromethane. The GSH metabolic pathway appeared to be activating toxicity rather than detoxifying.

In two further experiments by Chellman et al. (1986b), 36 and 45 mice were exposed by inhalation to 1,500 ppm chloromethane for 2 weeks, 5 days/week, 6 hours/day, with or without daily pretreatment with BSO. In the two experiments using this protocol, 10 of 36 (28%) and 5 of 45 (11%) of the mice died by the end of the first day (6 hours) of exposure to 1,500 ppm chloromethane. In contrast, none of the BSO-pretreated mice died after the first exposure. The authors concluded that pretreatment with BSO, and hence GSH depletion, protected mice from the lethal effects of chloromethane. This provided further evidence that the GSH metabolic pathway activated toxicity rather than detoxified.

Jiang et al. (1985) characterized cerebellar lesions resulting from an acute inhalation exposure to chloromethane in female C57BL/6 mice. Ten mice each were exposed to room air or 1,500 ppm chloromethane for 2 weeks, 5 days/week, 6 hours/day. Two mice died, and several had motor incoordination. Only one exposure concentration was used, but the study was designed to study the neurological and kidney effects specifically, and therefore, used an exposure regimen known to produce these effects. The authors concluded that the brain lesions seen after exposure to chloromethane were probably not a direct consequence of renal lesions.

Landry et al. (1985) evaluated the neurologic effects of continuous versus intermittent chloromethane exposure in female C57BL/6 mice Groups of 12 mice each were exposed to chloromethane in whole body inhalation chambers for 11 days either continuously (C) 22 hours/day at 0, 15, 50, 100, 150, 200, or 400 ppm or intermittently (I) 5.5 hours/day at 0, 150, 400, 800, 1,600, or 2,400 ppm. At 2,400-I ppm, the condition of the mice gradually deteriorated until they were killed in a moribund condition after 8 or 9 days of exposure. No deaths occurred in the 1,600-I ppm mice or in mice receiving lower intermittent exposures. The 400-C ppm exposed mice died or were sacrificed by day 4, and the 200-C ppm group by day 5, due to severe toxicity. Mice exposed to 150-C ppm were sacrificed in moribund condition by day 10.5. No deaths occurred in the mice exposed to ≤ 100-C ppm. The authors concluded that exposure duration affected susceptibility to chloromethane-induced neurotoxicity, with those continuously exposed exhibiting a non-proportionate greater susceptibility. The authors speculated that the greater susceptibility was due to a combination of glutathione depletion, the formation of a toxic metabolic intermediate, and the effects of nocturnal exposure.

Wolkowski-Tyl et al. (1983a) assessed the teratogenicity of an inhalation exposure to chloromethane in female Fischer 344 rats and B6C3F_1 mice. Groups of 33 mice per exposure level were exposed to 0, 100, 500 or 1,500 ppm chloromethane in whole-body exposure chambers, 6 hours daily on gestation days (Gd)

6-17. Actual chloromethane concentrations in the chambers were 0.05 (the ambient level; for the 0 dose), 102 (100 ppm), 479 (500 ppm), 1,492 (1500 ppm). At 1,492 ppm, there was severe maternal toxicity resulting in tremors, hunched appearance, difficulty righting, disheveled fur, bloody urine, and granular cell degradation in cerebellum with selective necrosis of neurons in the internal granular layer. All females in this group were sacrificed on gestation days 11-14 prior to the completion of exposure to Gd 17; two females died prior to necropsy (as early as Gd 9 after only 4 days of exposure). The authors concluded that in $B6C3F_1$ mice, an inhalation exposure to 1,492 ppm chloromethane resulted in severe maternal toxicity; exposure to 102 and 479 ppm chloromethane did not produce maternal toxicity. No chloromethane-related deaths were observed in female rats.

Wolkowski-Tyl et al. (1983b) assessed the reproductive and developmental effects of an inhalation exposure to chloromethane in C57BL/6 females mated to C3H males to produce $B6C3F_1$ offspring. After mating, 74-77 females were exposed to chloromethane at concentrations of 0, 250, 500, or 750 ppm on Gd 6-17. At 750 ppm, six dams were found dead and one was found moribund on Gd 15-18. The authors concluded that an inhalation exposure to chloromethane during Gd 6-17 resulted in maternal toxicity at 750 ppm, but not at 500 or 250 ppm. Exposure of pregnant mice to 250 ppm chloromethane produced neither maternal nor fetal toxicity nor teratogenicity.

Chellman et al. (1987) investigated the role of chloromethane-induced testicular and epididymal inflammation in the induction of sperm cytotoxicity and preimplantation loss in male Fischer 344 rats. The rats were exposed to 3,056 ppm chloromethane 6 hours/day for 5 consecutive days, with or without concurrent treatment with 3-amino-l-[m-(tri-fluoromethyl)phenyl]-2-pyrazoline (BW755C), an anti-inflammatory agent. None of the animals died during the course of exposure.

Working et al. (1985a) studied the effects of an inhalation exposure to chloromethane on germ cell viability in male Fischer 344 rats. Forty males each were exposed to 0, 1,000, or 3,000 ppm chloromethane for 5 days, 6 hours/day. No males died during the 5-day treatment period or 8-week breeding period.

In an evaluation of the toxicologic and oncogenic effects of inhaled chloromethane in male and female Fischer 344 rats and $B6C3F_1$ mice, 120 animals per sex per exposure level were exposed to chloromethane in whole body inhalation exposure chambers at target concentrations of 0 (control), 50, 225, or 1,000 ppm, 6 hours/day, 5 days/week. Necropsies were completed at 6, 12, 18, or 24 months after the initial exposure (n=10, 10, 20, 80 for rats; and n=10, 10, 10, 90 for mice; respectively). Actual measured concentrations

averaged over the 24-month exposure period were 0.3±4, 51±9, 224±16, and 997±65 ppm. During the acute exposure time frame (≤ 14 days), chloromethane exposure had no effect on the survival curves of male or female rats or mice at the exposure levels received. During the intermediate exposure time frame (15-364 days) there was some increased mortality beginning at 10 months in female mice exposed to 1,000 ppm chloromethane, but no effect on the survival of male mice or male or female rats. During the second half of the study (i.e., the chronic exposure of ≥365 days), there was increased mortality in 1,000 ppm exposed male mice beginning at 17 months with a large increase in mortality by 19 months. For 1,000 ppm female mice, increased mortality began at 10 months and continued to rise by 20 months. The 1,000 ppm mice groups were terminated at 21 months (2 males) and 22 months (18 females) due to high mortality. Chloromethane had no effect on the survival of male or female rats (CIIT 1981).

No deaths occurred in male dogs (4 per group) exposed to ≥400 ppm chloromethane for 90 days (McKenna et al. 1981b). Female dogs were not tested.

The LC_{50} values and all reliable LOAEL values for death in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.2 Systemic Effects

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

Respiratory Effects. Case reports generally have not described respiratory effects in humans exposed to chloromethane. No effects on pulmonary function were observed in volunteers who participated in a study of neurological and neurobehavioral effects of acute inhalation exposure of up to 150 ppm chloromethane (Stewart et al. 1980). This study, however, had several limitations such as small sample size, multiple dosing schemes, and a confusing protocol. Specifically, groups of two to four men and two to four women were exposed to 10, 100, or 150 ppm or to concentrations that were increased from 50-150 ppm in the same group for 1, 3, or 7.5 hours per day over 2-5 days per week for 1 or 2 weeks. Several subjects, both male and female, dropped out of the study before some of the experiments were completed, and other subjects were added. Furthermore, the same subjects were used for different protocols during different weeks of the study. Despite the limitations, chloromethane exposure did not appear to have any effect on pulmonary function.

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	Rat (Sprague-Dawley)	2 or 3 d 24 hr/d				1000 (6-8/10 died by day 12 post- exposure to 1000 ppm for 72 hours)	Burek et al. 1981
2	Rat (Fischer- 344)	2-5 d 6 hr/d				5000 M (1/5 died)	Chellman et al. 1986a
3	Rat (Fischer- 344)	9 days 6 hr/d				3500 F (killed in extremis) 5000 M (killed in extremis)	Morgan et al. 1982
4	Mouse (B6C3F1)	6 hr				2200 M (LC_{50})	Chellman et al. 1986b
5	Mouse	2 wk 5 d/wk 6 hr/d				1500 M (5/45 died on first day, no subsequent deaths)	Chellman et al. 1986b
6	Mouse (B6C3F1)	6 hr				2500 M (14/15 died)	Chellman et al. 1986b
7	Mouse (C57BL/6)	2 wk 5 d/wk 6 hr/d				1500 (2/10 died)	Jiang et al. 1985
8	Mouse (C57BL/6)	11 d 5.5 hr/d				2400 F (killed in extremis)	Landry et al. 1985
9	Mouse (C57BL/6)	11 d 22 hr/d				150 F (killed in extremis)	Landry et al. 1985
10	Mouse (C3H) (C57Bl/6) (B6C3F1)	12 d 6 hr/d				1000 M (1/5 died by day 11) 2000 F (all died by day 5)	Morgan et al. 1982
11	Mouse (B6C3F1)	12 d 6 hr/d Gd 6-17				1492 F (all animals terminated early; 2 died prior to necropsy)	Wolkowski-Tyl et al. 1983a

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
12	Mouse (57BL/6)	12 d 6 hr/d Gd 6-17				750 F (6 died, 1 moribund of 75 dams)	Wolkowski-Tyl et al. 1983b
	Systemic						
13	Human	4hr	Gastro		29000M (nausea, vomiting)		Battigelli and Perini 1955
14	Human	1 d	Gastro		39000M (nausea, vomiting)		Jones 1942
15	Human	1-2 wk 2-5 d/wk 1, 3 or 7.5 hr/d	Resp	150			Stewart et al. 1980
			Cardio	150			
			Hemato	150			
16	Rat (Sprague-Dawley)	2 or 3 d 24 hr/d	Resp	2000			Burek et al. 1981
			Hemato	2000			
			Hepatic	500	1000 (fatty infiltration of liver)		
			Renal	500		1000 (increased BUN, tubular cell necrosis)	
			Bd Wt	200	500 (9-15% decrease that was regained by 12 days postexposure)	1000 (29-30% decrease, persistent in males)	
17	Rat (Fischer- 344)	12 d 4-5 d/wk 6 hr/d	Hepatic		3500M (decreased liver non-protein sulfhydryl content)		Chapin et al. 1984

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
18	Rat (Fischer- 344)	5 d 6 hr/d	Hepatic		5000 M (cloudy swelling of hepatocytes, obliteration of sinusoids)		Chellman et al. 1986a
			Renal			5000 M (necrosis of proximal convoluted tubules)	
			Endocr		5000 M (vacuolation of cell cytoplasm in the adrenal cortex)		
			Bd Wt			5000 M (20% loss of body weight)	
19	Rat	5 d 6 hr/d	Bd Wt	3056			Chellman et al. 1987
20	Rat (Fischer- 344)	9 days 6 hr/d	Gastro		5000 (diarrhea)		Morgan et al. 1982
			Hepatic		2000 F (minimal hepatocyte 3500 M degeneration)		
			Renal			2000 M (degeneration and necrosis 3500 F of proximal convoluted tubules)	
			Endocr	2000	3500 (fatty degeneration of adrenals)		
21	Rat (Fischer- 344)	13 d 6 hr/d Gd 7-19	Bd Wt	1492 F			Wolkowski-Tyl et al. 1983a
22	Rat (Fischer- 344)	5 d 6 hr/d	Bd Wt	1000 M	3000 M (16% decr. body weight)		Working et al. 1985a
23	Mouse (B6C3F1)	6 hr	Hepatic		1500 M (50 fold increase in ALT)		Chellman et al. 1986b
24	Mouse (NS)	2 wk 5 d/wk 6 hr/d	Renal		1500 (cell regeneration as indicated by 3 fold increased thymidine incorporation)		Chellman et al. 1986b

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
25	Mouse (C57BL/6)	2 wk 5 d/wk 6 hr/d	Renal		1500 F (slight degeneration of proximal tubules)		Jiang et al. 1985
26	Mouse (C57BL/6)	11 d 5.5 hr/d	Hemato	1600 F		2400 F (hemoglobinuria; enlarged spleen, low packed cell volume)	Landry et al. 1985
			Hepatic	800 F		1600 F (23% decr. rel. liver weight)	
			Renal	1600 F	2400 F (slight multifocal degeneration and regeneration of tubules; increased relative kidney weight)		
			Bd Wt	1600 F	2400 F (16% decr. body weight)		
			Other	800 F	1600 F (decreased food consumption indicated by decreased ingesta at necropsy)		
27	Mouse (C57BL/6)	11 d 22 hr/d	Hepatic	50 F	100 F (decreased hepatocyte size; glycogen depletion)	150 F (necrosis)	Landry et al. 1985
			Renal	150 F			
			Bd Wt	100 F	150 F (12% decr. body weight)	200 F (32% decr. body weight)	
			Other		150 F (decreased food consumption indicated by diminished amount of feces)		
28	Mouse (C3H) (C57Bl/6) (B6C3F1)	12 d 6 hr/d	Hepatic	1000 M 2000 F		2000 M (degeneration, necrosis)	Morgan et al. 1982
			Renal	500		1000 M (tubular basophilia; hematuria)	

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
29	Mouse (57BL/6)	12 d 6 hr/d Gd 6-17	Hepatic	250 F	500 F (increased absolute and maternal liver weight)		Wolkowski-Tyl et al. 1983b
			Bd Wt	250 F		750 F (41% decrease in maternal total weight gain)	
30	Dog (Beagle)	3 d 23.5 hr/d	Resp	500 M			McKenna et al. 1981a
			Cardio	500 M			
			Gastro	500 M			
			Hemato	500 M			
			Musc/skel	500 M			
			Hepatic	500 M			
			Renal	500 M			
			Endocr	500 M			
			Dermal	500 M			
			Ocular	500 M			
			Bd Wt	500 M			
31	Cat (NS)	3 d 23.5 hr/d	Resp	500 M			McKenna et al. 1981a
			Cardio	500 M			
			Gastro	500 M			
			Hemato	500 M			
			Musc/skel	500 M			
			Hepatic	500 M			
			Renal	500 M			
			Endocr	500 M			
			Dermal	500 M			
			Ocular	500 M			
	Neurological						
32	Human	4 hr				29000 M (vertigo, tremors, weakness)	Battigelli and Perini 1955
33	Human	1 d				39000 (ataxia, headache, convulsions)	Jones 1942

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
34	Human	3 hr			200 (4% decrement in performance)		Putz-Anderson et al. 1981a
35	Human	1-2 wk 2-5 d/wk 1, 3 or 7.5 hr/d		150			Stewart et al. 1980
36	Rat (Sprague-Dawley)	2 or 3 d 24 hr/d		500		1000 (lethargy)	Burek et al. 1981
37	Rat (Fischer- 344)	5 d 6 hr/d				5000 M (tremors, ataxia, forelimb/hindlimb paralysis, degeneration of cerebellar granule cells)	Chellman et al. 1986a
38	Rat (Fischer- 344)	9 d 6 hr/d				5000 (hindlimb paralysis, forelimb incoordination, cerebellar lesions)	Morgan et al. 1982
39	Mouse	2 wk 5 d/wk 6 hr/d				1500 M (multiple degenerative and necrotic foci in cerebellar granular cell layer)	Chellman et al. 1986b
40	Mouse (B6C3F1)	6 hr				2500 M (cerebellar damage indicated by tremors, ataxia, and forelimb/hindlimb paralysis)	Chellman et al. 1986b
41	Mouse (C57BL/6)	2 wk 5 d/wk 6 hr/d				1500 F (motor incoordination, coagulative necrosis and edema in cerebellar granule cells)	Jiang et al. 1985
42	Mouse (C57BL/6)	11 d 5.5 hr/d		150 F	400F (slight cerebellar granule cell degeneration)	800 F (poor motor coordination)	Landry et al. 1985
43	Mouse (C57BL/6)	11 d 22 hr/d		50[b] F		100 F (cerebellar granule cell degeneration	Landry et al. 1985

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
44	Mouse (C3H) (C57Bl/6) (B6C3F1)	12 d 6 hr/d		500		1000 (moderate cerebellar degeneration, ataxia)	Morgan et al. 1982
45	Mouse (B6C3F1)	12 d 6 hr/d Gd 6-17		479 F		1492 F (tremors, difficulty righting, degradation and selective necrosis of cerebellar granular cells)	Wolkowski-Tyl et al. 1983a
46	Mouse (57BL/6)	12 d 6 hr/d Gd 6-17		250 F	500F (ataxia)	750 F (tremors, convulsions, hyperactivity, ataxia, and piloerection)	Wolkowski-Tyl et al. 1983b
47	Dog (Beagle)	3 d 23.5 hr/d		200 M		500 M (slight, multifocal lesions in brain and spinal cord; vacuolization, swollen axons, loss of axons)	McKenna et al. 1981a
48	Cat (NS)	3 d 23.5 hr/d		500 M			McKenna et al. 1981a
	Reproductive						
49	Rat (Sprague-Dawley)	2 or 3 d 24 hr/d		200 M		500 M (sperm granulomas, decreased sperm in the tubule lumen, interstitial edema, coagulated proteinaceous obstruction of lumen)	Burek et al. 1981
50	Rat (Fischer- 344)	12 d 4-5 d/wk 6 hr/d				3500 M (delayed spermiation, seminiferous epithelium vacuolation, and bilateral epididymal granulomas)	Chapin et al. 1984
51	Rat (Fischer- 344)	2-5 d 6 hr/d				5000 M (exfoliation of pachytene spermatocytes & early stage spermatids; granuloma in epididymis)	Chellman et al. 1986a

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
52	Rat (Fischer- 344)	5 d 6 hr/d				3009 M (preimplantation loss due to testicular toxicity)	Chellman et al. 1986c
53	Rat	5 d 6 hr/d				3056 M (decr. testes weight; delayed spermiation; decreased sperm production; incr. in abnormal sperm; decr. in % motile and % intact sperm)	Chellman et al. 1987
54	Rat (Fischer- 344)	9 days 6 hr/d				2000 M (reduction in spermatids, separation of spermatocytes)	Morgan et al. 1982
55	Rat (Fischer- 344)	13 d 6 hr/d Gd 7-19		1492 F			Wolkowski-Tyl et al. 1983a
56	Rat (Fischer- 344)	5 d 6 hr/d			1000M (decreased fertility)	3000 M (severely reduced fertility)	Working and Bus 1986
57	Rat (Fischer- 344)	5 d 6 hr/d		1000 M		3000 M (postimplantation loss in mates, and persistent decreased fertility)	Working et al. 1985a
58	Rat (Fischer- 344)	5 d 6 hr/d		1000 M	3000M (reversible disruption of spermatogenesis)		Working et al. 1985b
59	Mouse (B6C3F1)	12 d 6 hr/d Gd 6-17		479 F			Wolkowski-Tyl et al. 1983a
60	Mouse (57BL/6)	12 d 6 hr/d Gd 6-17		500 F			Wolkowski-Tyl et al. 1983b
61	Dog (Beagle)	3 d 23.5 hr/d		500 M			McKenna et al. 1981a
62	Cat (NS)	3 d 23.5 hr/d		500 M			McKenna et al. 1981a

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
Developmental							
63	Rat (Fischer- 344)	13 d 6 hr/d Gd 7-19		479		1492 (retarded skeletal development; decreased fetal body weight and crown-rump length in females)	Wolkowski-Tyl et al. 1983a
64	Mouse (B6C3F1)	12 d 6 hr/d Gd 6-17		102		479 (heart defects in fetuses)	Wolkowski-Tyl et al. 1983a
65	Mouse (B6C3F1)	12 d 6 hr/d Gd 6-17		250		500 (heart defect in fetuses)	Wolkowski-Tyl et al. 1983b
INTERMEDIATE EXPOSURE							
Death							
66	Mouse (B6C3F1)	12 mo 5 d/wk 6 hr/d				997 F (increased mortality)	CIIT 1981
Systemic							
67	Rat (Fischer- 344)	6 mo 5 d/wk 6 hr/d	Resp	224 F	997 F (incr. rel. lung wt. from interstitial pneumonia) 51 M (incr. rel. lung wt. from interstitial pneumonia)		CIIT 1981
			Cardio	997			
			Gastro	997			
			Hemato	997			
			Musc/skel	997			
			Hepatic	224 M 997 F	997M (incr. rel. liver weight)		
			Renal	997			
			Endocr	997			
			Bd Wt	224	997 (10-11% decreased body weight)		

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
68	Rat (Fischer- 344)	12 mo 5 d/wk 6 hr/d	Resp	997			CIIT 1981
			Cardio	997			
			Gastro	997			
			Hemato	997			
			Musc/skel	997			
			Hepatic	224 M 997 F	997M (increased ALT levels)		
			Renal	997			
			Endocr	997			
			Bd Wt	224	997M (18% decreased body weight gain)		
69	Rat (Fischer- 344)	20 wk 5-7 d/wk 6 hr/d	Bd Wt	472	1502F (10-19% decreased body weight gain)	1502 M (20% decreased body weight gain)	Hamm et al. 1985
70	Rat (Sprague-Dawley)	90 d 5 d/wk 6 hr/d	Resp	400			McKenna et al. 1981b
			Cardio	400			
			Gastro	400			
			Hemato	400			
			Musc/skel	400			
			Hepatic	400			
			Renal	400			
			Dermal	400			

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
71	Rat (Fischer- 344)	90 d 5 d/wk 6 hr/d	Resp	1473			Mitchell et al. 1979
			Cardio	1473			
			Hemato	1473			
			Hepatic	1473 M 741 F	1473F (incr. rel. liver weight)		
			Renal	741 M 1473 F	1473M (incr. rel. kidney weight)		
			Dermal	1473			
			Bd Wt	368 M 741 F	741 M (11% decr. body weight) 1473 F (13% decr. body weight)		
72	Mouse (B6C3F1)	12 mo 5 d/wk 6 hr/d	Resp	997			CIIT 1981
			Cardio	224 F 997 M	997F (incr. rel. heart weight)		
			Hemato	997			
			Musc/skel	997			
			Hepatic	224	997F (incr. rel. liver weight)	997 M (incr. ALT, necrosis, cytomegaly, karyomegaly, polykaryocytes)	
			Renal	225 M 997 F	997M (renal tubuloepithelial hyperplasia; decreased absolute weight)		
			Endocr	997			
			Bd Wt	224	997 (decreased body weight in the 7-15% range)		

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
73	Mouse (B6C3F1)	6 mo 5 d/wk 6 hr/d	Resp	997			CIIT 1981
			Cardio	997			
			Hemato	997			
			Musc/skel	997			
			Hepatic	224 F	997 F (incr. rel. liver weight) 51[c] M (incr. ALT, no histological changes)	997 M (incr. ALT, necrosis, karyomegaly, polykaryocytes)	
			Renal	224	997 M (decr. abs. kidney wt) 997 F (incr. rel. kidney wt)		
			Endocr	997			
			Bd Wt	224 M	997 M (10% decr. body weight)		
74	Mouse (CD-1)	90 d 5 d/wk 6 hr/d	Resp	400			McKenna et al. 1981b
			Cardio	400			
			Gastro	400			
			Hemato	400			
			Musc/skel	400			
			Hepatic	150	400 (incr. rel. liver weight)		
			Renal	400			
			Dermal	400			
75	Mouse (B6C3F1)	90 d 5 d/wk 6 hr/d	Resp	1473			Mitchell et al. 1979
			Cardio	1473			
			Hemato	1473			
			Musc/skel	1473			
			Hepatic	741	1473 (increased ALT)		
			Renal	1473			
			Ocular			368 (mucopurulent conjunctivitis)	
			Bd Wt	1473			

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
76	Dog (Beagle)	90 d 5 d/wk 6 hr/d	Resp	400 M			McKenna et al. 1981b
			Cardio	400 M			
			Gastro	400 M			
			Hemato	400 M			
			Musc/skel	400 M			
			Hepatic		50M (swollen hepatocytes)		
			Renal	400 M			
			Dermal	400 M			
			Ocular	400 M			
	Immunological/Lymphoreticular						
77	Rat (Fischer- 344)	6 mo 5 d/wk 6 hr/d		997			CIIT 1981
78	Rat (Fischer- 344)	12 mo 5 d/wk 6 hr/d		997			CIIT 1981
79	Rat (Sprague-Dawley)	90 d 5 d/wk 6 hr/d		400			McKenna et al. 1981b
80	Mouse (B6C3F1)	6 mo 5 d/wk 6 hr/d		224	997	(lymphoid depletion of spleen; thymic lymphoid necrosis)	CIIT 1981
81	Mouse (B6C3F1)	12 mo 5 d/wk 6 hr/d		997			CIIT 198m1
82	Mouse (CD-1)	90 d 5 d/wk 6 hr/d		400			McKenna et al. 1981b
83	Dog (Beagle)	90 d 5 d/wk 6 hr/d		400 M			McKenna et al. 1981b

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
Neurological							
84	Human	2-3 wk or more 5 d/wk 8-16 hr/d				265 (impairment of memory, gait, balance, speech, and vision)	Scharnweber et al. 1974
85	Rat (Fischer- 344)	12 mo 5 d/wk 6 hr/d		997			CIIT 1981
86	Rat (Fischer- 344)	6 mo 5 d/wk 6 hr/d		997			CIIT 1981
87	Rat (Sprague-Dawley)	90 d 5 d/wk 6 hr/d		400			McKenna et al. 1981b
88	Rat (Fischer- 344)	90 d 5 d/wk 6 hr/d		1473			Mitchell et al. 1979
89	Mouse (CD-1)	90 d 5 d/wk 6 hr/d		400			McKenna et al. 1981b
90	Mouse (B6C3F1)	90 d 5 d/wk 6 hr/d		1473			Mitchell et al. 1979
91	Dog (Beagle)	90 d 5 d/wk 6 hr/d		400 M			McKenna et al. 1981b
Reproductive							
92	Rat (Fischer- 344)	6 mo 5 d/wk 6 hr/d		224		997 M (degeneration & atrophy of seminiferous tubules; sperm granulomas)	CIIT 1981

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
93	Rat (Fischer- 344)	12 mo 5 d/wk 6 hr/d		224		997 M (degeneration & atrophy of seminiferous tubules; sperm granulomas)	CIIT 1981
94	Rat (Fischer- 344)	20 wk 5-7 d/wk 6 hr/d		150	475M (decreased male fertility, litters per copulation plug)	1500 M (sterility, atrophy of the seminiferous tubules, epididymal granulomas)	Hamm et al. 1985
95	Rat (Fischer- 344)	10 wk 5-7 d/wk 6 hr/d		150	475 (decreased F1 generation male fertility and number of litters in F1 females)		Hamm et al. 1985
96	Rat (Sprague-Dawley)	90 d 5 d/wk 6 hr/d		400			McKenna et al. 1981b
97	Rat (Fischer- 344)	90 d 5 d/wk 6 hr/d		1473			Mitchell et al. 1979
98	Mouse (B6C3F1)	12 mo 5 d/wk 6 hr/d		997			CIIT 1981
99	Mouse (CD-1)	90 d 5 d/wk 6 hr/d		400			McKenna et al. 1981b
100	Dog (Beagle)	90 d 5 d/wk 6 hr/d		400 M			McKenna et al. 1981b
	Developmental						
101	Rat (Fischer- 344)	20 wk 5-7 d/wk 6 hr/d		1502			Hamm et al. 1985

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
CHRONIC EXPOSURE							
Death							
102	Mouse (B6C3F1)	24 mo 5 d/wk 6 hr/d				997 (increased mortality)	CIIT 1981
103	Mouse (B6C3F1)	18 mo 5 d/wk 6 hr/d				997 (increased mortality)	CIIT 1981
Systemic							
104	Rat (Fischer- 344)	24 mo 5 d/wk 6 hr/d	Resp	997			CIIT 1981
			Cardio	997			
			Gastro	997			
			Hemato	997			
			Musc/skel	997			
			Hepatic	224 M 997 F	997M (increased relative liver weight)		
			Renal	997			
			Endocr	997			
			Bd Wt	224	997 (14-15% decreased body weight gain)		
105	Rat (Fischer- 344)	18 mo 5 d/wk 6 hr/d	Resp	997			CIIT 1981
			Cardio	997			
			Gastro	997			
			Hemato	997			
			Musc/skel	997			
			Hepatic	997			
			Renal	997			
			Endocr	997			
			Bd Wt	224 M 997 F	997M (10% decreased body weight gain)		

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
106	Mouse (B6C3F1)	18 mo 5 d/wk 6 hr/d	Resp	997			CIIT 1981
			Cardio	224 F 997 M	997F (incr. rel. heart weight)		
			Hemato	997			
			Musc/skel	997			
			Hepatic	224	997F (incr. rel. liver weight)	997 M (incr. ALT, centrilobular degeneration, karyomegaly, cytomegaly)	
			Renal	224 M 997 F	997M (renal hyperplasia)		
			Endocr	997			
			Bd Wt	224	997 (12-19% decreased body weight)		
107	Mouse (B6C3F1)	24 mo 5 d/wk 6 hr/d	Resp	997			CIIT 1981
			Cardio	51 F 997 M	224F (incr. rel. heart weight)		
			Hemato	997			
			Musc/skel	997			
			Hepatic	51 M 224 F	224M (increased ALT)	997 (necrosis, cytomegaly, karyomegaly, polykaryocytes)	
			Renal	224	997 (renal hyperplasia)		
			Endocr	997			
			Bd Wt	224	997 (15-19% decreased body weight)		
Immunological/Lymphoreticular							
108	Rat (Fischer- 344)	24 mo 5 d/wk 6 hr/d		997			CIIT 1981

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
109	Rat (Fischer- 344)	18 mo 5 d/wk 6 hr/d		997			CIIT 1981
110	Mouse (B6C3F1)	18 mo 5 d/wk 6 hr/d		224	997 (splenic lymphoid depletion)		CIIT 1981
111	Mouse (B6C3F1)	24 mo 5 d/wk 6 hr/d		224		997 (splenic atrophy and lymphoid depletion)	CIIT 1981
	Neurological						
112	Rat (Fischer- 344)	18 mo 5 d/wk 6 hr/d		997			CIIT 1981
113	Rat (Fischer- 344)	24 mo 5 d/wk 6 hr/d		997			CIIT 1981
114	Mouse (B6C3F1)	24 mo 5 d/wk 6 hr/d			51 (swelling and degeneration of axons in spinal cord)	997 (tremor, paralysis, hindlimb rigidity, cerebellar granular cell atrophy)	CIIT 1981
115	Mouse (B6C3F1)	18 mo 5 d/wk 6 hr/d			51[d] (axonal swelling and slight degeneration of axons in spinal cord)	997 (tremor, paralysis; mild reduction in number of cerebellar neurons in the granular cell layer)	CIIT 1981
	Reproductive						
116	Rat (Fischer- 344)	24 mo 5 d/wk 6 hr/d		224 M		997 M (degeneration and atrophy of seminiferous tubules; sperm granulomas)	CIIT 1981
117	Rat (Fischer- 344)	18 mo 5 d/wk 6 hr/d		224 M		997 M (degeneration and atrophy of seminiferous tubules; sperm granulomas)	CIIT 1981

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
119	Mouse (B6C3F1)	18 mo 5 d/wk 6 hr/d		224 M		997 M (testicular degeneration and atrophy)	CIIT 1981
118	Mouse (B6C3F1)	24 mo 5 d/wk 6 hr/d		224 M		997 M (testicular degeneration and atrophy)	CIIT 1981
	Cancer						
120	Mouse (B6C3F1)	24 mo 5 d/wk 6 hr/d				997 M (CEL: renal cortex adenomas and adenocarcinomas, papillary cystadenomas, and papillary cystadenocarcinomas)	CIIT 1981
121	Mouse (B6C3F1)	12 mo 5 d/wk 6 hr/d				997 M (CEL: renal cortex adenoma)	CIIT 1981

Table 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
122	Mouse (B6C3F1)	18 mo 5 d/wk 6 hr/d				997 M (CEL: renal cortical adenoma)	CIIT 1981

[a]The number corresponds to entries in Figure 2-1.

[b] Used to derive an acute inhalation minimal risk level (MRL). No adjustment was made for continuous exposure and a human equivalent concentration was derived. Uncertainty factor of 100 (10 for intraspecies variability, 10 for interspecies variability) applied resulting in an MRL of 0.5 ppm.

[c]Used to derive an intermediate inhalation MRL. No adjustment was made for continuous exposure and a human equivalent concentration was derived. Uncertainty factor of 300 (3 for a minimal LOAEL to NOAEL, 10 for intraspecies variability, 10 for interspecies variability) applied resulting in an MRL of 0.2 ppm (rounded to one significant figure from 0.17 ppm).

[d]Used to derive a chronic inhalation MRL. No adjustment was made for continuous exposure and a human equivalent concentration was derived. Uncertainty factor of 1000 (10 for LOAEL to NOAEL, 10 for intraspecies variability, 10 for interspecies variability) applied resulting in an MRL of 0.05 ppm (rounded to one significant figure from 0.051 ppm).

ALT = alanine aminotransferase; AST = aspartate aminotransferase; Bd Wt = body weight; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); decr. = decreased; Endocr = endocrine; F = female; Gastro = gastrointestinal; Gd = gestational day; Hemato = hematological; hr = hour(s); incr. = increased; LC_{50} = lethal concentration, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; NS = not specified; RBC = red blood cell; rel. = relative; Resp = respiratory; WBC = white blood cell; wk = week(s)

Figure 2-1. Levels of Significant Exposure to Chloromethane - Inhalation

Acute (≤14 days)

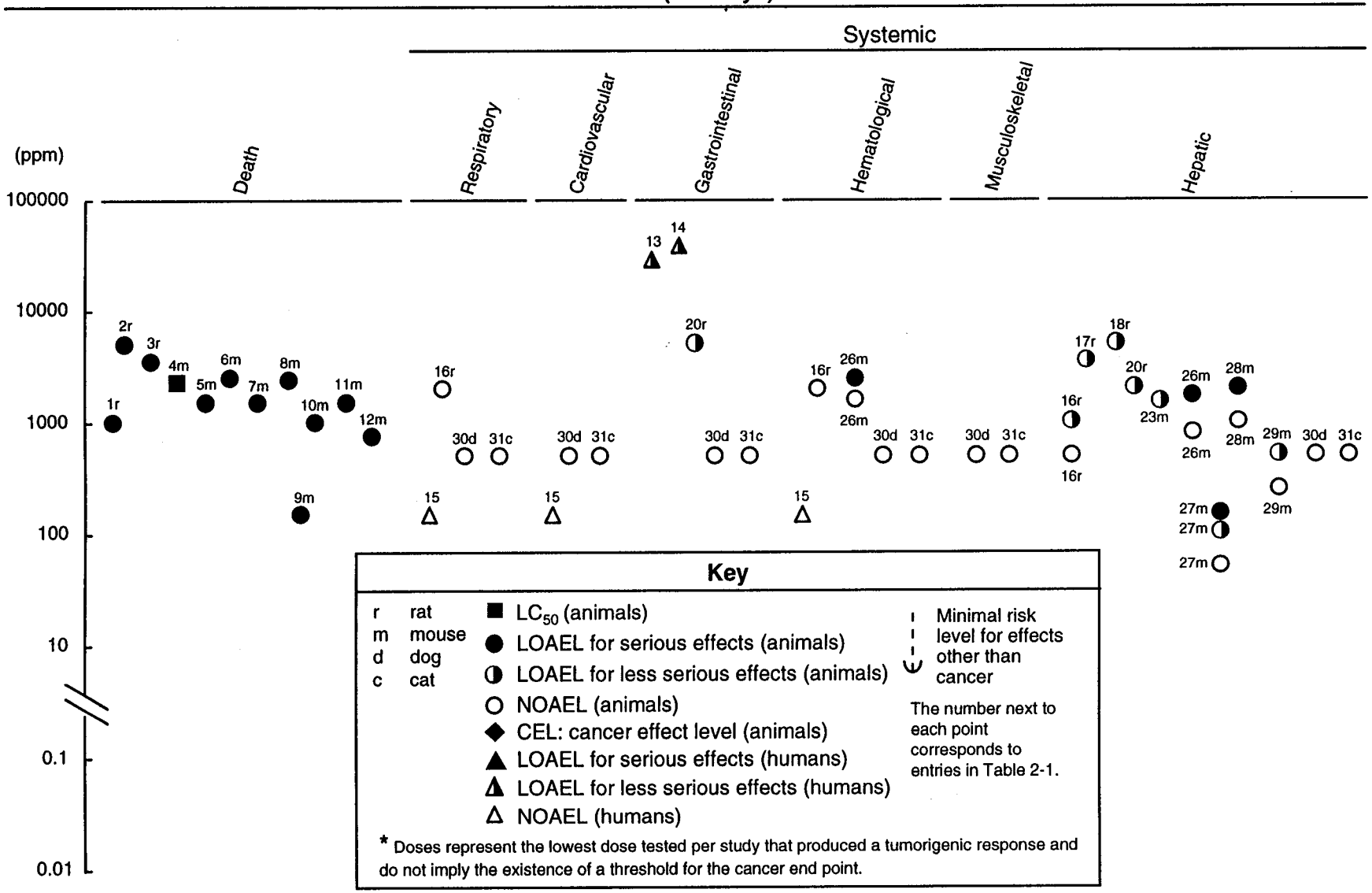


Figure 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (cont.)

Acute (≤14 days)

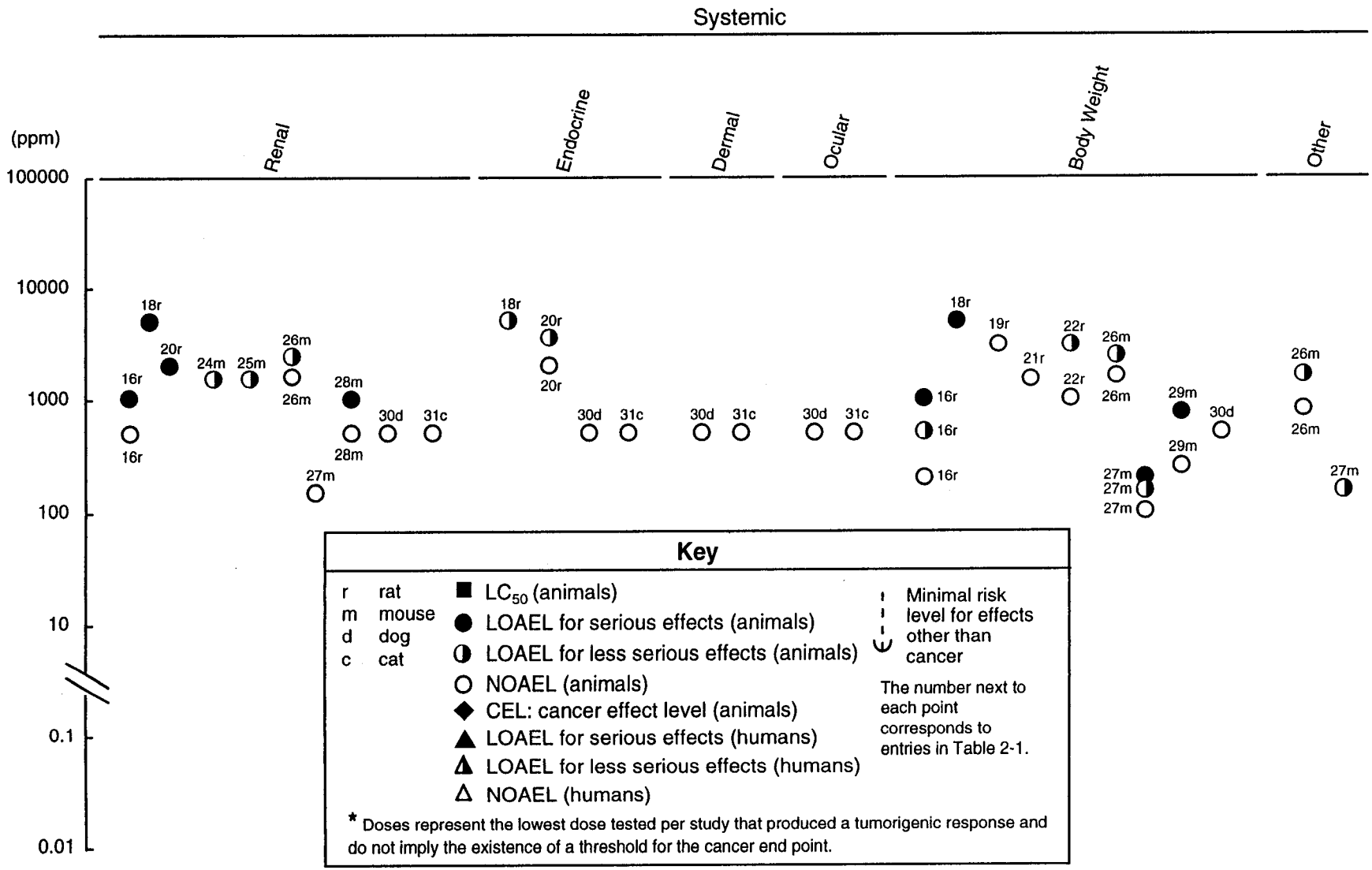


Figure 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (cont.)

Acute (≤14 days)

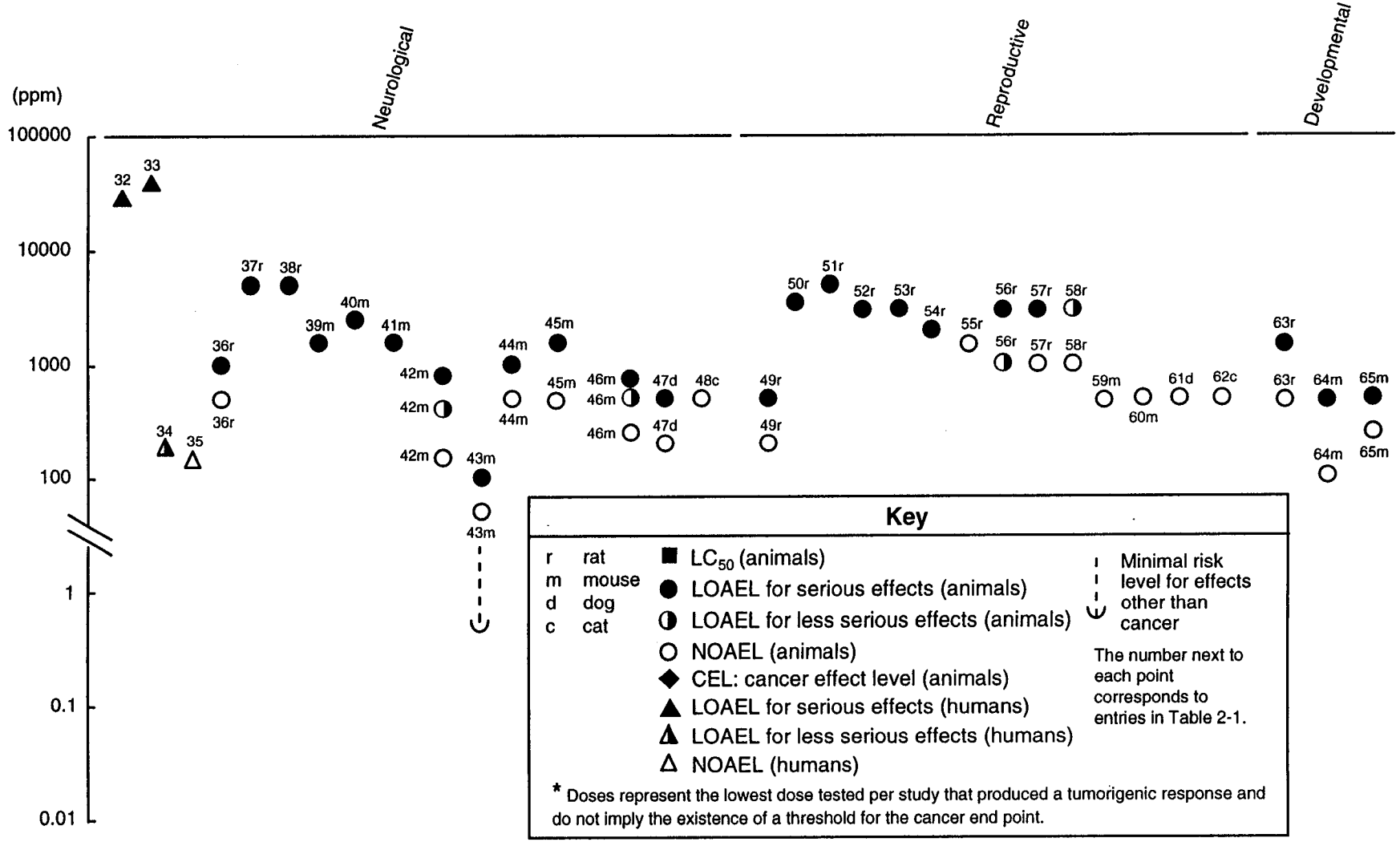


Figure 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (cont.)

Intermediate (15-364 days)

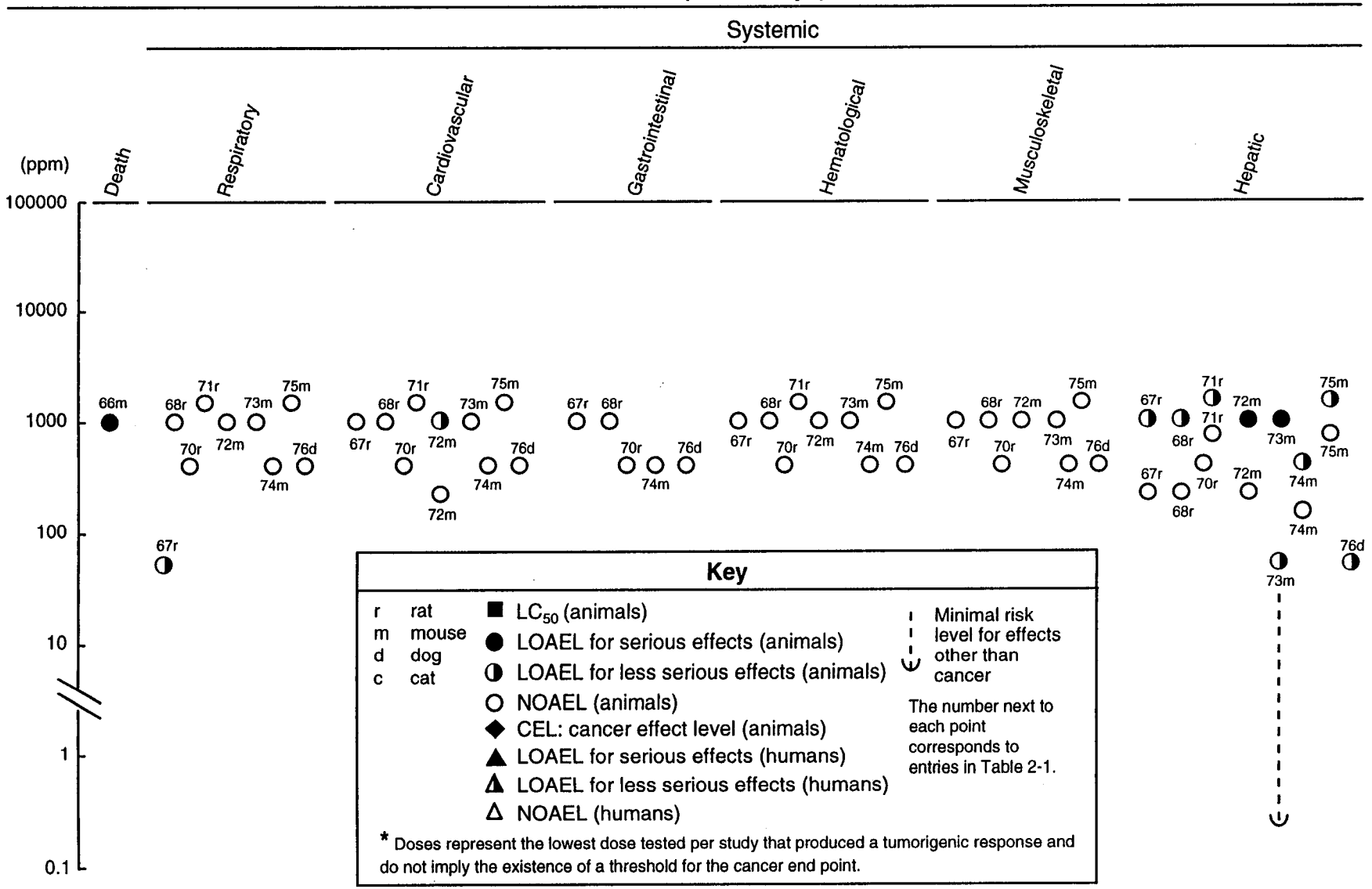


Figure 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (cont.)

Intermediate (15-364 days)

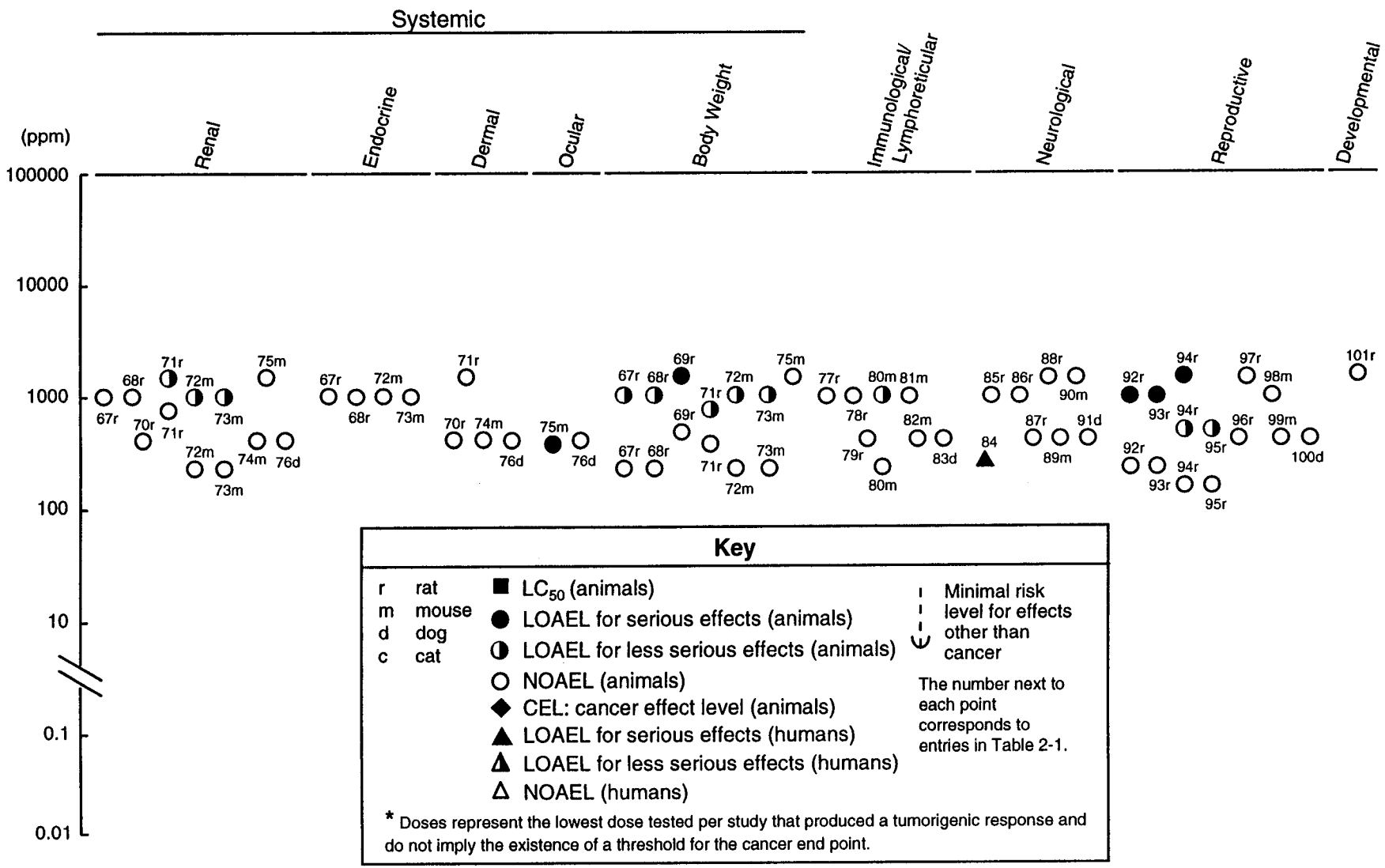


Figure 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (cont.)

Chronic (≥365 days)

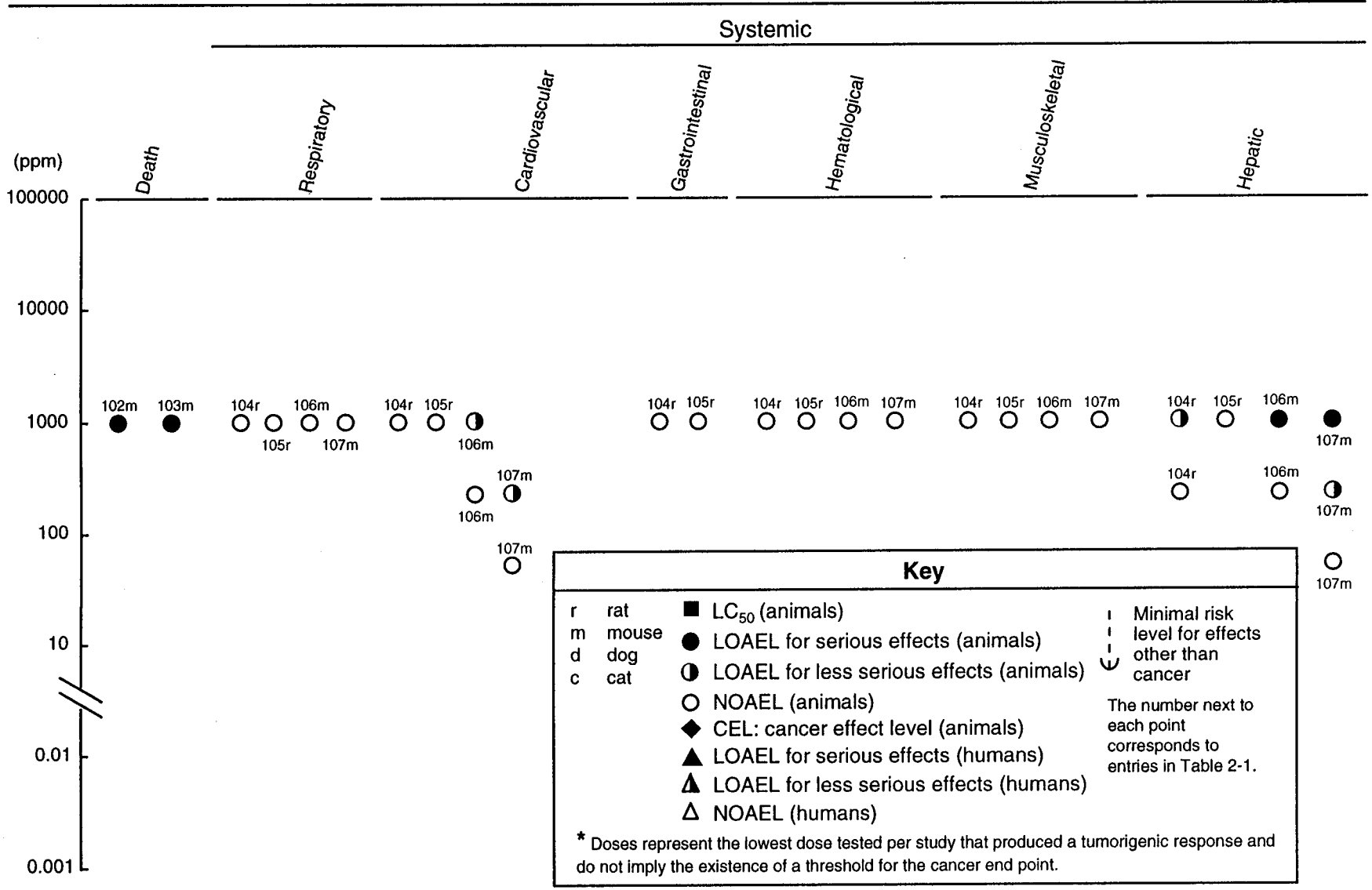
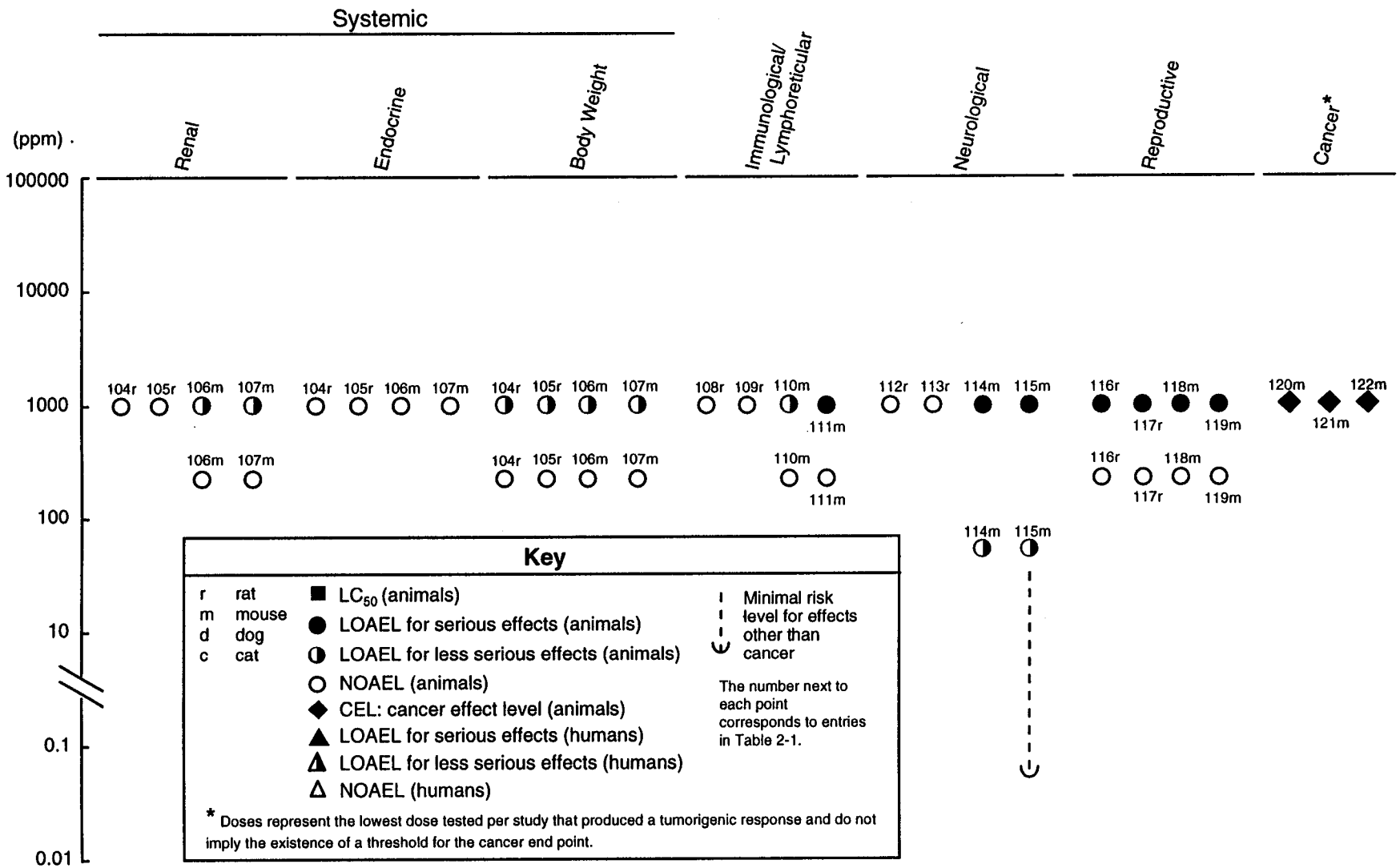


Figure 2-1. Levels of Significant Exposure to Chloromethane - Inhalation (cont.)

Chronic (≥365 days)



Acute exposure of dogs to 15,000 ppm caused an initial rise in heart rate and blood pressure, followed by markedly reduced respiration, decreased heart rate, and a progressive fall in blood pressure until the dogs died within 4-6 hours (von Oettingen et al. 1949, 1950). These effects may have resulted from vasodilation due to depression of the central nervous system. Pulmonary congestion was a common finding among the various species exposed to chloromethane until death (Dunn and Smith 1947; Smith and von Oettingen 1947a). As discussed above in Section 2.2.1.1, however, limitations of these reports preclude precise determination of concentration-duration-response relationships. More recent studies using very pure chloromethane (99.5-99.9%) failed to find any exposure-related histopathological lesions in the lungs of dogs and cats exposed acutely to 500 ppm chloromethane (McKenna et al. 1981a), rats exposed acutely to 2,000 ppm (Burek et al. 1981), male dogs exposed to 400 ppm, and rats and mice exposed to up to 1,500 ppm chloromethane for intermediate durations (McKenna et al. 1981b; Mitchell et al. 1979).

Dodd et al. (1982) examined the effects of an inhalation exposure to chloromethane on tissue nonprotein sulfhydryl (NPSH) content in male Fischer 344 rats. Groups of four animals each were exposed to chloromethane at concentrations of 0, 100, 500, or 1,500 ppm for 6 hours. Additional groups of four were exposed to 500 ppm chloromethane for periods of 1, 2, or 4 hours. Other groups of four were pretreated with Aroclor-1254 (metabolic inducer) or SKF-525A (metabolic inhibitor) prior to exposure to 500 ppm chloromethane [duration not noted]. The animals were sacrificed at various time points (0-18 hours) after exposure, at which time blood, liver, lung, and one kidney were collected for subsequent NPSH determinations. NPSH content of liver, kidney, and lung were decreased in a concentration-related manner. At 1,500 ppm, NPSH levels were 30% of control values in lungs immediately following exposure. At 500 ppm, levels were 55% of control values. No differences in NPSH content of the organs were observed after exposure to 100 ppm chloromethane compared with control. Lung NPSH levels returned to control values within 18 hours of exposure. A duration-related decrease was observed when rats were exposed to 500 ppm chloromethane for 1, 2, 4, or 6 hours. Pretreatment with Aroclor 1254 (inducer of microsomal enzymes) did not alter the decreases in tissue NPSH seen after exposure to chloromethane alone. Pretreatment with SKF-525A (inhibitor of microsomal enzymes) may have interfered with the ability of chloromethane to decrease NPSH in some tissues. Treatment with chloromethane significantly increased the activity of glutathione-S-alkyltransferase, and pretreatment with Aroclor 1254 did not alter the increase. The toxicological significance of this effect is not clear.

Male and female Fischer 344 rats and B6C3F_1 mice were exposed to chloromethane in whole body inhalation exposure chambers at target concentrations of 0 (control), 50, 225, or 1,000 ppm, 6 hours/day,

5 days/week. Necropsies were completed at 6, 12, 18, or 24 months after the initial exposure. At 6 months, relative lung weight was significantly increased at 50, 225, and 1,000 ppm in male rats and at 1,000 ppm in female rats. One male and 4 female rats at 1,000 ppm, 1 female at 225 ppm, and 2 males and 1 female at 50 ppm had minimal to moderate interstitial pneumonia with lymphocytic peribronchiolitis and perivasculitis. The interstitial lesions consisted of macrophage and lymphocytic infiltration. Also present were alveolar cell hyperplasia and mild alveolar luminal infiltrates consisting of large macrophages, lymphocytes, and in some areas, a few neutrophils. Five females at 1,000 ppm had areas of minimal subacute tracheitis (this lesion also occurred in 1 control male rat). At 12, 18, or 24 months, no chloromethane-related lung effects were observed. No effects on lungs were observed at any time point in mice. These respiratory effects were transitory, and the authors did not consider the effects to be associated with exposure to chloromethane (CIIT 1981).

Cardiovascular Effects. Cardiovascular effects of chloromethane have been described in case reports of humans exposed occupationally or accidentally due to refrigerator leaks (Gummert 1961; Hansen et al. 1953; Kegel et al. 1929; McNally 1946; Spevak et al. 1976; Verriere and Vachez 1949). These effects include electrocardiogram abnormalities, tachycardia and increased pulse rate, and decreased blood pressure. The precise concentrations and durations of exposure are not known. A retrospective epidemiological study of workers exposed to chloromethane in a butyl rubber manufacturing plant found no statistical evidence that the rate of death due to diseases of the circulatory system was increased in the exposed population when compared with U.S. mortality rates (Holmes et al. 1986). In a study of neurological and neurobehavioral effects of acute inhalation exposure in volunteers, no abnormalities of cardiac function or electrocardiograms were found at concentrations up to 150 ppm (Stewart et al. 1980).

The long-term cardiotoxic effects from an acute exposure to chloromethane were also studied by Rafnsson and Gudmundsson (1997) who found an excess mortality rate from cardiovascular disease. Seventeen crew members (males) were exposed for 2 days in 1963 to chloromethane that leaked from a refrigerator on board an Icelandic fishing trawler (exposure levels were not reported). The refrigerator was located under the sleeping quarters of the crew. This study followed a cohort of 24 men on board the vessel (6 officers and 18 deckhands) at 32 years postexposure. The reference group was selected from three registries of seamen. The Icelandic registries for seamen are some of the most comprehensive and complete available. The reference group contained five times as many individuals as the study group, and was controlled for age, occupation, and social class. The authors assumed simultaneous control for lifestyle factors including smoking habits and diet. The authors report excess mortality from all causes of death associated with acute

exposure to chloromethane (Mantel-Haenszel point estimate=2.2, 95%; CI=1.3-3.1), and a clear excess mortality from cardiovascular disease (M-H=2.1, 95%; CI= 1.2-3.8). This excess was more prominent among the deckhands who had received the highest exposure to chloromethane from the leaking refrigerator. The Risk ratios were elevated for all causes of death (RR=2.5, 95%; CI=l.0-5.7) as well as for cardiovascular disease (RR=3.9, 95%; CI=1.0-14.4). The study is weakened by the assumption of a simultaneous control for lifestyle factors including smoking habits and diet, and by the relatively small numbers of individuals with significant exposure. The authors also do not discuss the potential influence of the documented neurological deficits in this cohort on cardiovascular function (Gudmundsson 1977), and no definite mechanism of action was found in the literature. The authors suggest, however, that additional study on chloromethane's potential cardiovascular toxicity is warranted (Rafnsson and Gudmundsson 1997).

Scharnweber et al. (1974) presented 6 case studies of workers who were exposed to relatively low levels (200-400 ppm) of chloromethane for at least 2-3 weeks before onset of symptoms. Two cases occurred after "prolonged" (not otherwise specified) exposure to 8 hour time-weighted average (TWA) levels up to 300 ppm. Four cases occurred after work exposure on the order of 265 ppm (g-hour TWA) after 2-3 weeks of 12-16 hour days. One of the workers having prolonged exposure to 8-hour TWA levels up to 300 ppm experienced moderate hypertension (160/120 mm Hg).

Dogs exposed acutely to 15,000 ppm had an initial rise in heart rate and blood pressure, followed by markedly reduced respiration, decreased heart rate, and a progressive fall in blood pressure until death, which occurred within 4-6 hours (von Oettingen et al. 1949, 1950). These effects may have resulted from vasodilation due to depression of the central nervous system. Chloromethane exposure does not appear to result in histopathological lesions in the heart, as demonstrated by acute studies in male dogs and cats exposed to 500 ppm chloromethane (McKenna et al. 1981a), by intermediate duration studies in male dogs exposed to 400 ppm, and in rats and mice exposed to up to 1,500 ppm chloromethane (McKenna et al. 1981b; Mitchell et al. 1979).

Male and female Fischer 344 rats and $B6C3F_1$ mice were exposed to chloromethane in whole body inhalation exposure chambers at target concentrations of 0 (control), 50, 225, or 1,000 ppm, 6 hours/day, 5 days/week. Necropsies were completed at 6, 12, 18, or 24 months after the initial exposure. No cardiovascular effects were observed in male or female rats at any time point. No cardiovascular effects were observed in male mice. At 12 and 18 months, 1000 ppm female mice had increased relative heart

weight, and at 24 months, 225 ppm female mice had increased relative heart weight. These effects were considered to be chloromethane-related, but no associated histopathological lesions were observed (CIIT 1981).

Gastrointestinal Effects. Numerous case reports of humans exposed to chloromethane have described symptoms of nausea and vomiting (Baird 1954; Baker 1927; Battigelli and Perini 1955; Borovska et al. 1976; Hansen et al. 1953; Kegel et al. 1929; Mackie 1961; Jones 1942; Raalte and van Velzen 1945; Spevak et al. 1976; Verriere and Vachez 1949). In all cases, these symptoms were accompanied by central nervous system toxicity, which was usually severe. It is not clear, therefore, if the nausea and vomiting were secondary to the neurotoxic effects of chloromethane. Two of the reports (Battigelli and Perini 1955; Jones 1942) provided exposure concentration data.

Morgan et al. (1982) investigated the lesions induced by an inhalation exposure to chloromethane in C3H, C57BL/6, and B6C3F_1 mice and in Fischer 344 rats. Ten rats per sex were exposed to chloromethane for 5 days, 6 hours/day with a break in exposure for 2 days, and then a further 4 days of exposure. Rats were exposed to 0, 2,000, 3,500, or 5,000 ppm. Five mice per sex were exposed to chloromethane for 12 days, 6 hours/day. Mice were exposed to 0, 500, 1,000, or 2,000 ppm. Animals were sacrificed 18 hours after the last exposure or immediately after exposure if found to be moribund. Within 2 days of treatment, male and female rats in the 5,000 ppm group developed foul-smelling diarrhea. Gastrointestinal effects were not observed in mice.

Histopathological examination of animals exposed to various concentrations of chloromethane for acute, intermediate, or chronic durations did not show evidence of gastrointestinal damage (CIIT 1981; McKenna et al. 1981a, 1981b).

Hematological Effects. No hematological effects were found in volunteers who participated in a study of neurological and neurobehavioral effects of acute inhalation exposure of up to 150 ppm chloromethane (Stewart et al. 1980). Case reports of human overexposure have also generally been negative for hematological effects.

No long-term effect on the hematological system from an acute exposure was reported by Gudmundsson (1977). Seventeen crew members (males) were exposed for 2 days in 1963 to chloromethane that leaked from a refrigerator on board an Icelandic fishing trawler (no estimates of exposure levels were reported).

The refrigerator was located under the sleeping quarters of the crew. Thirteen years later (i.e., in 1976) 10 of the 11 survivors were examined (one lived in a foreign country and could not be located). All 10 were employed; 8 were employed at sea. The mean age of the 10 patients examined was 38.3 years (range 30-50 years). All 10 patients had normal hemoglobin, white cell count, differential leukocyte count, erythrocyte sedimentation rate, and serum creatinine.

Spleen enlargement, suggestive of extramedullary hematopoiesis, and hemoglobinuria, suggestive of intravascular hemolysis, were found in mice exposed intermittently to a high concentration (2,400 ppm) of chloromethane for 11 days (Landry et al. 1985). These effects were not seen when mice were exposed continuously to a lower concentration (150 ppm) (Landry et al. 1985). Male mice were not used in this study. No exposure-related effects on hematological parameters were found in male dogs or cats exposed continuously for 3 days to 500 ppm (McKenna et al. 1981a), or in rats exposed continuously for 3 days to 2,000 ppm (Burek et al. 1981). In addition, male dogs exposed to 400 ppm, rats and mice exposed to 1,500 ppm for 90 days (McKenna et al. 198 lb; Mitchell et al. 1979), and rats and mice exposed for 6, 12, 18, or 24 months to up to 1,000 ppm (CIIT 1981) did not have hematological effects.

Musculoskeletal Effects. Case reports generally have not described muscular or skeletal effects in humans exposed to chloromethane.

No adverse muscular or skeletal effects related to chloromethane exposure were observed in dogs and cats exposed acutely to 500 ppm chloromethane (McKenna et al. 1981 a), male dogs exposed to 400 ppm, and rats and mice exposed to 21,500 ppm chloromethane for intermediate durations (McKenna et al. 1981 b; Mitchell et al. 1979) or rats and mice exposed to up to 1,00 ppm chloromethane for chronic durations (CIIT 1981).

Hepatic Effects. Case reports of humans exposed to chloromethane have described clinical jaundice (Kegel et al. 1929; Mackie 1961; Weinstein 1937). A case of jaundice and cirrhosis of the liver was attributed to chloromethane exposure in a man who had been a refrigeration engineer for 10 years and had frequently been exposed to chloromethane vapors (Wood 1951). There was no reason to believe that these liver effects were due to other causes such as infective hepatitis or alcohol consumption.

Hepatic effects have also been observed in animals exposed to chloromethane, and mice appear to be more susceptible than rats. Rats exposed to l,000-1,500 ppm for acute, intermediate, or chronic durations had

either no liver effects or relatively mild to moderate changes, such as loss of normal areas of basophilia, cloudy swelling, increased liver weight, fatty infiltration, and increased serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum bilirubin (Burek et al. 1981; CIIT 1981; Mitchell et al. 1979; Morgan et al. 1982). No necrosis was seen. Acute, intermediate, or chronic exposure of mice to 1,000-l ,500 ppm generally resulted in necrosis and degeneration (CIIT 1981; Landry et al. 1985; Mitchell et al. 1979; Morgan et al. 1982). Although no significant liver effects were observed in male dogs and cats (McKenna et al. 1981 a, 1981 b), the exposure concentrations (400 or 500 ppm) may not have been high enough to produce liver toxicity in these species.

Chapin et al. (1984) investigated the cellular targets and the mechanism of reproductive tract lesions induced by inhaled chloromethane in male Fischer 344 rats. The animals were exposed to 3500 ppm chloromethane or air (controls) for 5 days, 6 hours/day, were subsequently not exposed for 3 days, and then exposed again for 4 days. Rats were killed on days 5, 7, 9, 11, 13, 15, 19, and 70 after starting exposure. To test for the effects of lower feed consumption in exposed rats, four weight-matched naive animals for each time interval were pair-fed identical amounts of feed to that consumed by the exposed animals and killed in the same manner. Tissue non-protein sulfhydryl (NPSH) content was measured in testes, caput and caudal epididymides, liver and heart blood. Liver NPSH content was significantly depleted within 1 hour of exposure (1.33 versus 5.44 μmol/g tissue; $p<0.05$).

Chellman et al. (1986a) studied the effects of 3-amino-l-[m-(trifluoromethyl)phenyl]-2-pyrazoline (BW755C), a potent anti-inflammatory agent, on chloromethane-induced lethality and reproductive toxicity in male Fischer 344 rats. Rats were exposed to 5,000 ppm chloromethane for 5 days, 6 hours/day, with or without treatment with BW755C (10 mg/kg, intraperitoneally 1 hour pre- and postexposure). Rats exposed to 5,000 ppm chloromethane, 6 hours/day for 5 days exhibited cloudy swelling of hepatocytes in the liver with subsequent obliteration of the sinusoids. Rats exposed to both chloromethane and BW755C had only very subtle, if any, lesions. The results are surprising because the liver lesions were not inflammatory in nature. The authors concluded that protection from chloromethane-induced injury by BW755C was not simply the result of altered metabolism because BW755C had no effect on tissue distribution or excretion of ^{14}C-chloromethane and administration of BW755C did not decrease hepatic glutathione content. The protection afforded by BW755C may have been related to an inhibition of leukotriene and prostaglandin synthesis.

Dodd et al. (1982) examined the effects of an inhalation exposure to chloromethane on tissue nonprotein sulfhydryl (NPSH) content in male Fischer 344 rats. Groups of four animals each were exposed to chloromethane at concentrations of 0, 100, 500, or 1,500 ppm for 6 hours. Additional groups of four were exposed to 500 ppm chloromethane for periods of 1, 2, or 4 hours. Other groups of four were pretreated with Aroclor-1254 (metabolic inducer) or SKF-525A (metabolic inhibitor) prior to exposure to 500 ppm chloromethane (duration not noted). The animals were sacrificed at various time points (0 to 18 hours) after exposure, at which time blood, liver, lung, and one kidney were collected for subsequent NPSH determinations. NPSH content of liver was decreased in a concentration-related manner. At 1,500 ppm, NPSH levels were 17% of control values immediately following exposure. At 500 ppm, NPSH levels were 41% of control values. No differences in NPSH content were observed after exposure to 100 ppm chloromethane compared with control. Liver NPSH levels returned to control values within 8 hours of treatment. Pretreatment with Aroclor 1254 (inducer of microsomal enzymes) did not alter the decreases in liver NPSH seen after exposure to chloromethane alone. Pretreatment with SKF-525A (inhibitor of microsomal enzymes) may have interfered with the ability of chloromethane to decrease NPSH in some tissues. Treatment with chloromethane significantly increased the activity of glutathione-S-alkyltransferase, and pretreatment with Aroclor 1254 did not alter the increase. The toxicological significance of this effect is not clear.

Chellman et al. (1986b) investigated the role of glutathione in the mediation of chloromethane-induced toxicity in the liver, kidney and brain of male $B6C3F_1$ mice. Animals were exposed for 6 hours to 1,500 ppm chloromethane, with and without pretreatment with buthionine-S,R-sulfoximine (BSO), diethyl maleate (DEM), or fasting to deplete glutathione (GSH). The mice were sacrificed 18 hours after completion of exposures, blood samples were collected, and the serum was analyzed for alanine aminotransferase (ALT) to measure liver toxicity. There was a 50-fold increase in ALT activity in exposed mice without pretreatment. Fasting or pretreatment with BSO or DEM resulted in ALT values which were similar to those of controls. Therefore, depletion of GSH protected mice from hepatic toxicity of chloromethane.

Jager et al. (1988) investigated the effects of an inhalation chloromethane exposure on tissue levels of glutathione-S-transferase (GST) and formaldehyde dehydrogenase (FDH) in male and female Fischer 344 rats and $B6C3F_1$ mice. Activities of GST were 2-3 times higher in livers of male $B6C3F_1$ mice, compared with those of female mice, and with rats of both sexes. In kidneys, GST activities of male mice were about 7 times lower than those found in the liver. The activity of FDH was higher in mouse liver (both sexes)

than in rat liver. More formaldehyde was produced in the liver of male, as compared to those of female mice. After a single, g-hour exposure to 1,000 ppm chloromethane in males or female mice, formaldehyde levels were not observed to increase in livers or kidneys (ex vivo). Lipid peroxidation was significantly and markedly increased in the liver of male and female mice, and to a lesser extent in the kidney, from the single exposure to chloromethane.

Landry et al. (1985) observed mild hepatic effects in mice intermittently exposed to 400 to 2,400 ppm (glycogen depletion, no hepatic degeneration or necrosis). Only the 1,600 ppm mice had significantly increased liver absolute (22%) and relative (23%) weight. Mice continuously exposed to 400 ppm died or were sacrificed by day 4, and by day 5 for a 200 ppm group, due to severe toxicity. Mice continuously exposed to 150 ppm were sacrificed in moribund condition by day 10.5. Decreased food consumption was indicated by diminished amount of feces and scratched food under the cages of the 150 or 200 ppm groups. The 150 ppm exposure resulted in a significant decrease in absolute liver weight (13%), but not relative weight. Mice had a decreased hepatocyte size (due to glycogen depletion) at 100 ppm with focal necrosis at 150 ppm and greater.

Morgan et al. (1982) investigated the lesions induced by an inhalation exposure to chloromethane in C3H, C57BL/6, and B6C3F_1 mice and in Fischer 344 rats. Ten rats per sex were exposed to chloromethane for 5 days, 6 hours/day with a break in exposure for 2 days, and then a further 4 days of exposure. Rats were exposed to 0, 2,000, 3,500, or 5,000 ppm. Animals were sacrificed 18 hours after the last exposure or immediately after exposure if found to be moribund. All exposed groups except 2,000 ppm males had high incidences (8/10 to 10/10) of minimal hepatocellular lesions, consisting of loss of normal area of cytoplasmic basophilia. Five mice per sex were exposed to chloromethane for 12 days, 6 hours/day at levels of 0, 500, 1,000, or 2,000 ppm. Animals were sacrificed 18 hours after the last exposure or immediately after exposure if found to be moribund. Hepatocellular degeneration consisting of necrosis, hyaline accumulation in bile ducts, vacuolization, and glycogen depletion was observed. The lesions resembled those usually described for carbon tetrachloride and chloroform. Necrosis was confined to male C57BL/6 and B6C3F_1 mice exposed to 2,000 ppm. The other lesions occurred to varying degrees in other groups and were of minimal severity. No liver lesions were observed in controls.

Wolkowski-Tyl et al. (1983b) assessed the reproductive and developmental effects of an inhalation exposure to chloromethane in C57BL/6 females mated to C3H males to produce B6C3F_1 offspring. After mating, 74-77 females were exposed to chloromethane at concentrations of 0, 250, 500, or 750 ppm on

Gd 6-17. Surviving dams were weighed and sacrificed on gestation day 18. A significant increase in maternal absolute liver weight (9%) and relative liver weight (6%) was observed in the 500 ppm mice. A nonsignificant decrease was observed in the 750 ppm dams.

Male and female Fischer 344 rats and B6C3F_1 mice were exposed to chloromethane in whole body inhalation exposure chambers at target concentrations of 0 (control), 50, 225, or 1,000 ppm, 6 hours/day, 5 days/week (CIIT 1981). Necropsies were completed at 6, 12, 18, or 24 months after the initial exposure. Increased ALT associated with exposure-related liver lesions was seen in male mice exposed to 1,000 ppm chloromethane at all time points. The lesions were centrilobular and characterized by mild to moderate hepatocellular degeneration often associated with vacuolization of most of the cytoplasm, individual hepatocellular necrosis, cytomegaly and karyomegaly, and numerous hepatocytes containing eosinophilic, intranuclear inclusion material. Increased ALT was also seen in 50 and 225 ppm males but no histopatholgical changes to the liver were observed at these exposure levels. Increased ALT in female mice exposed to 50, 225, and 1,000 ppm at 6 and 12 months was observed, but no histopathological changes were observed in females at any of the dose levels. ALT levels returned to normal at 18 and 24 months in female mice. Females that became moribund or that were exposed to 1,000 ppm for the longer 18- and 24-month exposure periods had liver lesions similar to those found in the males, but with less frequency and severity. Statistically significant increases in relative liver weight were observed in both male and female mice at 1,000 ppm. Male and female rats did not have the histopathological liver lesions seen in mice. Male rats did generally have increased relative liver weights at 1,000 ppm. No effect on ALT levels was observed in rats.

McKenna et al. (1981b) exposed CD-l mice to 99.9% pure chloromethane. Complete histological examination performed on the control and 400 ppm groups. In the liver, there was a significant increase in relative liver weight in 400 ppm females and a trend in 400 ppm males and 150 ppm males and females. The increase was accompanied by equivocal lesions (change in tinctorial properties of liver cells, possibly due to decrease vacuolization). The lesions were subtle and reversible and not considered adverse. McKenna et al. (1981b) also exposed Beagle dogs to 99.9% pure chloromethane. There were no effects on ALT or AST, but hepatocytes were swollen in 2 of 4 dogs at 400 ppm, 1 of 4 dogs at 150 ppm, 2 of 4 dogs at 50 ppm, and 0 of 4 controls. No other liver effects were observed, and the toxicological significance of these effects are unclear.

The lowest concentration for dose-related hepatic effects is the LOAEL of 51 ppm for increased ALT in male mice (CIIT 1981). This LOAEL is used as the basis for an intermediate inhalation MRL of 0.2 ppm, calculated as described in the footnote to Table 2-1 and in Appendix A. This MRL is presented in Figure 2-1.

Renal Effects. Case reports of humans exposed to chloromethane have described such indicators of renal toxicity as albuminuria, increased serum creatinine and blood urea nitrogen, proteinuria, and anuria (Kegel et al. 1929; Mackie 1961; Spevak et al. 1976; Verriere and Vachez 1949). Exposure concentrations at which these effects occurred are not known.

Sprague-Dawley rats exposed to chloromethane at 1,000 ppm for 72 hours had slightly increased blood urea nitrogen (BUN), but this effect only occurred significantly in females. Abnormal urinalysis parameters indicative of renal failure occurred in both sexes of rats exposed to 1,000 or 2,000 ppm for 48 or 72 hours. Histological examination revealed renal tubular cell necrosis, increased lipid accumulation in tubule cells at 1,000 ppm for both exposure periods, and evidence of regeneration after the recovery period. Greatly increased (statistically significant) BUN in 2,000 ppm male and female rats sacrificed at 48 hours indicated kidney failure (Burek et al. 1981).

Chellman et al. (1986a) exposed male Fischer 344 rats to 5,000 ppm chloromethane for 5 days, 6 hours/day resulting in necrosis of the proximal convoluted tubules. Dodd et al. (1982) exposed male Fischer 344 rats to chloromethane at 0, 100, 500, or 1,500 ppm for 6 hours. Nonprotein sulfhydryl (NPSH) content of kidney was decreased in a concentration-related manner. Kidney NPSH levels returned to control values within 8 hours of treatment. The toxicological significance of this effect is not clear.

Morgan et al. (1982) investigated the lesions induced by an inhalation exposure to chloromethane in C3H, C57BL/6, and B6C3F_1 mice and in Fischer 344 rats. Rats were exposed to 0, 2,000, 3,500, or 5,000 ppm for 5 days, 6 hours/day with a break in exposure for 2 days, and then a further 4 days of exposure. Mice were exposed to 0, 500, 1,000, or 2,000 ppm for 12 days, 6 hours/day. Two types of kidney lesions were seen, basophilia of renal tubules and degeneration and necrosis of renal proximal convoluted tubules. The degeneration was found mainly in the 2,000 ppm groups in both males and females of all strains. The basophilia, presumed to be regeneration, was found mainly in the 1,000 ppm group. Hematuria occurred in mice exposed to 1,000 and 2,000 ppm, but it was not clear whether it was due to renal damage or lesions elsewhere in the urogenital tract. In the rat kidneys, there was a dose-related increased incidence and

severity of degeneration of proximal tubules. No basophilia in renal tubules occurred in rats as was seen in mice. The authors speculated that the basophilia in mice is a proliferative response related to the induction of kidney tumors seen in mice and not rats.

Chellman et al. (1986b) investigated the role of glutathione in the mediation of chloromethane-induced toxicity in the liver, kidney and brain of male $B6C3F_1$ mice. Mice exposed to 1,500 ppm chloromethane 6 hours/day, 5 days/week for 2 weeks had no significant changes in kidney weight, glomerular filtration rate, urinary excretion of glucose and protein, or urinary concentrating ability. Histologically, the only effect of chloromethane exposure was a slight increase in the number of basophilic cortical tubules. Incorporation of tritiated thymidine into deoxyribonucleic acid (DNA) was 3-fold greater in kidneys of chloromethane exposed male mice than controls. Incorporation of tritiated thymidine was not significantly elevated in mice exposed and pretreated with BSO. BSO alone had no effect on DNA synthesis. In female mice, incorporation of tritiated thymidine into DNA was S-fold greater in kidneys of chloromethaneexposed versus controls. Therefore, depletion of GSH protected mice from increased DNA synthesis induced by chloromethane. The increased DNA synthesis may result from a compensatory proliferation in response to cell death. Although cell death was not observed in kidneys histologically, basophilic foci are consistent with regenerative cellular response following cell death.

Jager et al. (1988) investigated the effects of a chloromethane inhalation exposure on tissue levels of glutathione-S-transferase (GST) and formaldehyde dehydrogenase (FDH) in male and female Fischer 344 rats and $B6C3F_1$ mice. Activities of GST in kidneys of male mice were about 7 times lower than those found in the liver. About 50% more formaldehyde was produced in the male mouse kidney, compared to the female kidney (indicative of higher levels of P-450 in the male kidney). No DNA-protein crosslinks in the kidney and only some evidence of single-strand breaks was observed in male $B6C3F_1$ mice exposed to 1,000 ppm chloromethane for 4 days, 6 hours/day. After a single, 8 hour exposure to 1,000 ppm chloromethane in male or female mice, formaldehyde levels were not observed to increase in livers or kidneys (ex vivo). Lipid peroxidation was significantly and markedly increased in the liver of male and female mice, and to a lesser extent in the kidney, from the single exposure to chloromethane.

Female C57BL/6 mice exposed to 1,500 ppm chloromethane for 2 weeks, 5 days/week, 6 hours/day showed a slight degeneration of proximal convoluted tubules and proteinaceous material in tubular lumen. The renal and brain lesions in the study were unrelated in terms of severity; therefore, the authors

concluded that the brain lesions seen after exposure to chloromethane were probably not a direct consequence of renal lesions (Jiang et al. 1985).

Landry et al. (1985) evaluated the neurologic effects of continuous versus intermittent chloromethane exposure in female C57BL/6 mice. Mice were exposed to chloromethane in whole body inhalation chambers for 11 days either continuously for 22 hours/day at 0, 15, 50, 100, 150, 200, or 400 ppm or intermittently for 5.5 hours/day at 0, 150, 400, 800, 1,600, or 2,400 ppm. Kidney effects were only observed in the intermittently exposed mice at 2,400 ppm. The effects consisted of a slight multifocal degeneration and regeneration of tubules, and an eosinophilic staining cast within the tubules. The 2,400 ppm mice had a nonsignificant increase in relative kidney weight. No histopathological lesions were observed in the kidney, thus the increased weight does not appear to represent an adverse effect.

Beagle dogs and cats exposed to 200 or 500 ppm chloromethane for 23.5 hours/days for 3 days had no significant differences in clinical chemistry or urinalysis parameters. A comprehensive histological examination revealed no exposure-related lesions in any system other than neurological. This was a good comprehensive study, but is limited by the number of animals (3) per group (McKenna et al. 1981a). Beagle dogs were also exposed to 0, 50, 150, and 400 ppm for 6 hours/day, 5 days/week for 90 days. There were no exposure-related gross or histopathological lesions in the kidneys and no effect on BUN (McKenna et al. 1981b). This was a comprehensive study, but is limited by the number of animals (4) per group.

Sprague-Dawley rats were exposed to 0, 50, 150, or 400 ppm chloromethane 6 hours/day, 5 days/week, for 90 days. There was no effect on BUN, but urinary specific gravity was decreased in males at 400 ppm and females at 150 ppm. This decrease was not associated with gross histologic pathology, and therefore, the toxicological significance of this effect is unclear. CD-l mice were exposed to the same regimen with no apparent effects on the kidneys (McKenna et al. 1981b).

Fischer 344 rats exposed to 0, 375, 750, and 1,500 ppm for 6 hours/day, 5 days/week, for 13 weeks developed a significant increase in relative left kidney weight for the 1,500 ppm males. There were no clinically significant hematological, clinical chemistry, or urinalysis abnormalities so the significance of this effect is unclear (Mitchell et al. 1979).

$B6C3F_1$ mice were exposed to 0, 375, 7.50, and 1,500 ppm for 6 hours/day, 5 days/week, for 13 weeks. No exposure-related histopathological lesions of the kidneys, and no clinically significant effects on hematological and urinalysis indices were observed. Relative kidney weight was increased in 1,500 ppm males, but no histopathological lesions were associated with the increase (Mitchell et al. 1979).

Male and female Fischer 344 rats and $B6C3F_1$ mice were exposed to chloromethane in whole body inhalation exposure chambers at target concentrations of 0 (control), 50, 225, or 1,000 ppm, 6 hours/day, 5 days/week. Necropsies were completed at 6, 12, 18, or 24 months after the initial exposure. Increased relative kidney weights were noted in female mice at 1,000 ppm, while decreased absolute kidney weights were seen in males at 1,000 ppm; there was no apparent reason for the sex difference. The authors interpreted the decrease in absolute kidney weight in male mice as biologically significant. Males exposed to 1,000 ppm developed renal tubuloepithelial hyperplasia and karyomegaly that became progressively worse, followed by the development of renal adenomas and adenocarcinomas. Females did not develop these lesions until after 18 months and to a much lesser extent. Male and female rats had varying levels of increased relative kidney weights throughout the study, but these were not associated with clinical, gross, or histopathological findings; thus, the toxicological significance of these effects is unclear (CIIT 1981).

Endocrine Effects. No studies were located regarding endocrine effects in humans after inhalation exposure to chloromethane.

Some effects have been observed in high-level, acute exposure animal studies. Male Fischer 344 rats exposed to 5,000 ppm chloromethane for 5 days, 6 hours/day developed vacuolar degeneration in the cell cytoplasm of the adrenal cortex in the outer region of the zona fasciculata (Chellman et al. 1986a). Fatty droplets were seen in the epithelial cells of the zona fasciculata in the adrenals of Fischer 344 rats exposed to 3,500 and 5,000 ppm chloromethane for 5 days, 6 hours/day with a break in exposure for 2 days, and then a further 4 days of exposure; the severity of this lesion increased with dose (Morgan et al. 1982).

Results are generally negative with lower level or longer duration exposures. No chloromethane-related effects on the endocrine organs were observed from acute exposures up to 500 ppm in Beagle dogs or cats (McKenna et al. 198 la), or from intermediate and chronic exposures up to 1,000 ppm in mice or rats (CIIT 1981).

Dermal Effects. No studies were located regarding dermal effects in humans after inhalation exposure to chloromethane.

No dermal effects were observed from acute chloromethane exposures up to 500 ppm in Beagle dogs or cats (McKenna et al. 1981a), or from intermediate exposures up to 400 ppm in Sprague-Dawley rats or CD-l mice (McKenna et al. 1981b), up to 1,500 ppm in Fischer 344 rats.(Mitchell et al. 1979), or up to 400 ppm in Beagle dogs (McKenna et al. 1981b).

Ocular Effects. Case reports of humans exposed to chloromethane have described such symptoms as blurred and double vision (Baker 1927; Borovska et al. 1976; Gummert 1961; Kegel et al. 1929; Mackie 1961). These symptoms probably reflect effects on the nervous system rather than effects on the eye itself. Ophthalmological examination of male cats and Beagle dogs exposed to 500 ppm continuously for 3 days (McKenna et al. 1981a), dogs exposed to 400 ppm for 90 days (McKenna et al. 1981b), or of rats and mice exposed to 1,000 ppm for up to 24 months (CIIT 1981) failed to reveal eye lesions. However, mucopurulent conjunctivitis with total destruction of the eye in some cases was found in mice exposed to ≥375 ppm for 6 hours/day, 5 days/week, for 90 days (Mitchell et al. 1979). These lesions were attributed to exposure because no lesions were found in controls; however, the failure of longer-term studies to detect eye lesions at higher concentrations makes the findings of Mitchell et al. (1979) questionable. If the eye lesions were due to chloromethane exposure, the effect was probably due to direct contact of the vapor with the eye, rather than a consequence of inhalation.

Body Weight Effects. No studies were located regarding body weight effects in humans after inhalation exposure to chloromethane.

A consistent systemic effect of chloromethane exposure in animals is reduced body weight gain, which was observed in rats and mice exposed to chloromethane for acute, intermediate, and chronic durations (Burek et al. 1981; CIIT 1981; Landry et al. 1985; Mitchell et al. 1979). Landry et al. (1985) evaluated the neurologic effects of continuous versus intermittent chloromethane exposure in female C57BL/6 mice. Groups of 12 mice each were exposed to chloromethane in whole body inhalation chambers for 11 days either continuously for 22 hours/day at 0, 15, 50, 100, 150, 200, or 400 ppm or intermittently for 5.5 hours/day at 0, 150, 400, 800, 1,600, or 2,400 ppm. Mice were weighed prior to exposure, on exposure days 4 and 8, and at necropsy. The 400 ppm exposed mice died or were sacrificed by day 4, and

the 200 ppm group by day 5, due to severe toxicity. Mice exposed to 150 ppm were sacrificed in moribund condition by day 10.5. Continuous exposure to chloromethane resulted in significantly decreased body weight in the 200 ppm group (33%) by day 4 compared to the controls, and in the 150 ppm group by day 4 (16%) persisting to the sacrifice at day 10.5 (12%). A nonsignificant decrease was seen in the 100 ppm group and no effects on body weight were seen at 50 ppm.

Other Systemic Effects. No studies were located regarding other systemic effects in humans after inhalation exposure to chloromethane.

The only other systemic effect reported in animal studies was a decrease in food consumption in the Landry et al. (1985) study. This study evaluated the neurologic effects of continuous versus intermittent chloromethane exposure in female C57BL/6 mice exposed to chloromethane in whole body inhalation chambers for 11 days either continuously (C) for 22 hours/day at 0, 15, 50, 100, 150, 200, or 400 ppm or intermittently (I) for 5.5 hours/day at 0, 150, 400, 800, 1,600, or 2,400 ppm. There was a significant degree of inanition in the 200-C and 400-C ppm mice prior to necropsy with decreased carcass size, amount of abdominal fat, amount of ingesta in the gastrointestinal tract, and small, pale livers.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans after inhalation exposure to chloromethane.

In animals, lymphoid depletion of the spleen and splenic atrophy were observed in mice exposed to 1,000 ppm chloromethane for up to 2 years (CIIT 1981). The lymphoid depletion was first observed in mice killed after 6 months of exposure, while the splenic atrophy was observed in mice killed after 18 months. This LOAEL value for immunological effects in mice is recorded in Table 2-l and plotted in Figure 2-l for both intermediate and chronic duration categories. The lower exposure level in this study (225 ppm) cannot be considered the most reliable NOAEL for immunological effects, however, because more sensitive tests for immune function were not conducted. In addition, cats exposed continuously to chloromethane for 3 days had higher incidences of immunologically-related brain lesions than did control cats (McKenna et al. 1981a). The lesions, however, were consistent with infection or post-vaccinal reaction (the cats were vaccinated for panleukopenia by the supplier). Exacerbation of viral-induced

central nervous system disease could not be ruled out. It is not known whether the exacerbation would represent an immunological effect.

Landry et al. (1985) exposed female C57BL/6 mice to chloromethane for 11 days either continuously for 22 hours/day at 0, 15, 50, 100, 150, 200, or 400 ppm or intermittently for 5.5 hours/day at 0, 150, 400, 800, 1,600, or 2400 ppm. The absolute and relative weight of the thymus was significantly decreased at the 1,600 ppm (40% and 39%, respectively) and 2,400 ppm intermittent exposures (89% and 87%, respectively). There was no exposure-related histopathology in the thymus, but the decreased relative thymus weight is generally considered to be evidence of possible immunotoxicity. There was decreased absolute and relative thymus weight at 15 (23% and 22%, respectively), 50 (21% and 21%), 150 ppm (71% and 69%) continuous exposures, but not at 100 ppm. The decrease at 150 ppm was considered to be exposure-related, but the decreases at 15 and 50 ppm were not because they were within normal historical range.

In contrast to the results of the Landry et al. (1985) study, exposure to chloromethane at levels up to 400 ppm for 6 hours/day, 5 days/week for 90 days resulted in no observed exposure-related adverse effects to the organs and tissues of the immune system of Sprague-Dawley rats, CD-1 mice, or male Beagle dogs (McKenna et al. 1981b). Thus, the potential for chloromethane-induced immunotoxicity remains unresolved.

2.2.1.4 Neurological Effects

Numerous case reports of humans exposed to chloromethane vapors as a result of industrial leaks and defective refrigerators have described neurological effects (Baird 1954; Baker 1927; Battigelli and Perini 1955; Borovska et al. 1976; Gummert 1961; Hansen et al. 1953; Hartman et al. 1955; Jones 1942; Kegel et al. 1929; MacDonald 1964; McNally 1946; Raalte and van Velzen 1945; Spevak et al. 1976; Wood 1951). In general, symptoms develop within a few hours after exposure and include fatigue, drowsiness, staggering, headache, blurred and double vision, mental confusion, tremor, vertigo, muscular cramping and rigidity, sleep disturbances, and ataxia. These symptoms may persist for several months, and depression and personality changes may develop. In some cases, complete recovery eventually occurs. In other cases of more severe poisoning, convulsion, coma, and death may ensue; or neurological effects may persist. Microscopic examination of the brain of an individual who died following chloromethane exposure revealed

accumulation of lipoid-filled histiocytes in the leptomeninges of the hemispheres, hyperemia of the cerebral cortex, and lipoid droplets in the adventitia cells of the capillaries throughout the brain (Kegel et al. 1929).

Battigelli and Perini (1955) report two cases of workers in a cooling plant who were exposed to a leak of chloromethane while repairing refrigeration system with an estimated exposure of >29,000 ppm. Both workers developed symptoms of vertigo, tremors, dulled senses, nausea, vomiting, and abdominal pain. The symptoms appeared 3-4 hours after the inhalation exposure. Disturbances began to recede about 6 hours postexposure and disappeared completely by 1 day postexposure.

A case was reported by Lanham (1982) of a man and wife who developed symptoms of blurred vision, fatigue, vertigo, tremor, and abnormal gait several days after storing insulating boards made of Styrofoam in the basement of their house. Air levels of chloromethane measured by 3 different devices were above 200 ppm.

Seven men had acute exposures to chloromethane while repairing refrigeration systems. Four of the cases provided sufficient information to estimate an exposure level of 39,000, 50,000,440,000, and 600,000 ppm, respectively. Common symptoms were ataxia, staggering, headache, drowsiness, anorexia, blurred and double vision, convulsions, nausea, and vomiting (Jones 1942).

Putz-Anderson et al. (1981b) assessed the behavioral effects of inhaled chloromethane when administered alone at 0 or 200 ppm, or in combination with alcohol or caffeine. Chloromethane exposures in volunteers lasted 3.5 hours. Patients were subjected to three performance tests (visualvigilance, dual task, and time discrimination (designed to test human attention or alertness) prior to and during the treatment period. Venous blood and alveolar air concentrations of chloromethane were obtained prior to and 90 minutes after beginning chloromethane exposures. Chloromethane alone had no effect. Alcohol caused a significant impairment in performance, but there was no difference in alcohol-induced impairment when chloromethane was given with alcohol. Caffeine alone improved performance, but there was no effect on improvement when chloromethane was given with caffeine. There was much variation in alveolar air and blood levels of chloromethane.

Putz-Anderson et al. (198 la) assessed the behavioral effects of inhaled chloromethane, alone or in combination with oral diazepam (a central nervous system depressant), in 56 men and women. Chloromethane was administered alone at concentrations of 0, 100, or 200 ppm, or in combination with

10 mg orally administered diazepam. Chloromethane exposures lasted 3 hours. Patients were subjected to three performance tests (visual vigilance, dual task, and time discrimination; designed to test human attention or alertness) prior to and during the treatment period. Venous blood and alveolar air concentrations of chloromethane were obtained prior to and 90 minutes after beginning chloromethane exposures. Due to a limited number of patients, data from the 100 ppm chloromethane group was excluded from the analysis. For all tests, the control group (no chloromethane or diazepam) had a 2.73% decline in performance between the precontrol and control test (i.e., a control for the fatigue effect). The net impairment resulting from exposure to 200 ppm chloromethane was a marginally significant 4% (total impairment 6.7% minus the 2.73% negative control). The net impairment of diazepam alone was 10.1%. The net impairment of the combined chloromethane and diazepam was 13.5%. The authors concluded that the effects of chloromethane exposure were minimal and were not potentiated by concomitant diazepam exposure.

Spevak et al. (1976) describe a case of chloromethane poisoning among four family members (one brother [age 64] and three sisters [ages 50, 52, and 60]). All were exposed to fluid and vapors leaking from a refrigerator for approximately 1 hour while cleaning the spill. Approximately 4 hours after their exposure, all four subjects felt weak and had abdominal pains, vomiting, hiccups, and severe headaches; which they thought was due to food poisoning. All subjects lost consciousness until the next day. Neighbors told the subjects that a doctor visited them and administered some medication, but the identity of the medication was unknown. By 2 days after the exposure, the symptoms had not disappeared, and all four were admitted to the hospital with clinical signs of drunkenness, confusion, somnolence, ataxia, and dysarthria. Nervous system damage progressed with cerebellar symptoms of nystagmus in all four patients, and adiadochokinesis developing in one of the women. All subjects had disturbances of the cranial nerves (optic, oculomotor, and facial), as well as speech disturbances, tremors, and elevated reflexes. Tachycardia, faint heart sounds and slightly elevated blood pressure were also noted. The most severely affected subject (one of the sisters who also had the longest exposure) suffered from jaundice, conjunctival hemorrhages, and epigastric tenderness; however, her liver and spleen were not enlarged. The brother had the shortest exposure and had a normal skin color. Biochemical analysis of blood and urine revealed increases in indirect bilirubin in all three sisters and serum creatinine for all four patients. Blood urea was increased only for the most severely affected sister. All other hematology and blood chemistry data were normal including number of red and white blood cells, platelets, and reticulocytes; red cell osmotic fragility test; coagulation factors; serum electrophoresis, cholesterol, alkaline phosphatase, ALT, AST, and fibrinogen; and blood glucose, blood ammonia, bone marrow smears, blood pH, and blood gases.

Electroencephalograms were also normal. The three sisters received symptomatic treatment with isotonic glucose, B complex vitamin, and oxygen. The treatment resulted in a disappearance of all symptoms of intoxication except ataxia. The brother refused treatment. Symptoms of kidney damage disappeared after two weeks, and the outcome of the intoxication was, in the words of the physicians, good in all cases (Spevak et al. 1976).

Stewart et al. (1980) found no exposure-related neurological abnormalities, abnormal EEG, effect on cognitive test, or significant subjective response from acute exposures up to 150 ppm in volunteers. This study, however, had several limitations such as small sample size, multiple dosing schemes, and a confusing protocol. Specifically, groups of two to four men and two to four women were exposed to 10, 100, or 150 ppm or to concentrations that were increased from 50-1.50 ppm in the same group for 1, 3, or 7.5 hours per day over 2-5 days per week for 1 or 2 weeks. Several subjects, both male and female, dropped out of the study before some of the experiments were completed, and other subjects were added. The same subjects were also included in different protocols during different weeks of the study.

Gudmundsson (1977) reports on a 20-month and 13-year follow-up after an acute high level exposure to chloromethane. Seventeen crew members (males) were exposed for 2 days in 1963 to chloromethane that leaked from a refrigerator on board an Icelandic fishing trawler (no estimates of exposure levels were reported). The refrigerator was located under the sleeping quarters of the crew. This case history describes both the acute phase of the illness and a follow-up of the survivors at 20 months and 13 years postexposure. Fifteen of the seventeen crew members exposed to chloromethane showed signs of intoxication.
In the acute phase of the illness, nine patients exhibited abnormal neurological signs. Four died, one within 24 hours of the exposure. Two patients developed severe depression and committed suicide 11 and 18 months later, respectively. The fourth patient was assessed as 75% disabled due to severe neurological and psychiatric disturbances, and died 10 years postexposure at the age of 34. Autopsy revealed recent coronary occlusion (not necessarily connected with the primary illness). At 20 months postexposure, 7 patients had neurological symptoms (not specified), and 8 had psychiatric complaints primarily psychoneurosis and depression. Five survivors stated they had a reduced tolerance to alcohol. Thirteen years later (i.e., in 1976) 10 of the 11 survivors were examined (one lived in a foreign country and could not be located). The mean age of the 10 survivors examined was 38.3 years (range 30-50 years). All 10 were employed; 8 were employed at sea. Neurological deficits included fine tremor of the hands in three survivors, paralysis of accommodation in two, and signs of peripheral neuropathy in two. Five survivors had no abnormal neurological signs. Six survivors had marked neurotic and depressive symptoms. Two

complained of decreased libido and two complained of severe headache. Alcohol may be a confounding factor. Nine survivors complained of a markedly reduced tolerance for alcohol, and the same number complained of early fatigue and decreased stamina. Excessive alcohol consumption was admitted by four survivors. Alcohol may contribute to the peripheral neuropathy. Regarding the progress or reversibility of the symptoms, one patient who had considerable muscle atrophy and fasiculations 20 months after the accident had improved by 13 years postexposure, but still exhibited signs of anterior horn damage. In two survivors, the paralysis of accommodation remained unchanged, but in one there was a complete regression. In conclusion, all survivors of the acute chloromethane exposure suffered from mild to permanent neurological and/or psychiatric sequelae directly attributable to chloromethane neurotoxicity.

Some information on longer term exposures is available. MacDonald (1964) presented eight case reports of chloromethane poisoning in a polymer plant. Symptoms of blurring vision, mental confusion, headache, loss of coordination, and dizziness were common. More severely intoxicated individuals experienced nausea and vomiting. Personality changes, depression and irritability were reported by many of the cases. The symptoms persisted for months. It was not possible to determine the LOAEL.

Schamweber et al. (1974) presented 6 case studies of workers who were exposed to relatively low levels (200-400 ppm) of chloromethane for at least 2-3 weeks before onset of symptoms. Two cases occurred after “prolonged” (not otherwise specified) exposure to S-hour TWA levels up to 300 ppm. Four cases occurred after work exposure on the order of 265 ppm (S-hour TWA) after 2-3 weeks of 12-16 hour days. A 54-year-old worker initially suffered from confusion, blurry vision, erratic driving, difficulty in eating and swallowing, headache, and disturbance of balance. Three weeks after hospitalization, the patient still complained about headache and had a staggered gait. Memory difficulties persisted for 2 months. Patient improved at three months, but still had tremors and nervousness. A second B-year-old worker had delirium, confusion, disorientation, and combativeness. Two months after hospitalization, the patient still had poor memory and nervousness. Three months later, the patient was well enough to return to work. A 33-year-old foam worker had blurred vision, increased tiredness, nervousness, and stuttering that resolved after a 6-week recovery period. Other foam workers developed similar symptoms with impairment in memory, gait, and speech (tongue swelling, slurring) and vision (diplopia, blurred), slight to moderate increase in blood pressure, and an EEG with a predominance of slow waves in the beta range that resolved from 1 to 3 months after removal from exposure. The authors concluded that an 8-hour TWA of 200 ppm or greater is necessary for development of chronic chloromethane intoxication based on these and other industrial experiences.

Repko et al. (1977) performed a study on the effects of chloromethane from exposures to workers. Seventy-three behavioral measures of task performance, four indices of exposure, eight indicators of neurological function, and a clinical EEG were obtained. The exposed population was derived from several fabricating plants. Ambient air concentrations of chloromethane ranged from 7.4 to 70 ppm, with means from each plant ranging from 8.46 to 58.72 ppm. The overall mean was 33.57 ppm. Mean concentration of chloromethane in breath ranged from 2.67 to 24.19 ppm, with a mean of 13.32 ppm. Correlations were found between the duration of exposure and breath concentration, duration and ambient concentration, concentration in air and concentration in breath, chloromethane in air and hematocrit, urine pH and hematocrit, and duration and hematocrit. There were no significant differences in neurological tests or EEGs. In the behavioral battery, effects on cognitive time-sharing and finger tremor were found, but correlation coefficients indicated that chloromethane in breath is not a sensitive indicator of performance deficit. Workers showed a general tendency toward poorer performance as chloromethane levels in air increased. The authors concluded that occupational exposure to chloromethane below 100 ppm produces subtle, quantifiable behavioral effects, but that data on the threshold at which chloromethane begins to produce these changes in functional capacity are not currently available. A limitation of this study was the inability to achieve perfect matching as to sex, race, age, and level of education.

Chloromethane exposure also results in neurological effects in animals. Rats, mice, rabbits, guinea pigs, dogs, cats, and monkeys exposed to chloromethane until death all displayed signs of severe neurotoxicity, including paralysis and convulsions (Smith and von Oettingen 1947a, 1947b). As discussed in Section 2.2.1.1, these studies have several limitations that preclude determination of concentration-duration-response relationships, but the results do demonstrate the universal response of animals to the neurotoxic effects of chloromethane.

More recent animal studies support the neurotoxic potential of chloromethane, with sufficiently high levels of acute inhalation exposure leading to ataxia, tremors, limb paralysis and incoordination, and cerebellar lesions consisting of degeneration of the granular layer. Mice appear to be more sensitive than rats, with similar but more severe responses at lower exposure concentrations.

After 48 continuous hours of chloromethane exposure at 1,000 ppm, Sprague-Dawley rats were lethargic compared to the controls, and their condition worsened to sick or moribund by the end of a 72-hour exposure. The 2,000 ppm exposure eventually led to death. There were no effects on brain weight, and no

exposure-related gross or histopathological lesions in the brain. No effects were seen at 500 ppm for up to 72 hours of exposure (Burek et al. 1981).

Male Fischer 344 rats exposed to 5,000 ppm chloromethane alone for 5 days, 6 hours/day had more pronounced signs of central nervous system toxicity (tremors, ataxia, forelimb/hindlimb paralysis) than those receiving chloromethane plus pre-and post-treatment with the potent anti-inflammatory agent, BW755C (10 mg/kg, intraperitoneally 1 hour pre- and postexposure). Chloromethane alone caused a degeneration of cerebellar granule cells, while rats exposed to chloromethane and BW755C did not exhibit this effect. The result was surprising because this brain lesion is not usually associated with inflammation. The authors concluded that protection from chloromethane-induced injury by BW755C was not simply the result of altered metabolism because BW755C had no effect on tissue distribution or excretion of ^{14}C-chloromethane, and administration of BW755C did not decrease hepatic glutathione content. The protection of BW755C may have been related to an inhibition of leukotriene and prostaglandin synthesis (Chellman et al. 1986a).

Fischer 344 rats were exposed to 0, 2,000, 3,500, or 5,000 ppm chloromethane for 6 hours/day, 5 days/week, for 2 weeks. On day 5, hind limb paralysis was observed in two males and one female in the 5,000 ppm group. After the fifth day, 13 animals were killed in extremis (5,000 ppm:6 males, 5 females; 3,500 ppm:2 females). By the second week, the rats appeared to tolerate the exposures much better, but one 5,000 ppm female had convulsive seizures during the last exposure. Histological examination of the brain and thoracic spinal cord revealed minimal to moderate degeneration of cerebellar internal granular layer in two females and three males exposed to 5,000 ppm. The lesions were identical to those seen in mice. There were no lesions in the spinal cord. The authors concluded that this study confirmed the existence of species, sex, and strain differences in susceptibility to chloromethane-induced toxicity. No neurological or histopathological lesions were reported for the 3,500 ppm group. The 3,500 ppm dose is not designated a NOAEL due to the absence in the report of an explicit statement that no neurotoxicity occurred at 3,500 ppm and the severity of this effect reported for the 5,000 ppm mice. C3H, C57BL/6, or B6C3F$_1$ mice were exposed to chloromethane for 12 days, 6 hours/day. Mice were exposed to 0,500, 1,000, or 2,000 ppm. Some of the mice that died had moderate to severe ataxia. Histologically, there were no brain lesions at 500 ppm in any strain. Cerebellar degeneration was seen as follows: C3H mice (none); C57BL/6 mice, 3 of 5 males and 5 of 5 females exposed to 1,000 ppm and 0 of 5 males and 4 of 4 females exposed to 2,000 ppm; B6C3F$_1$ mice, 2 of 5 females exposed to 2,000 ppm. The lesions were most severe in 2,000 ppm C57BL/6 females, followed by 1,000 ppm C57BL/6 males. The cerebellar lesions consisted

of focal degeneration of the granular layer, which affect posture and coordination. The authors concluded that this study confirmed the existence of species, sex, and strain differences in susceptibility to chloromethane-induced neurotoxicity (Morgan et al. 1982).

Chellman et al. (1988a) investigated the role of glutathione in the mediation of chloromethane-induced toxicity in the brain of male $B6C3F_1$ mice. Mice exposed to 1,500 ppm chloromethane for 6 hours/day, 5 days/week, for 2 weeks developed multiple degenerative, necrotic foci in the internal granule cell layer of the cerebellum; in some areas the foci involved the whole thickness of the granular cell layer. Cerebellar degeneration consisted of granule cells with pyknotic nuclei and clear, swollen perikarya. Tremors, ataxia, and forelimb/hindlimb paralysis were seen in chloromethane-exposed mice prior to death, and were associated with cerebellar damage. Cerebellar damage was not observed in chloromethane-exposed mice pretreated with a glutathione depleter. The authors concluded that the depletion of GSH protected mice from cerebellar damage due to exposure to chloromethane. Based on this result, the mechanism of neurotoxicity may involve conjugation of chloromethane with glutathione in the liver, followed by biliary excretion and enterohepatic circulation of the glutathione conjugate, or possibly a cysteine conjugate, and further metabolism by kidney and/or gut flora beta-lyase to methanethiol. Methanethiol produces similar central nervous system symptoms (tremors, convulsion, coma) as seen in animals or humans acutely intoxicated with chloromethane (Chellman et al. 1986b).

Jiang et al. (1985) characterized the cerebellar lesions resulting from an acute inhalation exposure of 1,500 ppm chloromethane to female C57BL/6 mice for 2 weeks, 5 days/week, 6 hours/day. Two mice died, and several had motor incoordination. All exposed mice had varying degrees of cerebellar degeneration located mainly in the ventral paraflocculus, but also occurring in dorsal paraflocculus. Granule cells were mainly affected, with two distinct types of lesions: (1) nuclear and cytoplasmic condensation of scattered granule cells with slight hydropic swelling of astrocytes (also seen to a lesser extent in controls); and (2) focal malacia with varying degrees of watery swelling of groups or extensive areas of granule cells, nuclear condensation, karyorrhexis, and necrosis. The second type of lesion was more prevalent. Purkinje cells were largely unaffected by the malacic process, and the inflammatory response was minimal. Electron microscopy showed that the damage in the areas of malacia (the type 2 lesion above) ranged in severity from edema of granule cell perikarya to severe edema and almost complete destruction of all tissue components. Involvement of cell types other than granule cells occurred only in the most severely affected areas (i.e., Purkinje cells were well preserved while astrocytes adjacent to Purkinje cells [the Bergmann’s glia] showed moderate to severe cytoplasmic distention by translucent edema fluid).

The biochemical mechanism for the induced defects in granule cell fluid/electrolyte balance is unknown. Only one exposure concentration was used, but the study was designed to examine the neurological and kidney effects specifically, and therefore, used an exposure regimen known to produce these effects. Based on the severity of the kidney effects, the authors concluded that the observed brain lesions were probably not a direct consequence of renal lesions; rather, the mechanism may be associated with metabolic changes in granule cells.

Landry et al. (1985) observed decreased performance on the rotating rod at an 800 ppm and greater intermittent exposure (5.5 hours/day for 11 days) when tested at 4 days, but persisting to day 8 only in the 2,400 ppm mice (with considerably greater deficit in this group). Histological lesions consisted of slight cerebellar granule cell degeneration in some of the mice exposed to 400, 800, or 1,600 ppm. In the 2,400 ppm group, all of the mice were affected to a slight degree. Mice exposed continuously for 22 hours/day for 11 days had similar effects at exposure levels of 100 ppm. The apparent greater sensitivity to continuous exposure may be related to the conversion of chloromethane to an active metabolite, decreased respiration at concentrations that are intolerable when exposure is continuous, and/or diurnal susceptibility. Diurnal susceptibility (i.e., in this case lower sensitivity during the daytime intermittent exposure) could result from the lower activity of mice during the daytime and the lower respiratory minute volume.

Pregnant $B6C3F_1$ mice exposed to 1,500 ppm chloromethane in whole-body exposure chambers, 6 hours/day on Gd 6-17 developed tremors, hunched appearance, difficulty righting, disheveled fur, bloody urine, and granular cell degradation in cerebellum with selective necrosis of neurons in the internal granular layer. All females in this group were sacrificed on Gd 11-14 prior to the completion of exposure to Gd 17; two females died prior to necropsy (as early as Gd 9, after only 4 days of exposure). These effects were not seen in the 479 ppm or lower exposure (Wolkowski-Tyl et al. 1983a).

C57BL/6 females were mated to C3H males to produce $B6C3F_1$ offspring. After mating, 74-77 females were exposed to chloromethane at concentrations of 0, 250, 500, or 750 ppm on Gd 6-17. Exposure to 500 ppm chloromethane resulted in ataxia in 6 of 74 females by Gd 18; exposure to 750 ppm resulted in hyperactivity, ataxia, piloerection, tremors and convulsions. The authors concluded that inhalation exposure to chloromethane during Gd 6-17 resulted in maternal toxicity at 750 ppm; teratogenic effects were seen at 500 and 750 ppm. Exposure of pregnant mice to 250 ppm chloromethane produced neither maternal nor fetal toxicity nor teratogenicity (Wolkowski-Tyl et al. 1983b).

Beagle dogs (n=3) exposed to 500 ppm chloromethane for 23.5 hours/days for 3 days had moderate to severe limb stiffness, tremors, salivation, and incoordination. These effects became less severe but persisted during a 4-week recovery. All 500 ppm dogs had neurological deficiencies based on clinical testing at 4 days after exposure, but nearly complete recovery on day 26 after exposure. Histological examination revealed brain and spinal cord lesions in all 3 dogs consisting of vacuolization, swollen eosinophilic axons, loss of axons, demyelinization and gitter cells. These changes were very slight and multifocal in the brain stem (medulla, pons, or both) and slight and multifocal in the lateral and ventral funiculi of the spinal cord. No lesions were observed in the cerebrum or cerebellum nor in the dorsal funiculi or grey matter of the spinal cord (McKenna et al. 1981a).

Cats (n=3) exposed to 500 ppm chloromethane for 23.5 hours/days for 3 days were less active than controls after 24 hours of exposure, but had no clinical signs after exposure. Cats did not undergo neurological tests. Histological lesions in cats were seen in l/3 control, l/3 at 200 ppm, and 3/3 at 500 ppm; and consisted of lesions in the brain occurring in a multifocal or random pattern in the white matter of the cerebrum, cerebellum and midbrain. In the spinal cord they primarily occurred in the lateral and ventral funiculi. The authors did not believe that these were treatment related but were instead consistent with infection or post-vaccinal reaction (cats were vaccinated for panleukopenia by supplier). The authors stated that exposure to 500 ppm may have resulted in an exacerbation of a viral-induced, spontaneously occurring disease process in the central nervous system of the cats. (McKenna et al. 1981a).

Intermittent exposures for longer durations also resulted in less severe neurotoxicity. $B6C3F_1$ mice or Fischer 344 rats exposed to 0, 375, 750, and 1,500 ppm for 6 hours/day, 5 days/week, for 13 weeks showed no exposure-related histopathological lesions of brain and spinal cord and no effect on brain weight (Mitchell et al. 1979). Beagle dogs, CD-l mice, or Sprague-Dawley rats exposed to as high as 400 ppm chloromethane for 6 hours/day, 5 days/week for 90 days showed no apparent neurological effects (McKenna et al. 1981b).

Longer-term higher-level exposures have, however, resulted in neurotoxicity in mice even if only for 6 hours/day. Male and female Fischer 344 rats and $B6C3F_1$ mice were exposed to chloromethane in whole body inhalation exposure chambers at target concentrations of 0 (control), 50, 225, or 1,000 ppm, 6 hours/day, 5 days/week for up to 24 months. Necropsies were completed at 6, 12, 18, or 24 months after the initial exposure. As early as 6 months, the absolute brain weight was reduced in male and female mice exposed to 1,000 ppm chloromethane; however, relative brain weights were not affected by chloromethane

exposure. Clinical signs of neurotoxicity (tremor, paralysis) were observed in both sexes (exposure level not specified, but most likely 1,000 ppm). By 18 months, decreased absolute brain weights were noted in females exposed to 1,000 ppm chloromethane. Clinical signs of neurotoxicity (tremor, paralysis) were seen in both sexes, along with abnormal functional test neurological results (restricted use of rear legs, abnormal gait, poor extensor thrust, leg rigidity), and cerebellar lesions (minimal to mild reduction in the number of neurons in the granular cell layer, most prominently in the sulci). Axonal swelling and degenerative changes of minimal severity were observed in the spinal nerves and cauda equina in the lumbar spinal cord of 3 of 7 male mice (1,000 ppm), 5 of 5 male and 10 of 10 female mice (225 ppm), 4 of 5 male and 10 of 10 female mice (50 ppm), and 1 of 5 male and 2 of 10 female mice (control). The neurotoxic lesions progressed in frequency and severity in mice to the end of the exposure period. In contrast to its effects in mice, chloromethane did not produce neurotoxicity in rats (i.e., negative clinical, pathological, and functional tests) at levels up to 1,000 ppm for 6 to 24 months in duration (CIIT 1981). The mechanisms underlying this dramatic difference in species susceptibility are not understood.

The highest NOAEL values and all reliable LOAEL values in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l. The 50 ppm concentration in mice exposed acutely (Landry et al. 1985) is the highest NOAEL below which no LOAEL exists. At 100 ppm, the mice had cerebellar lesions. Based on the NOAEL of 50 ppm, an acute inhalation MRL of 0.5 ppm was calculated as described in the footnote to Table 2-l and in Appendix A. The 51 ppm concentration in mice exposed chronically to chloromethane (CIIT 1981) is the lowest LOAEL (axonal swelling and slight degeneration of axons in the spinal cord). Based on this LOAEL, a chronic inhalation MRL of 0.05 ppm was calculated as described in the footnote to Table 2-1 and in Appendix A. These MRLs are presented in Figure 2-1.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to chloromethane.

Chloromethane has been shown to be a reproductive toxicant in a variety of animal studies. Sprague-Dawley rats exposed to 500 ppm for 48 hours had increased proteinaceous and cellular aggregates in the epididymis with interstitial edema (2/5 rats) and focal suppurative inflammation (l/5) immediately after the exposure. By 12 days postexposure, the lesions had increased in severity with the formation of sperm granulomas, decreased sperm in the tubule lumen, interstitial edema, coagulated proteinaceous

debris or inflammation leading to obstructive changes causing at least partial occlusion of the affected lumen, and unilateral testicular atrophy. The lesions were more severe in rats exposed to higher concentrations and/or for the longer duration. Mean absolute and relative testicular weight was decreased to 50% in rats exposed to 1,000 ppm for 72 hours; this effect was thought to be secondary to a severely obstructed epididymis. The decreased testes weight was not observed in 1,000 ppm rats exposed for 48 hours or in males exposed to 200 or 500 ppm for either duration (Burek et al. 1981).

Male Fisher 344 rats were exposed to 3,500 ppm chloromethane for 6 hours/day for 5 days, then a stop in exposure for 3 days, and then a restarting of the exposure for another 4 days. This regimen resulted in several testicular and epididymal lesions and interference with neuroendocrine control of spermatogenesis. The initial testicular effects were directed at either the late stage spermatids or the Sertoli cells with a resultant delay in spermiation. No testicular abnormalities were found at 5 days, but at 7 days one rat had scattered foci of disruption of seminiferous epithelium, and exfoliation of germinal cells. By day 9 all exposed rats had disruption of spermatogenesis, and by day 13 all had disruption and disorganization of seminiferous epithelium and epithelial vacuolation. At 70 days, 70-90% of seminiferous tubules were shrunken, contained whorls of Schiff's reagent-positive material, and had Sertoli cell nuclei near the basement membrane. The remainder showed varying degrees of recovery. All animals killed after 19 days displayed bilateral epididymal granulomas in regions 5 or 6 of the cauda epididymis. The nature and distribution of the inflammatory cells indicated that the primary neutrophilic response may have been against the tubular epithelium and not extravasated sperm. Serum testosterone showed a time dependent decrease during the 5 consecutive days of exposure (not seen in the pair-fed controls). Leydig cell and gonadotropin function was normal when challenged with hCG and LHRH; thus, the authors propose that chloromethane lowers circulating testosterone by acting in the brain to decrease circulating levels of gonadotrophic hormones. NPSH content was depleted in testis, caput and caudal epididymides samples, but not in heart blood. This effect is thus probably the result of enzyme-mediated conjugation of glutathione with chloromethane, and not a consequence of direct alkylation. The authors speculate that chloromethane conjugation with testicular and epididymal glutathione may result in depletion of glutathione, which serves in a variety of protective cellular functions (Chapin et al. 1984).

Rats exposed to 7,500 ppm chloromethane 6 hours/day for 2 days developed epididymal granulomas within 3 weeks after exposure (Chellman et al. 1986a). Effects of 7,500 ppm chloromethane on testes were not reported. Rats exposed to 5,000 ppm, 6 hours/day for 5 days developed sperm granulomas in the epididymides, and testicular lesions (exfoliation of pachytene spermatocytes and early stage spermatids).

No granulomas were found in rats treated concurrently with chloromethane and the anti-inflammatory agent, amino-l-[m-(trifluoromethyl)-phenyl]-2-pyrazoline (BW755C). There was also no evidence of epididymal or testicular lesions in rats treated with both 5,000 ppm chloromethane and BW755C. BW755C, therefore, protected rats against chloromethane toxicity. The authors concluded that protection from chloromethane-induced injury by BW755C was not simply the result of altered metabolism because BW755C had no effect on tissue distribution or excretion of ^{14}C-chloromethane, and administration of BW755C did not decrease hepatic glutathione content. The protection of BW755C may have been related to an inhibition of leukotriene and prostaglandin synthesis.

Chellman et al. (1986c) investigated the relationship between chloromethane-induced epididymal inflammation and the occurrence of dominant lethal mutations in male Fischer 344 rats. Chloromethane exposure at 3,009 ppm for 6 hours/day for 5 days resulted in a significant increase in pre-implantation loss in females mated with exposed males at weeks 2 and 3 postexposure, and BW755C did not protect against this effect. The authors concluded that pre-implantation losses were due to the cytotoxic effect of chloromethane on the testes. A subsequent study by the authors (see Chellman et al. 1987) showed reduced numbers and abnormal sperm from chloromethane induced testicular toxicity in male rats, leading to a failure to fertilize.

Chellman et al. (1987) also investigated the role of chloromethane-induced testicular and epididymal inflammation in the induction of sperm cytotoxicity and preimplantation loss in male Fischer 344 rats. Rats exposed to 3,056 ppm chloromethane 6 hours/day for 5 consecutive days had significantly decreased relative weight of seminal vesicles at week 1, epididymis at weeks 2 and 3, and testes at week 3; disruption of spermatogenesis (delayed spermiation, disorganization of seminiferous epithelium, and decreased number of mid- and late spermatids); and decreased sperm production per day at weeks 1, 2, and 3 postexposure. Epididymal examination revealed visible sperm granulomas and inflammation; a large amount of PAS-positive material in epididymis associated with greatly decreased number of sperm, increased number of abnormal sperm and cellular debris of testicular origin; reduced number of sperm, decreased percent motile sperm and percent intact sperm, and increased abnormal sperm in the vas deferens by week 3. Concurrent treatment with BW755C did not protect the rats from these testicular effects, but did protect the rats from the formation of sperm granulomas and inflammation in the epididymides. The authors concluded that chloromethane-induced sperm toxicity was due to toxicity to the testes, rather than the result of inflammation and granuloma formation in the epididymis. This testicular toxicity and

movement of damaged sperm out of the testes into the epididymis and vas deferens was probably responsible for fertilization failures and preimplantation losses seen by Working and Bus (1986).

Male Fischer 344 rats were exposed to chloromethane at 0, 2,000, 3,500, or 5,000 ppm for 5 days, 6 hours/day with a break in exposure for 2 days, and then a further 4 days of exposure. Histological examination of the testes and epididymides revealed testicular degeneration in all males of all exposed groups with a clear dose-related increase in severity. The testicular lesions consisted of a reduction in or lack of late stage spermatids, separation of spermatocytes, and early stage spermatids. The lumen of epididymal tubules contained greatly reduced numbers of sperm. There was a dose-related increase in eosinophilic, hyaline droplets and degenerating cells of unknown type (Morgan et al. 1982).

Pregnant Fischer 344 rats exposed to 1,492 ppm chloromethane 6 hours/day on Gd 7-19 had significantly depressed maternal food consumption and weight gain during exposure, but there were no statistically significant differences among the treatment groups for number of litters, percent litters with live fetuses, the number of corpora lutea, number of implantations, number or percent resorptions, number of live fetuses per litter, or fetal sex ratio. B6C3F_1 mice exposed to 1,492 ppm chloromethane for 6 hours/day on Gd 6-17 developed severe maternal toxicity resulting in tremors, hunched appearance, difficulty righting, disheveled fur, bloody urine, and granular cell degradation in cerebellum with selective necrosis of neurons in the internal granular layer. All females in this group were sacrificed on Gd 11-14 prior to the completion of exposure to Gd 17; two females died prior to necropsy (as early as Gd 9, after only 4 days of exposure). These effects were not seen in the 479 ppm group. There were no significant differences for exposures of 479 ppm or less for the number of litters, percent litters with live fetuses, the number of corpora lutea, number of implantations, number or percent resorptions, number of live fetuses per litter, or fetal sex ratio (Wolkowski-Tyl et al. 1983a).

Working and Bus (1986) assessed the effects of inhalation exposure to chloromethane on preimplantation loss to distinguish between cytotoxicity (i.e., fertilization rate) and genotoxicity in rats. Male Fischer 344 rats exposed to chloromethane at 3,000 ppm for 5 days, 6 hours/day were bred to no more than 2 females weekly during weeks l-4 and week 8 post-exposure. Males in the 1,000 ppm group were bred to no more than 2 females during week 3 post-exposure. Females were sacrificed lo-12 hours postmating, and embryos and ova were scored as fertilized or unfertilized. In an *in vitro* experiment, fertilized ova were examined in culture for cleavage. The combined fertilization rate in all females bred to control males was 88%. In females bred to the 1,000 ppm males, 80% of ova were fertilized. In females bred to the

3,000 ppm males, fertilization of ova was 39% at week 1 of mating, 3.4% at week 2, 22.1% at week 3, 41% at week 4, and 72% at week 8. There were no significant differences in the cleavage rates of ova from females bred to controls (96.5%) or to males exposed to 1,000 or 3,000 ppm chloromethane (92.4-93.8%). The authors concluded that all preimplantation losses observed in previous studies (Working et al. 1985a) could be explained by a cytotoxic effect resulting in failure of fertilization and not a genotoxic effect resulting in early embryonic death (Working and Bus 1986).

Working et al. (1985a) studied the effects of inhalation exposure to chloromethane on germ cell viability in male Fischer 344 rats. At 17 weeks after exposure to 3,000 ppm chloromethane for 6 hours/day for 5 days, 30% of the males had sperm granulomas in one or both epididymides; none were noted in the 1,000 ppm or control groups. Exposure to 3,000 ppm chloromethane also resulted in a slight increase (9.5%) in postimplantation loss only at week 1 postexposure (sperm exposed in epididymis or vas deferens), but increased preimplantation losses at week 1 (31.4%), peaking at week 2 (93.6%) then declining to 14.1% by week 8 postexposure. Fertility in males exposed to 3,000 ppm chloromethane was significantly decreased by postexposure week 2 and remained depressed throughout the study period. The authors concluded that a cytotoxic rather than genotoxic mechanism may play a role in the observed preimplantation losses. They further speculated that inflammation-derived reactive metabolites (e.g., superoxide anion) could damage DNA or sperm in epididymis (Working et al. 1985a).

Fischer 344 rats exposed to 3,000 ppm chloromethane at 6 hours/day for 5 days had decreased testicular weight from the third post-exposure week with a steady decline to 50% by week 8, and a recovery by week 16. Histologically, sperm granulomas in epididymides were observed in 50% of the exposed rats. Disruption of spermatogenesis in testes, decreased number of sperm, increased number of abnormal sperm, and decreased sperm motility were also observed. Recovery was nearly complete by week 16. The authors concluded that inhalation of high concentrations of chloromethane produce a prolonged cytotoxicity in testes leading to oligospermia due initially to depletion of postmitotic stages of spermatogenic cells, and ultimately to the killing of spermatogonial stem cells. The resultant decreased fertility was not permanent. The inflammation of the epididymis may account for depressed motility and increased numbers of abnormal sperm, but a genotoxic effect could not be ruled out on the basis of this study (Working et al. 1985b).

Exposure to chloromethane up to 750 ppm had no effect on reproductive parameters in C57BL/6 females mated to C3H males to produce $B6C3F_1$ offspring, such as the percentage of pregnant females, the number of implantations/litter, number of resorptions/litter, or the number of dead/litter. The authors concluded

that inhalation exposure to chloromethane during Gd 6-17 resulted in maternal toxicity only at 750 ppm and teratogenic effects at 500 and 750 ppm. Exposure of pregnant mice to 250 ppm chloromethane produced neither maternal nor fetal toxicity nor teratogenicity (Wolkowski-Tyl et al. 1983b).

Beagle dogs or cats exposed to 500 ppm chloromethane for 23.5 hours/days for 3 days and observed for 4 weeks (dogs) or two weeks (cats) postexposure showed no changes in weights of testes or development of histopathological lesions in the testes (McKenna et al. 1981a). No exposure-related gross or histopathological lesions in reproductive organs and no changes in testes weight occurred from exposures up to 400 ppm for 6 hours/day 5 days/week for 90 days in CD-l mice, Beagle dog, or Sprague-Dawley rat (McKenna et al. 1981b) or up to 1,473 ppm in Fisher 344 rats (Mitchell et al. 1979).

Han-n-n et al. (1985) examined whether an inhalation exposure to chloromethane affected the reproductive status of Fischer 344 rats exposed to 1,500 ppm chloromethane 6 hours/day, 5 days/week for 10 weeks premating, and then for 7 days/week during a 2-week mating period. Male rats exhibited seminiferous tubule atrophy (lo/lo) and granulomas in the epididymis (3/10) following exposure. No treatment effects were noted for litter size, sex ratio, pup viability, pup survival, or pup growth, and there was no significant difference in fertility between exposed and nonexposed females. In the F_0 recovery study, males exposed to 1,500 ppm chloromethane experienced a partial recovery of fertility, while males exposed to 475 ppm chloromethane experienced a full recovery. There were no F_1 litters from the 1,500 ppm group. Chloromethane had no statistically significant effect on fertility in the second generation (F_1 for 151 and 472 ppm exposures), but there was a dose related trend towards fewer litters and fewer males proven fertile in the 475 ppm group. Litters in the 475 ppm group had a significantly decreased percentage of males and significantly less male and female F_2 pup growth only during postnatal days 14 to 21. The significance of these affects are unknown (Han-m et al. 1985). The study did not mate unexposed males with exposed females. Such a mating with females exposed to 1,500 ppm would be necessary to rule out an effect on female fertility. Reduced fertility may be due to a cytotoxic effect on the testes (Working et al. 1985a, 1985b).

Male and female Fischer 344 rats and B6C3F_1 mice were exposed to chloromethane in whole body inhalation exposure chambers at target concentrations of 0 (control), 50, 225, or 1,000 ppm, 6 hours/day, 5 days/week for 6, 12, 18, or 24. At 12 months, there were no exposure-related lesions in reproductive organs of mice exposed to chloromethane at concentrations up to 1,000 ppm., but lesions developed in the later months. Seven of 43 males exposed to 1,000 ppm, and that died or were sacrificed between 18 and

21 months, had testicular germinal cell degeneration, giant cell formation, and tubular atrophy, compared with l/20 controls sacrificed at 24 months. Lesions developed earlier in the rat. By 6 months of exposure in rats, one male rat from the 1,000 ppm group had bilateral, diffuse degeneration and atrophy of the seminiferous tubules. This lesion significantly increased in this group at later sacrifices. At 12 months, gross and histological examination of testes and epididymides of males revealed germinal epithelial degeneration and atrophy of seminiferous tubules (4/10 males exposed to 1,000 ppm chloromethane). Chloromethane exposure had no effect on testis or ovary weights. At 18 months, gross and histological examination of testes and epididymides of male rats exposed to 1,000 ppm revealed germinal epithelial degeneration and atrophy of seminiferous tubules. Exposure to chloromethane had no effect on testes or ovary weights. Sperm granulomas were seen in two 1,000 ppm male rats at the 6-month sacrifice, in one male each at 50 and 225 ppm at 18 month, and in one male at 1,000 ppm at 24 months. None were seen at 12 months. The authors stated that it is possible that the sperm granulomas were induced early but resolved at later times, or that the lesion was spontaneous, but it is not possible to definitively attribute the lesions to chloromethane exposure on the basis of the results of this study. By 24 months, all male rats, including controls, had interstitial cell hyperplasia or adenomas associated with aging, which precluded detection of further exposure-related seminiferous tubule degeneration and atrophy. Absolute and relative testes weights were decreased in the 1,000 ppm group. There was a concentration-related decrease in bilateral compressive degeneration and atrophy and increase in unilateral compressive degeneration and atrophy (caused by testicular tumors), which correlated with decreased interstitial cell tumor size. This observation was supported by the testicular weight decreases observed in 1,000 ppm exposed male rats (CIIT 1981).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation exposure to chloromethane.

Maternal toxicity, evidenced by decreased body weight gain and retarded development of 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 of metatarsals and phalanges of the anterior limbs, thoracic centra in the pubis of the pelvic girdle, and metatarsals of the hindlimbs.

Wolkowski-Tyl et al. (1983a) also found increased incidences of heart malformations in the fetuses of mouse dams exposed by inhalation to 480 ppm chloromethane during Gd 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 Gd 11.5-125, 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. (1983) 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.

The highest NOAEL and all reliable LOAEL values for developmental effects in mice and rats are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after inhalation exposure to chloromethane. In animals, chloromethane exposure has resulted in dominant lethal mutations in the sperm of male rats (Chellman et al. 1986c; Rushbrook 1984; Working et al. 1985a). Experiments on the mechanism of the postimplantation loss observed in the females mated to the exposed males indicated that the dominant lethal effect may be secondary to epididymal inflammation, rather than a direct genotoxic effect of chloromethane (Chellman et al. 1986c). Chloromethane did not result in unscheduled DNA synthesis in hepatocytes, spermatocytes, or tracheal epithelial cells when male rats were exposed to 3,500 ppm, 6 hours per day for 5 days, but did produce a marginal increase in unscheduled DNA synthesis in hepatocytes when rats were exposed to 15,000 ppm for 3 hours (Working et al. 1986).

Jager et al. (1988) have shown that the formation of formaldehyde (via P-450 activity) was 10 times higher in male mouse liver than in male kidney. Male mouse liver also produced formaldehyde at about twice the amount produced by female liver, and male kidney about 50% more than female kidney. This led to the hypothesis that male mice renal tumors resulted from increased production of formaldehyde and increased numbers of formaldehyde-induced DNA lesions. Glutathione depletion also removes the cofactor for formaldehyde dehydrogenase (FDH), the enzyme that inactivates formaldehyde. Jager et al. (1988), however, did not observe increased formaldehyde levels in mouse liver or kidney after a single 8-hour exposure to 1,000 ppm chloromethane, or an increase in DNA protein cross links (DPC), a typical formaldehyde-induced lesion, after exposure to 1,000 ppm for 6 hours per day for 4 days. Ristau et al. (1989), however, did observe an increase in DPC in the renal tissue of male but not female B6C3F_1 mice exposed to chloromethane at 1,000 ppm for 8 hours. DNA-protein crosslinks were not observed in liver. In a follow-up study, Ristau et al. (1990) showed a rapid removal of DPC whereas single strand breaks appeared to accumulate. Both types of lesions were ascribed to the action of formaldehyde. Ristau et al. (1989) assayed for DPC immediately after a single 8-hour exposure, whereas Jager et al. (1988) dosed over a 4-day period. Delays from exposure to assays that allow rapid repair of formaldehyde-induced DPCs could possibly explain why Jager et al. (1988) did not observe an increase. Both the DPCs and the incomplete and delayed repair of chloromethane-induced DNA lesions may contribute to the formation of renal tumors. Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

A retrospective epidemiology study of male workers exposed to chloromethane in a butyl rubber manufacturing plant produced no statistical evidence that the rates of death due to cancer at any site were increased in the exposed population when compared with U.S. mortality rates (Holmes et al. 1986). No specific exposure levels were given in this study.

Rafnsson and Gudmundsson (1997) report on excess mortality from cancer in a long-term follow-up after an acute high-level exposure. Seventeen crew members (males) were exposed for 2 days in 1963 to chloromethane that leaked from a refrigerator on board an Icelandic fishing trawler (no estimates of exposure levels were reported). The refrigerator was located under the sleeping quarters of the crew. Gudmundsson (1977) reported mild to permanent neurological and/or psychiatric sequelae at 20 months and 13 years postexposure. This study evaluated a cohort of 24 men on board the vessel at 32 years postexposure (6 officers and 18 deckhands including the surviving crew members who had the highest

exposure). The reference group was selected from three registries of seamen. The Icelandic registries for seamen are some of the most comprehensive and complete available. The reference group contained five times as many individuals as the study group, and was controlled for age, occupation, and social class. The authors report an excess mortality from all causes associated with chloromethane exposure (Mantel-Haenszel point estimate=2.2, 95%; CI=1.3-3.1). An elevated mortality from all cancers was also reported (M-H=15, 95%; CI=0.3-5.6) and for lung cancer (M-H=2.7, 95%; CI=0.l-52.6). Because the reference group matched for age, occupation, and social class, the authors assumed simultaneous control for lifestyle factors including smoking habits and diet. Conclusions from this study are limited because of this assumption. Indirect effects of the neurological deficits in this cohort on cancer susceptibility or lifestyle factors were also not discussed.

A high incidence of renal tumors was found in male mice that were exposed to 1,000 ppm chloromethane and died or were killed at 12 months or later in a 2-year oncogenicity study (CIIT 1981). Tumors consisted of renal cortex adenomas and adenocarcinomas, papillary cystadenomas, tubular cystadenomas, and papillary cystadenocarcinomas. No evidence of carcinogenicity was found in female mice or in male or female rats exposed to concentration of 1,000 ppm or less in this study. The cancer effect levels from this study are recorded in Table 2-l and plotted in Figure 2-1.

2.2.2 Oral Exposure

2.2.2.1 Death

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

2.2.2.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, renal, endocrine, dermal, ocular, or body weight effects in humans or animals after oral exposure to chloromethane.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to chloromethane.

Only one animal study was located in which chloromethane was administered orally. In this study, the hepatotoxic effects of chloroform, carbon tetrachloride, dichloroethane, and chloromethane were compared (Reynolds and Yee 1967). Rats were given chloromethane in mineral oil by gavage at a single dose of 420 mgkg. Only the livers were examined for effects, but no liver necrosis was found in the rats given chloromethane. Higher doses of chloromethane were not administered because of the known anesthetic and lethal effects of the compound. The NOAEL from this study is recorded in Table 2-2 and plotted in Figure 2-2.

No studies were located regarding the following health effects in humans or animals after oral exposure to chloromethane:

2.2.2.3 Immunological and Lymphoreticular Effects

2.2.2.4 Neurological Effects

2.2.2.5 Reproductive Effects

2.2.2.6 Developmental Effects

2.2.2.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

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

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans or animals after dermal exposure to chloromethane.

Table 2-2. Levels of Significant Exposure to Chloromethane - Oral

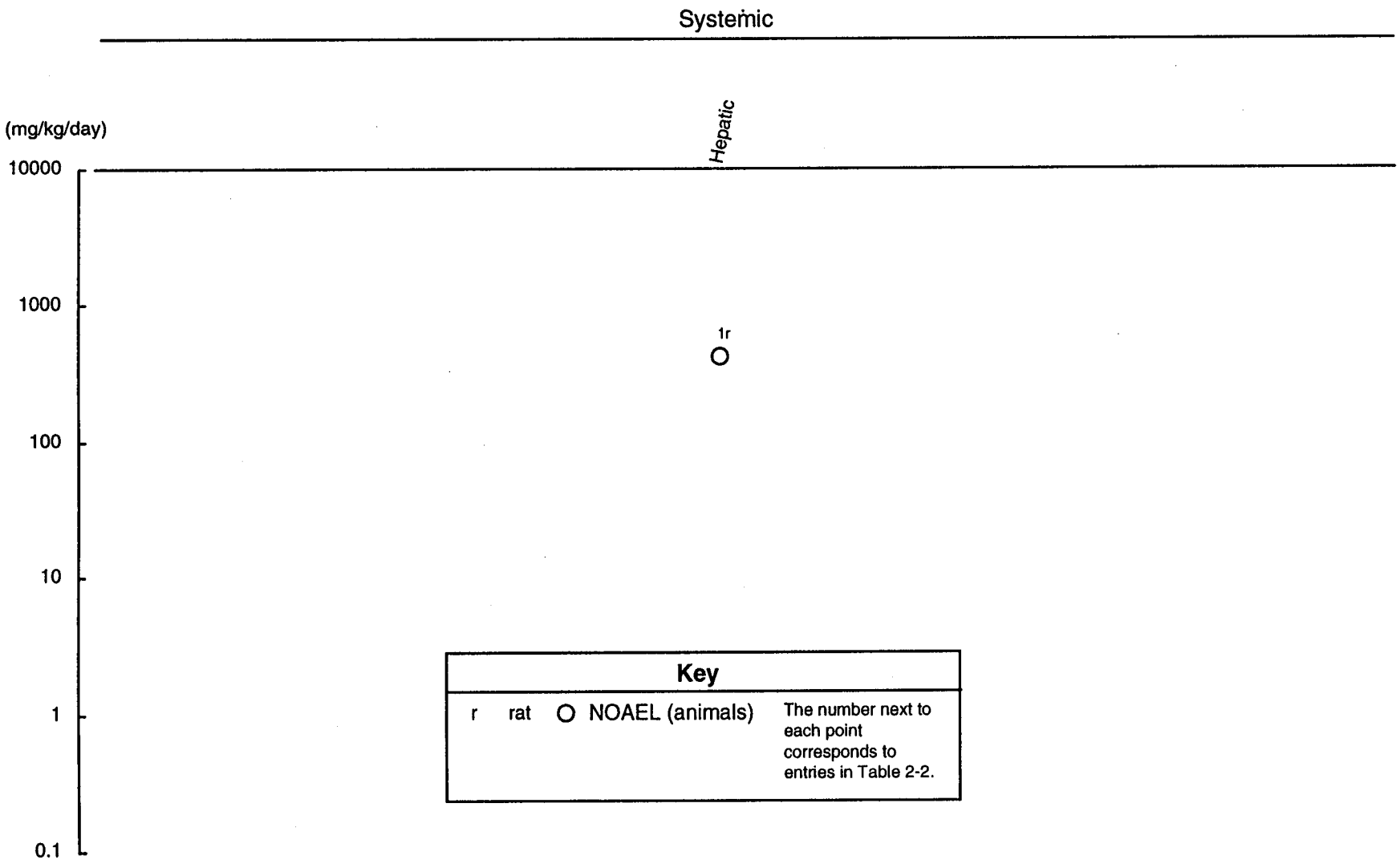
Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	ACUTE EXPOSURE						
	Systemic						
1	Rat (Charles River)	once (GO)	Hepatic	420			Reynolds and Yee 1967

[a]The number corresponds to entries in Figure 2-2.

(GO) = gavage in oil; LOAEL = lowest-observable-adverse-effect level; NOAEL = no-observable-adverse-effect level

Figure 2-2. Levels of Significant Exposure to Chloromethane - Oral

Acute (≤14 days)



2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, or body weight effects in humans or animals after dermal exposure to chloromethane.

Ocular Effects. No studies were located regarding ocular effects in humans after dermal exposure to chloromethane.

A limited number of animal studies report ocular effects, but the results are mixed. Beagle dogs and cats were exposed by inhalation to 0, 200, or 500 ppm chloromethane 23.5 hours/day for 3 days, and were observed for 4 weeks (dogs) or 2 weeks (cats) postexposure before sacrifice. No ocular effects were observed in dogs from direct contact with chloromethane gas. On postexposure day 13, examination of the cat eye revealed focal opacity of the cornea consistent with a temporally persistent papillary membrane in the left eye of a control cat and a 200 ppm cat. These lesions were not considered to be treatment related (McKenna et al. 1981a).

Mitchell et al. (1979) reported mucopurulent conjunctivitis with total destruction of the eye in $B6C3F_1$ mice exposed to 375, 750, or 1,500 ppm for 6 hours/day, 5 days/week, for 13 weeks. No eye lesions were observed in controls. These lesions were attributed to exposure because no lesions were found in controls; however, the failure of longer-term studies to detect comparable eye lesions at higher concentrations makes the findings of Mitchell et al. (1979) questionable.

Beagle dogs exposed to 400 ppm chloromethane for 6 hours/day, 5 days/week for 90 days had no exposure-related gross or histopathological lesions in the eyes from direct contact with chloromethane gas (McKenna et al. 1981b).

Male and female Fischer 344 rats and $B6C3F_1$ mice were exposed to chloromethane at target concentrations of 0, 50, 225, or 1,000 ppm, 6 hours/day, 5 days/week. Ophthalmic exams were performed at baseline and at sacrifice. At 6 months, cornea1 cloudiness or opacity without conjunctivitis was noted in control rats (2 of 10 male rats and 1 of 10 females), at 50 ppm (1 of 10 males at 12 months), and at 225 ppm (1 of 10 females at 18 months). The significance of this lesion is not clear because there was no dose-related incidence pattern at later sacrifices. At 12 months, a cornea1 lesion described as a haze

elliptically patterned over a central portion of the eye was seen in control rats (1 of 10 males and l/ of 10 females), at 50 ppm (8 of 10 males and 6 of 10 females), at 225 ppm (9 of 10 males and 7 of 10 females), and at 1,000 ppm group (9 of 10 males and 9 of 10 females). This lesion was only seen at 12 months and was distinctly different from the cornea1 cloudiness or opacity seen at 6 or 18 months. This cornea1 haze may have been the result of chemical effects upon the eyes in which the lacrimal function was compromised by intercurrent disease (an outbreak of sialodacryo-adenitis [SDA] was histopathologically diagnosed at 12 months). At 18 months in rats, the incidence of cornea1 cloudiness in exposed male rats was similar to that of control males. In females, the incidence of cornea1 cloudiness increased with dose: controls (2/20), at 50 ppm (4/20), at 225 ppm (12/20), and at 1,000 ppm (12/20). No significant difference in ocular lesions were observed in rats at 24 months. In mice, at 6 months, an acute, focal scleritis was observed in 3 of 10 males and 1 of 10 females in the 1,000 ppm group. This lesion was always associated with a neutrophilic inflammatory infiltrate which was present at the corneoscleral junction. At 12, 18, and 24 months, there were no statistically significant ocular lesions observed in mice (CIIT 1981).

The highest NOAEL and all reliable LOAEL values for ocular effects in mice and rats are recorded in Table 2-3.

No studies were located regarding the following effects in humans or animals after dermal exposure to chloromethane.

2.2.3.3 Immunological and Lymphoreticular Effects

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5

Table 2-3. Levels of Significant Exposure to Chloromethane - Dermal

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference
ACUTE EXPOSURE						
Systemic						
Dog (Beagle)	3 d 23.5 hr/d	Ocular	500M ppm			McKenna et al. 1981a
Cat (NS)	3 d 23.5 hr/d	Ocular	500M ppm			McKenna et al. 1981a
INTERMEDIATE EXPOSURE						
Systemic						
Rat (Fischer- 344)	6 mo 5 d/wk 6 hr/d	Ocular	997 ppm			CIIT 1981
Rat (Fischer- 344)	12 mo 5 d/wk 6 hr/d	Ocular		51 ppm (corneal haze)		CIIT 1981
Mouse (B6C3F1)	12 mo 5 d/wk 6 hr/d	Ocular	997 ppm			CIIT 1981
Mouse (B6C3F1)	6 mo 5 d/wk 6 hr/d	Ocular	224 ppm		997 ppm (acute focal scleritis)	CIIT 1981
Mouse (B6C3F1)	90 d 5 d/wk 6 hr/d	Ocular			368 ppm (mucopurulent conjunctivitis)	Mitchell et al. 1979
Dog (Beagle)	90 d 5 d/wk 6 hr/d	Ocular	400M ppm			McKenna et al. 1981b

Table 2-3. Levels of Significant Exposure to Chloromethane - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL	LOAEL		Reference
				Less Serious	Serious	
CHRONIC EXPOSURE						
Systemic						
Rat (Fischer- 344)	24 mo 5 d/wk 6 hr/d	Ocular	997 ppm			CIIT 1981
Rat (Fischer- 344)	18 mo 5 d/wk 6 hr/d	Ocular	51 M ppm 997 F ppm	224 F ppm (corneal cloudiness)		CIIT 1981
Mouse (B6C3F1)	18 mo 5 d/wk 6 hr/d	Ocular	997 ppm			CIIT 1981
Mouse (B6C3F1)	24 mo 5 d/wk 6 hr/d	Ocular	997 ppm			CIIT 1981

d = day(s); F = female; hr = hour(s); LOAEL = lowest-observable-adverse-effect level; M = male; mo = month(s); NOAEL = no-observable-adverse-effect level; NS = not specified; wk = week(s)

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to chloromethane.

2 . 3 TOXICOKINETICS

Chloromethane is readily absorbed from the lungs and rapidly reaches equilibrium with levels in blood and expired air approximately proportional to the exposure concentrations. At high concentrations, kinetic processes like metabolism or excretion may become saturated, limiting the rate of uptake. Differences in these processes may account for some of the observed differences in species uptake and distribution. It is not known what levels, if any, of chloromethane or its metabolites cross the placenta or enter the milk. There is also no information on differences between adults and children for the toxicokinetics of chloromethane.

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. Chloromethane is metabolized by conjugation with glutathione to yield S-methylglutathione, S-methyl-cysteine, and other sulfur-containing compounds. These compounds are excreted in the urine or can 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 CO_2. Alternatively, formaldehyde may be directly produced from chloromethane via a P-450 oxidative dechlorination.

The conjugation of chloromethane with glutathione is primarily enzyme catalyzed. In contrast to all other animal species investigated (rats, mice, bovine, pigs, sheep, and rhesus monkeys), human erythrocytes contain a glutathione transferase isoenzyme that catalyzes the conjugation of glutathione with chloromethane. There are two distinct human subpopulations based on the amount or forms of this transferase. They are, for practical purposes, known as fast metabolizers (i.e., lower body burdens and higher excretion rates) and slow metabolizers (i.e., higher body burdens and lower excretion rates). These two subpopulations are also called conjugators and nonconjugators. Determination of the relative proportion of these subpopulations to the whole has just begun, but early results indicate considerable variation among different ethnic groups. There is considerable interest in further evaluating the relationship between endogenous levels of glutathione transferase and susceptibility of subpopulations to

chloromethane-induced toxicity. There is no information available on differences in isoforms or levels of glutathione transferase or P-450 in children (i.e., a different metabolic profile) that would result in a significantly increased or decreased susceptibility to chloromethane toxicity compared to that observed in adults. Research that addresses this issue is needed.

Little is known about the toxicokinetics of chloromethane from the oral or dermal routes of exposure.

2.3.1 Absorption

2.3.1.1 inhalation Exposure

Chloromethane is absorbed readily from the lungs of humans following inhalation exposure. Alveolar breath levels of chloromethane reached equilibrium within 1 hour during a 3- or 3.5hour exposure of men and women (Putz-Anderson et al. 1981a, 1981b). Mean f SD alveolar 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 ± 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.

Blood and alveolar air levels of chloromethane also reached 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 3.7 μg/min/kg and the slow metabolizers absorbed 1.4 μg/min/kg.

In experiments in rats, uptake of chloromethane reached 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, 1983b). Absorbed doses were calculated as 67 mg/kg for rats exposed to 1,000 ppm and 3.8 mg/kg for rats exposed to 50 ppm (i.e., a ratio of 17.6 compared to a predicted ratio of 20 based on absorption being directly proportional to exposure concentration). The rate of uptake was 0.167 mg/min/kg for 1,000 ppm and 0.01 mg/min/kg for 50 ppm (ratio of 16.7). Where the uptake was not completely proportional to

exposure, the difference in the ratio of absorbed doses from the predicted ratios may be due to a lower respiratory minute volume in the rats exposed to 1,000 ppm or to different amounts remaining in the body at the end of exposure and how much is metabolized. Blood chloromethane concentrations reached equilibrium within 1 hour and were proportional to exposure concentration 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.

At relatively low exposure concentrations, absorption of chloromethane from the lungs appears to be proportional to exposure concentration in rats and humans, but at higher concentrations, kinetic processes like metabolism or excretion may become saturated, limiting the rate of uptake. In dogs, however, it appears that absorption is proportional to exposure concentration through a wide range of exposure levels.

2.3.1.2 Oral Exposure

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

2.3.1.3 Dermal Exposure

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

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution in humans after inhalation exposure to chloromethane.

After absorption of chloromethane, distribution of chloromethane and/or its metabolites is extensive in animals. Total uptake of radioactivity (as μmol ^{14}C-chloromethane equivalents/g wet weight) in whole tissue homogenates following exposure of rats to 500 ppm for 6 hours was 1.21 for lung, 4.13 for liver, 3.43 for kidney, 2.29 for testes, 0.71 for muscle, 0.57 for brain, and 2.42 for intestine (Kornbrust et al. 1982). 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 4.5 in liver, 4.1 in heart, and 3.7 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).

2.3.2.2 Oral Exposure

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

2.3.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals after dermal exposure to chloromethane.

2.3.3 Metabolism

Information regarding metabolism of chloromethane in humans is limited. 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 (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. There are two distinct subpopulations 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 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 subpopulations 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). The urinary excretion of S-methylcysteine in the volunteers exposed to chloromethane was variable, and was not significantly different in pre-

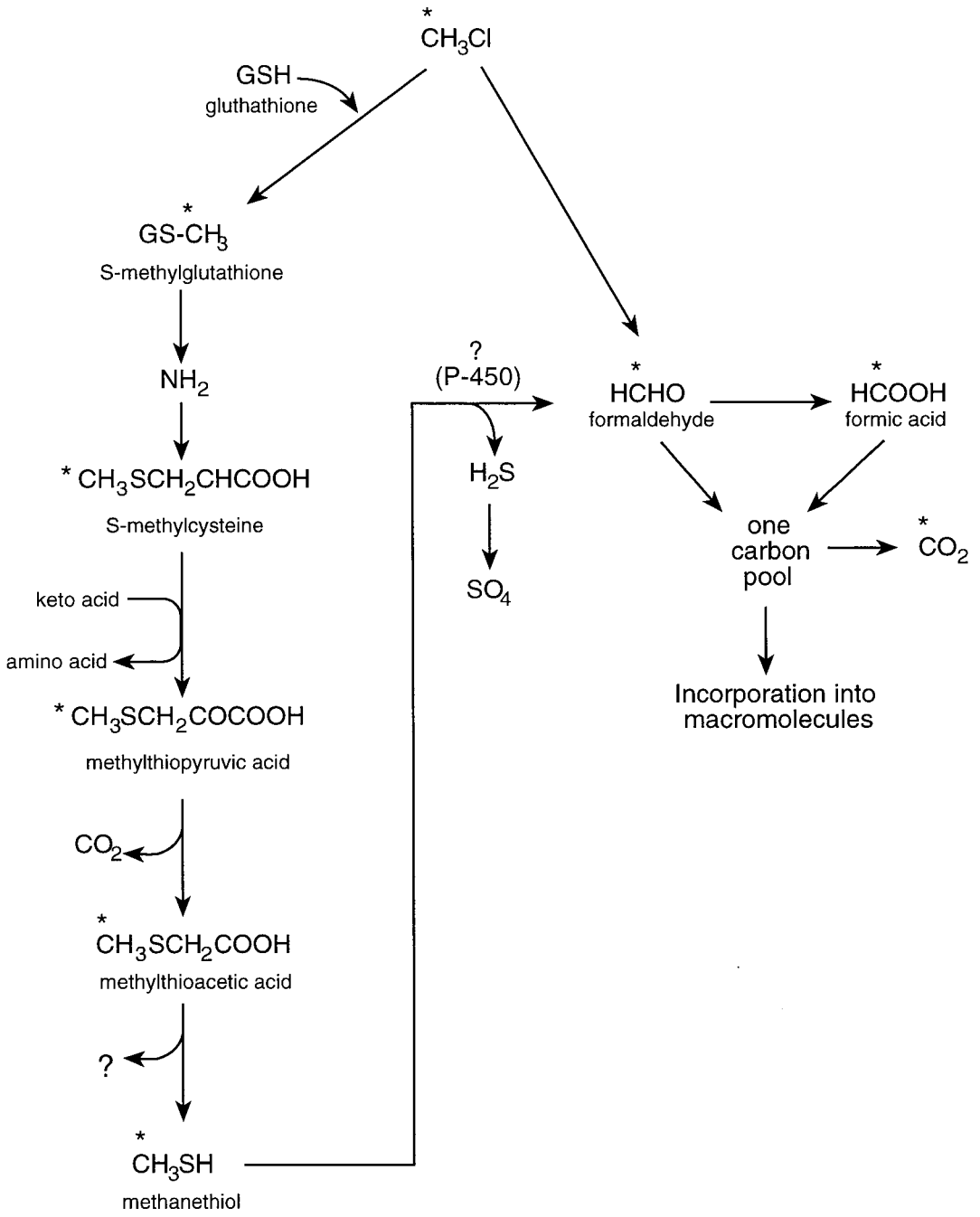
and postexposure levels. No change was detected in the S-methylcysteine concentration or in the total sulfhydryl concentration in the urine of 4 workers before and after a 7-hour shift in a styrene production plant by DeKok and Antheunius (1981) who concluded that S-methylcysteine is not a human metabolite of chloromethane. It is possible, however, that the workers examined by DeKok and Antheunius (1981) were slow eliminators.

Peter et al. (1989a, 1989b) 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 (nonconjugators). The results suggested that a minor form of human erythrocyte glutathione S-transferase is responsible for the unique metabolism of methyl chloride 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 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 nonconjugators of 30-40% reported for a German population (Peter et al. 1989a). A different study by Kempkes et al. (1996) found a frequency of 15% for nonconjugators 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.

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 2-3 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 (Dodd et al. 1982; Kornbrust and Bus 1984; Landry et al. 1983a, 1983b; Redford-Ellis and Gowenlock 1971a,

Figure 2-3. Proposed Scheme for the Metabolism of Chloromethane



*Indicates the position of the radioactive label

Source: Kornbrust and Bus 1983

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 CO_2 (Heck et al. 1982; Jager et al. 1988; Kornbrust and Bus 1983; Kornbrust et al. 1982). Formaldehyde may also be a direct product of chloromethane 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; Jager et al. 1988). Dekant et al. (1995) demonstrated oxidation of chloromethane to formaldehyde by cytochrome P-450 (2El) 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.

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.

2.3.4 Elimination and Excretion

2.3.4.1 Inhalation Exposure

Very little unchanged chloromethane is excreted in the urine. In volunteers exposed to chloromethane, Stewart et al. (1980) found no chloromethane in the urine, and urinary excretion was <0.01 %/min in another study (Morgan et al. 1970). The excretion patterns of chloromethane following prolonged exposure will differ from those observed in these experiments, which followed single breath exposure; therefore, these data are not useful for monitoring occupational exposure. 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 50-90 minutes, with differences possibly due to different metabolic rates. These results suggest that chloromethane is unlikely to accumulate in tissues during repeated intermittent exposures.

In rats exposed to chloromethane for 6 hours and dogs exposed for 3 hours at concentrations of 50 or 1,000 ppm, blood levels rose rapidly and reached equilibrium proportionate or nearly proportionate to exposure levels (Landry et al. 1983a). Blood concentrations declined rapidly in a biphasic, nonconcentration-dependent manner when exposure was stopped. The disappearance from blood was consistent with a linear 2-compartment open model. Half-lives for the a-phase were 4 minutes in rats, and 8 minutes ‘in dogs; half-lives for the β-phase were 15 minutes in rats and 40 minutes in dogs. The disappearance of chloromethane from blood probably represents metabolism rather than excretion of parent compound. As discussed above in Section 2.3.3 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.

2.3.4.2 Oral Exposure

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

2.3.4.3 Dermal Exposure

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

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

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

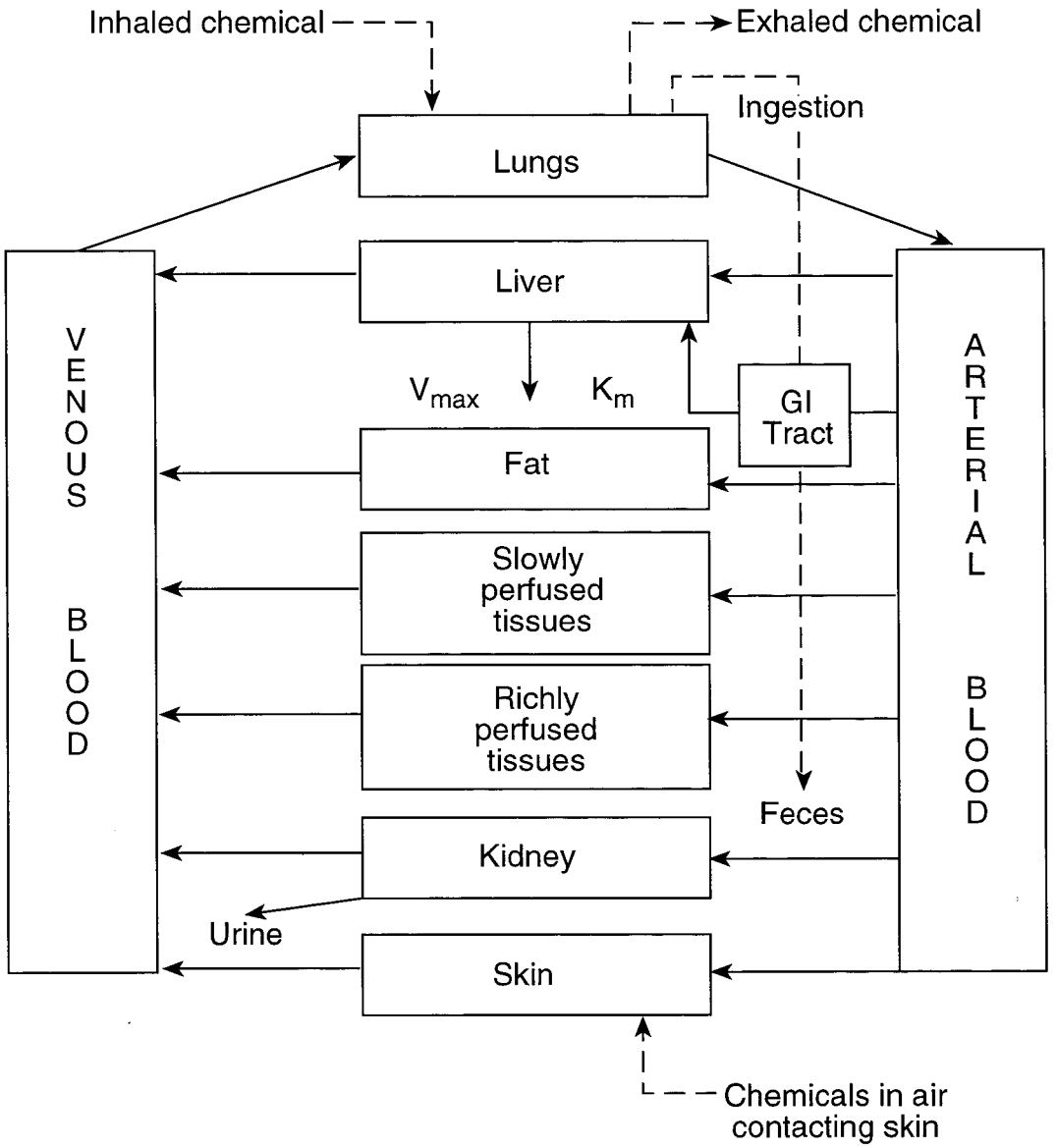
PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substancespecific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically-sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 2-4 shows a conceptualized representation of a PBPK model.

Figure 2-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

If PBPK models for chloromethane exist, 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.

No PBPK models for adults, children, or test animal models were located for chloromethane.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

As presented in Section 2.3.3, metabolism of chloromethane involves conjugation with glutathione to yield S-methylglutathione, S-methylcysteine, and other sulfur-containing compounds (Dodd et al. 1982; Kornbrust and Bus 1984; Landry et al. 1983a, 1983b; Redford-Ellis and Gowenlock 1971a, 1971b). These compounds can be excreted in the urine (Landry et al. 1983a), and S-methylglutathione may be further metabolized to methanethiol. Cytochrome P-450 dependent metabolism of methanethiol may yield formaldehyde and formic acid whose carbon atoms can then enter the one-carbon pool for incorporation into macromolecules or formation of CO_2 (Heck et al. 1982; Jager et al. 1988; Kornbrust and Bus 1983). Guengerich and Shimada (1991) suggest that the human cytochrome P-450 enzyme 2El is a major catalyst in the oxidation of chloromethane. Formaldehyde may also be a direct product of chloromethane via oxidative dechlorination. Methanethiol and formaldehyde, and lipid peroxidation due to glutathione depletion have been suggested as the toxic intermediates and mechanism responsible for the toxicity of chloromethane (Dekant et al. 1995; Jager et al. 1988; Kornbrust and Bus 1983, 1984; Ristau et al. 1989, 1990). There is no information available on differences in isoforms or levels of glutathione transferase or P-450 in children that would result in significantly different metabolic rates (i.e., increased or decreased susceptibility to chloromethane toxicity) than those observed in adults.

2.4.2 Mechanisms of Toxicity

Hepatic effects: While the exact mechanism for the hepatotoxic effects of chloromethane is unclear, chloromethane can elicit lipid peroxidation as a secondary consequence of glutathione depletion (Kornbrust and Bus 1984). Comparison of lipid peroxidation in the S-9 fraction from mouse and rat livers revealed much greater lipid peroxidation in mouse liver than in rat liver. Further evidence that the mechanism of

hepatotoxicity may involve lipid peroxidation comes from the finding that mice exposed to 2,500 ppm chloromethane expired ethane to an extent comparable to that produced by 2 mL/kg carbon tetrachloride, and developed moderate to severe hepatocellular hydropic degeneration.

Dodd et al. (1982) examined the effects of an inhalation exposure to chloromethane on tissue nonprotein sulfhydryl (NPSH) content in male Fischer 344 rats. NPSH content of liver, kidney, and lung were decreased in a chloromethane concentration-related manner. Pretreatment with Aroclor 1254 (an inducer of microsomal enzymes) did not alter the decreases in tissue NPSH seen after exposure to chloromethane alone. Pretreatment with SKF-525A (an inhibitor of microsomal enzymes) may have interfered with the ability of chloromethane to decrease NPSH in some tissues. Treatment with chloromethane significantly increased the activity of glutathione-S-alkyltransferase, and pretreatment with Aroclor 1254 did not alter the increase. The toxicological significance of this effect is not clear. These results support the hypothesis that chloromethane reacts enzymatically with glutathione (GSH), which is the most abundant NPSH, and the hypothesis that the reaction is not dependent upon the formation of a reactive intermediate by microsomal enzymes. Possible mechanisms for the toxicity of chloromethane related to glutathione depletion include: enhancement of the toxicity of chemicals that are detoxified via conjugation with GSH; prevention of GSH from acting as a cellular reducing agent, thereby interfering with a variety of physiological functions; or an increase in chloromethane-glutathione conjugates that are then further metabolized to putative toxic metabolite (e.g., formaldehyde or methanethiol).

Neurological effects: Chellman et al. (1986b) investigated the role of glutathione in the mediation of chloromethane-induced toxicity in the brain of male B6C3F_1 mice. Mice exposed to 1,500 ppm chloromethane for 6 hours/day, 5 days/week for 2 weeks, developed multiple degenerative, necrotic foci in the internal granule cell layer of the cerebellum; in some areas the foci involved the whole thickness of the granular cell layer. Cerebellar degeneration consisted of granule cells with pyknotic nuclei and clear, swollen perikarya. Tremors, ataxia, and forelimb/hindlimb paralysis were seen in chloromethane-induced lethality and were associated with chloromethane-induced cerebellar damage. Cerebellar damage was not observed in chloromethane-exposed mice pretreated with BSO, a glutathione depleter. The authors concluded that the depletion of GSH protected mice from cerebellar damage due to exposure to chloromethane. The mechanism may involve conjugation of chloromethane with glutathione in the liver, followed by biliary excretion and enterohepatic circulation of the glutathione conjugate or possibly a cysteine conjugate and further metabolism by kidney and/or gut flora beta-lyase to methanethiol.

Methanethiol produces similar central nervous system symptoms (tremors, convulsion, coma) as seen in animals or humans acutely intoxicated with chloromethane (Chellman et al. 1986b).

In the metabolic scheme proposed by Kornbrust and Bus (1983), chloromethane reacts with glutathione to form S-methylglutathione. Subsequent metabolism of S-methylglutathione produces methanethiol as an intermediate. Jiang et al. (1985) discuss the possibility of a relationship between degenerative effects in the kidney and granular layer lesions in the brain, which were also observed in mice. Granular cell necrosis is often seen in people who die of renal insufficiency (i.e., not due to chloromethane exposure). In the Jiang et al. (1985) mouse study, however, the severity of the brain and kidney lesions were unrelated, and the authors conclude that the brain lesions were probably not a direct consequence of the chloromethaneinduced kidney lesions.

Reproductive effects: Studies on the mechanism of chloromethane-induced testicular effects suggest that preimplantation loss is due to chloromethane cytotoxicity to the sperm in the testes at the time of exposure rather than genotoxic effects on the sperm (Chellman et al. 1986a, 1986c, 1987; Working and Bus 1986; Working and Chellman 1989; Working et al. 1985a, 1985b). Working et al. (1985a) previously had . provided results indicating that chloromethane-induced postimplantation loss results from an inflammatory response in the epididymis that indirectly produces genetic damage to the sperm rather than from a direct genotoxic effect of chloromethane. Inhibition of the chloromethane-induced epididymal inflammatory response with anti-inflammatory agent BW755C (Chellman et al. 1986c) was subsequently shown to reduce the amount of postimplantation loss (Chellman et al. 1986c).

Genotoxicitv: Chloromethane exposure consistently produced dominant lethal mutations in the sperm of rats, as measured by postimplantation 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 postimplantation 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 postimplantation loss, the dominant lethal mutations probably resulted secondary to the epididymal inflammatory response (Chellman et al. 1986c; Working and Chellman 1989). 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. 1979, 1981; Working et al. 1985a).

Renal tumors: Some proposed mechanisms for the carcinogenic effect (renal tumors) detected in male mice include glutathione depletion in the target tissue, increased lipid peroxidation, and formation of formaldehyde-induced DNA lesions (Bolt and Ganswendt 1993). Chloromethane can be metabolized to formaldehyde (Kornbrust and Bus 1982). Exposure to 1,000 ppm chloromethane depletes glutathione in the kidney to ≈5% of the pre-exposure levels (Bolt et al. 1986; Hallier et al. 1990), effectively removing the cofactor for the glutathione-dependent primary metabolic pathway for chloromethane. The alternate oxidative pathway leads directly to the formation of formaldehyde via cytochrome P-450. Jager et al. (1988) have shown that the formation of formaldehyde (via P-450 activity) was 10 times higher in male mouse liver than in male kidney. Male mouse liver also produced formaldehyde at about two times the amount of female liver, and male kidney about 50% more than female kidney. This led to the hypothesis that male mice tumors resulted from increased production of formaldehyde and increased numbers of formaldehyde-induced DNA lesions. Glutathione depletion also removes the cofactor for formaldehyde dehydrogenase (FDH), the enzyme that inactivates formaldehyde. Jager et al. (1988), however, did not observe increased formaldehyde levels in mouse liver or kidney after a single, 8-hour exposure to 1,000 ppm chloromethane, or an increase in DNA protein cross links (DPC), a typical formaldehydeinduced lesion, after exposure to 1,000 ppm for 6 hours per day for 4 days. Ristau et al. (1989), however, did observe an increase in DPC in the renal tissue of male but not female mice. In a follow-up study, Ristau et al. (1990) showed a rapid removal of DPC whereas single strand breaks appeared to accumulate. Both types of lesions were ascribed to the action of formaldehyde. Ristau et al. (1989) assayed for DPC immediately after a single 8-hour exposure, whereas Jager et al. (1988) dosed over a 4-day period. Delays from exposure to assay that allow rapid repair of formaldehyde-induced DPCs could possibly explain why Jager et al. (1988) did not observe an increase. Both the DPCs and the incomplete and delayed repair of chloromethane-induced DNA lesions may contribute to the formation of renal tumors. Morgan et al. (1982) also noted a proliferative response in male and female mouse proximal tubules following exposure to 1,000 ppm of chloromethane. This proliferative response could also contribute to the tumorigenicity of chloromethane in the males.

2.4.3 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. 1986a, 1986b; CIIT 1981). 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 much greater extent in mouse liver, kidney, and brain compared with rats (Kornbrust and Bus 1984). Pretreatment of mice with buthionine-S,R-sulfoxime (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 (Kornbrust and Bus 1984).

In humans, S-methylcysteine appears as a metabolite of chloromethane (see Section 2.3.3), 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 formaldehyde-induced DNA or tissue damage (Dekant et al. 1995; Jager et al. 1988; Ristau et al. 1989, 1990).

2.5 RELEVANCE TO PUBLIC HEALTH

Overview.

Information regarding health effects of chloromethane in humans and animals is available primarily for the inhalation route of exposure. Oral and dermal routes of exposure are of concern because chloromethane is ubiquitous in the environment. Because it is highly volatile, however, chloromethane rapidly moves from water or soil to the air (see Chapter 5). Issues relevant to children are explicitly discussed in Sections 2.6, Children’s Susceptibility, and 5.6, Exposures of Children.

The central nervous system is the major target of chloromethane toxicity in both humans and animals, as demonstrated by such signs and symptoms as dizziness, staggering, blurred vision, ataxia, muscle

incoordination, convulsions, and coma after acute exposure to high levels. High acute exposures can also result in death of humans and animals. The liver and kidney are also target organs for chloromethane toxicity in humans and animals from acute or longer-term exposure. Toxic manifestations seen in humans, but generally not in animals, include cardiovascular and gastrointestinal effects. These may be secondary to the neurotoxicity. Effects that have been observed in animals, but not reported in humans, include epididymal occlusion, testicular atrophy, infertility, sterility in males, carcinogenicity (e.g., kidney tumors in male mice), and possibly developmental effects (e.g., heart defects) in mice.

Species differences in susceptibility to chloromethane toxicity have been observed. Different P-450 activities between species, sexes, and tissues within the body affect the dehalogenation of chloromethane to formaldehyde, and can thus influence the level of formaldehyde-induced DNA or tissue damage. Rates of conjugation with glutathione differ and lead to differing levels of toxic metabolites. In animal studies, mice have been shown to be more sensitive than rats to the lethal effects of chloromethane, probably due to the higher rate of formation of the toxic metabolite, S-methylglutathione. S-methylcysteine appears as a metabolite of chloromethane in humans, so conjugation with glutathione probably also occurs in humans. There is no information available on differences in isoforms or levels of glutathione transferase or P-450 in children that would result in significantly different metabolic rates (i.e., increased or decreased susceptibility to chloromethane toxicity) than those observed in adults.

Minimal Risk Levels for Chloromethane.

Inhalation MRLs.

- An MRL of 0.5 ppm has been derived for acute-duration inhalation exposure (14 days or less) to chloromethane.

An acute MRL of 0.5 ppm was derived from a NOAEL of 50 ppm for no effect on motor coordination or damage to the cerebellar granule cells in a study by Landry et al. (1985). This study evaluated the neurologic effects of continuous versus intermittent chloromethane exposure in female C57BL/6 mice. The results support a good dose-response effect for cerebellar damage and motor incoordination. The NOAEL of 50 ppm was converted to a human equivalent dose by multiplying with the ratio of the blood:gas (air) partition coefficient for the mouse to the human value. The default value of 1.0 was used because the

coefficients are not known (see formula 4-48a, EPA 1994b). The resulting $NOAEL_{[HEC]}$ of 50 ppm was then divided by an uncertainty factor of 100 (10 for interspecies variability and 10 for human variability). The obtained MRL value is 0.5 ppm (see Appendix A).

Neurological effects have been described in numerous case reports of humans exposed to chloromethane vapors as a result of industrial leaks and leaks from defective home refrigerators (Baird 1954; Gudmundsson 1977; Hansen et al. 1953; Hartman et al. 1955; Jones 1942; Kegel et al. 1929; MacDonald 1964; McNally 1946; Raalte and van Velzen 1945; Rafnsson and Gudmundsson 1997; Spevak et al. 1976; Wood 1951). Depending on the extent of exposure and the availability of medical treatment, the signs and symptoms can range from staggering and blurred vision to coma, convulsions, and death. In some cases, mild to permanent neurological and/or psychiatric deficits have been reported 13 years after an acute high level exposure (Gudmundsson 1977).

Severe neurological signs (ataxia, tremors, limb paralysis, incoordination, convulsions) have also been observed in rats, mice, rabbits, guinea pigs, dogs, cats, and monkeys exposed acutely by inhalation to high concentrations of chloromethane (Burek et al. 1981; Chellman et al. 1986a, 1986b; Landry et al. 1985; McKenna et al. 1981a; Morgan et al. 1982; Smith and von Oettingen 1947b). Cerebellar lesions have been observed microscopically in guinea pigs and rats (Kolkmann and Volk 1975; Morgan et al. 1982). Mice are more susceptible than rats (CIIT 1981; Morgan et al. 1982), and more sensitive to neurological effects after continuous exposure to low concentrations than after intermittent exposure to higher concentrations of chloromethane (Landry et al. 1985). The greater sensitivity of mice to continuous exposure makes the mouse a good model for the neurotoxicological effects seen in humans.

- An MRL of 0.2 ppm has been derived for intermediate-duration inhalation exposure (15 to 364 days) to chloromethane.

An intermediate MRL of 0.2 ppm was derived from a LOAEL of 51 ppm for significantly increased serum alanine amino transferase levels (indicative of hepatotoxicity) in male mice at the 6 month time point in a 2-year study (377 I.U./L ± 124 versus 170 ±49 in controls). This LOAEL is a minimal LOAEL because no histopathological lesions were observed in the low- or mid-dose levels, but were observed at the high dose level. The objective of the study was to evaluate the toxicologic and oncogenic effects of inhaled chloromethane in male and female Fischer 344 rats and $B6C3F_1$ mice. The dose-response effect for liver toxicity was observed in male mice. Females also had increased ALT, but the increase was not associated

with treatment-related histopathological changes in the liver. Liver necrosis and other pathological changes in the liver of high dose male mice was also observed at 12, 18, and 24 months. No further adjustments in the LOAEL were made for a continuous exposure, and the comparable $LOAEL_{[ADJ]}$ of 51 ppm was then converted to a human equivalent dose by multiplying with the ratio of the blood:gas (air) partition coefficient for the mouse to the human value. The default value of 1.0 was used because the coefficients are not known (see formula 4-48a, EPA 1994b). The resulting $LOAEL_{[HEC]}$ of 51 ppm was then divided by an uncertainty factor of 300 (3 for the use of a minimal LOAEL, 10 for interspecies variability, and 10 for human variability) and rounded to one significant figure. The obtained MRL value is 0.2 ppm (see Appendix A).

Case reports of humans exposed to chloromethane vapors have described clinical jaundice and cirrhosis of the liver (Kegel et al. 1929; Ma&e 1961; Weinstein 1937; Wood 1951), but exposure concentrations were not known.

Hepatic effects have been observed in animals exposed by inhalation to chloromethane at concentrations > 1,000 ppm in acute, intermediate, and chronic duration experiments (Burek et al. 1981; Chellman et al. 1986a; CIIT 1981; Landry et al. 1985; Mitchell et al. 1979; Morgan et al. 1982). Milder liver effects occurred in mice exposed acutely to an intermittent but relatively high concentration than to a low but continuous concentration (Landry et al. 1985). The greater susceptibility to continuous exposure may result from relatively greater metabolism to a toxic intermediate or from diurnal susceptibility. Hepatic effects were more severe in mice (necrosis and degeneration) than in rats (cloudy swelling, fatty infiltration, increased ALT and AST with no necrosis). Furthermore, no hepatic lesions were observed in rats over the course of 2 years of inhalation exposure to 1,000 ppm, while mice similarly exposed had necrotic lesions after 6 months (CIIT 1981). The greater susceptibility of mice to the hepatotoxic effects of chloromethane may be related to the greater ability of chloromethane to conjugate with hepatic glutathione in mice than in rats (Dodd et al. 1982; Kornbrust and Bus 1984). The reaction of chloromethane with glutathione appears to be toxifying rather than detoxifying (Chellman et al. 1986b). While the exact mechanism for the hepatotoxic effects of chloromethane is unclear, chloromethane can elicit lipid peroxidation as a secondary consequence of depletion of glutathione (Kombrust and Bus 1984). Comparison of lipid peroxidation in the S-9 fraction from mouse and rat livers revealed much greater lipid peroxidation in mouse liver than in rat liver. The finding that mice exposed to 2,500 ppm chloromethane expired ethane to an extent comparable to that produced by 2 mL/kg carbon tetrachloride, and developed moderate to severe hepatocellular hydropic degeneration provide further evidence that the mechanism of hepatotoxicity may involve lipid peroxidation.

- An MRL of 0.05 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to chloromethane.

A chronic MRL of 0.05 ppm was derived from a LOAEL of 51 ppm for axonal swelling and degeneration . of axons of the spinal cord in mice after 18 months of exposure (CIIT 1981). This two year study evaluated the toxicologic and oncogenic effects of inhaled chloromethane in male and female Fischer 344 rats and $B6C3F_1$ mice. There was a consistent dose-response for neurological effects in male and female mice. At the high dose, there was a mild reduction in the number of neurons in the granular cell layer of the cerebellum with decreased width of the granular cell layer. In the high, mid, and low dose groups, axonal swelling and degeneration of minimal severity was observed in the spinal nerves and the cauda equina associated with the lumbar spinal cord. The LOAEL was converted to a human equivalent dose by multiplying the LOAEL with the ratio of the blood:gas (air) partition coefficient for the mouse to the human value. The default value of 1.0 was used because the coefficients are not known (see formula 4-48a, EPA 1994b). The resulting $LOAEL_{[HEC]}$ of 5.1 ppm was then divided by an uncertainty factor of 1,000 (10 for the use of a LOAEL, 10 for interspecies variability, and 10 for human variability) and rounded to one significant figure. The obtained MRL value is 0.05 ppm (see Appendix A).

As with support for the acute MRL, neurological effects have been described in numerous case reports of humans exposed to chloromethane vapors (Baird 1954; Gudmundsson 1977; Hansen et al. 1953; Hartman et al. 1955; Jones 1942; Kegel et al. 1929; MacDonald 1964; McNally 1946; Raalte and van Velzen 1945; Rafnsson and Gudmundsson 1997; Spevak et al. 1976; Wood 1951). Signs and symptoms can range from staggering and blurred vision to coma, convulsions, and death. Severe neurological signs (ataxia, tremors, limb paralysis, incoordination, convulsions) have also been observed in rats, mice, rabbits, guinea pigs, dogs, cats, and monkeys exposed acutely by inhalation to high concentrations of chloromethane (Burek et al. 1981; Chellman et al. 1986a, 1986b; Landry et al. 1985; McKenna et al. 1981a; Morgan et al. 1982; Smith and von Oettingen 1947b). Cerebellar lesions have been observed microscopically in guinea pigs and rats (Kolkmann and Volk 1975; Morgan et al. 1982).

Oral MRLs.

No acute, intermediate, or chronic-duration oral MRLs were derived for chloromethane because of lack of appropriate data on effects of oral exposure to chloromethane.

Death. Case reports of humans who have died from exposure to chloromethane involved the inhalation of fumes that leaked from home refrigerators or industrial cooling and refrigeration systems (Baird 19.54; Borovska et al. 1976; Gudmundsson 1977; Kegel et al. 1929; McNally 1946; Thordarson et al. 1965). Exposure concentrations were probably very high, perhaps >30,000 ppm, because the leaks occurred in rooms with little or no ventilation. Exposure to high concentrations, even as high as 600,000 ppm, result in neurological effects (Jones 1942), but need not result in death if exposure is discontinued and/or medical attention is received in time. Since the use of chloromethane as a refrigerant in refrigeration devices has declined, exposure from leaks is of less concern than in the past, although some old refrigerators containing chloromethane are probably still in use. Concentrations of chloromethane in the environment, even at hazardous waste sites, are not likely to be high enough to cause death.

Acute inhalation lethality data in animals indicate that high intermittent concentrations can be tolerated better than lower continuous concentrations (Burek et al. 1981; Jiang et al. 1985; Landry et al. 1985; Morgan et al. 1982). This phenomenon may be related to the conversion of chloromethane to a toxic metabolite or to diurnal susceptibility (Landry et al. 1985). Acute and chronic inhalation studies also indicated that mice are more sensitive than rats to the lethal effects of chloromethane (Chellman et al. 1986a, 1986b; CIIT 1981). The greater susceptibility of mice may be due to differences in the ability of chloromethane to react with glutathione in the two species. Chloromethane is conjugated with glutathione in liver, kidney, and brain to a much greater extent in mice than in rats (Kornbrust and Bus 1984). Pretreatment of mice with buthionine-S,R-sulfoximine (BSO), which depletes glutathione, thereby preventing its reaction with chloromethane, protected mice from the lethal effects of chloromethane (Chellman et al. 1986b). Thus, the reaction of chloromethane with glutathione to produce S-methyl-glutathione appears to be a toxifying rather than a detoxication mechanism (Chellman et al. 1986b). While the exact mechanism for the lethal effects of chloromethane is unclear, subsequent metabolism of S-methyl-glutathione may result in the formation of methanethiol and formaldehyde (Kornbrust and Bus 1983), which have been postulated to be toxic intermediates (Chellman et al. 1986b; Kornbrust and Bus 1982). Alternatively, chloromethane can elicit lipid peroxidation as a consequence of depletion of glutathione (Kombrust and Bus 1984). Conjugation of chloromethane with glutathione probably occurs in humans because S-methylcysteine appears to be a human metabolite (see Section 2.3.3). No information was located regarding the extent to which chloromethane reacts with glutathione in humans or the ability of chloromethane to elicit lipid peroxidation in humans. The clinical signs and histopathological lesions noted with death in humans are similar to those in animals, suggesting a commonality of mechanism, but it is difficult to determine which animal species best serves as a model for extrapolating results in humans.

Systemic Effects.

Respiratory Effects. Case reports generally have not described respiratory effects in humans exposed to chloromethane.

In dogs acutely exposed to lethal concentrations there was a marked reduced in respiration prior to death, but this effect was probably secondary to central nervous system depression (von Oettingen et al. 1949, 1950). Pulmonary congestion prior to death was a common finding among a variety of species (rats, mice, guinea pigs, rabbits, dogs, cats, and monkeys), but the study limitations precluded the determination of a good dose-response relationship (Dunn and Smith 1947; Smith and von Oettingen 1947a). More recent studies failed to find exposure-related histopathological lesions in the lungs of dogs and cats exposed acutely to 500 ppm chloromethane (McKenna et al. 198 la), rats exposed acutely to 2,000 ppm (Burek et al. 198 l), male dogs exposed to 400 ppm, and rats and mice exposed to up to 1,500 ppm chloromethane for intermediate durations (CIIT 1981; McKenna et al. 198 lb; Mitchell et al. 1979), or rats and mice exposed chronically to up to 1,000 ppm (CIIT 1981).

Cardiovascular Effects. Cardiovascular effects, such as electrocardiogram abnormalities, tachycardia and increased pulse rate, and decreased blood pressure; and gastrointestinal effects such as nausea and vomiting, have been described in case reports of humans exposed to chloromethane vapors occupationally or accidentally due to refrigerator leaks (Baird 1954; Baker 1927; Battigelli and Perini 1955; Borovska et al. 1976; Gummert 1961; Hansen et al. 1953; Kegel et al. 1929; Mackie 1961; McNally 1946; Jones 1942; Raalte and van Velzen 1945; Spevak et al. 1976; Verriere and Vachez 1949). These case reports also describe neurological effects; therefore, the cardiovascular and gastrointestinal effects may be secondary to the neurotoxic effects of chloromethane. Exposure concentrations were probably very high, perhaps >30,000 ppm, because the leaks occurred in rooms with little or no ventilation.

Rafnsson and Gudmundsson (1997) report a clear excess mortality from cardiovascular disease (Mantel-Haenszel point estimate=2.1, 95%; CI=1.2-3.8) in crew members (males) exposed for 2 days to chloromethane that leaked from a refrigerator on board an Icelandic fishing trawler (no estimates of exposure levels were reported). This excess was more prominent among deckhands who had received the highest exposure to chloromethane. The Risk ratios were elevated for all causes of death (RR=2.5, 95%; CI=l.0-5.7) as well as for cardiovascular disease (RR=3.9, 95%; CI=l.0-14.4). The study is weakened by an assumption of comparable lifestyle factors (including smoking habits and diet) between the cohort and the

reference group and by the relatively small size of the exposed cohort. The authors also do not discuss the potential influence of the documented neurological deficits in this cohort (Gudmundsson 1977) on cardiovascular function. The authors suggest, however, that additional study on chloromethane's potential cardiovascular toxicity is warranted.

Increased heart rate and blood pressure followed by decreased heart rate and blood pressure, possibly due to vasodilation resulting from depression of the central nervous system, occurred in dogs exposed by inhalation to high concentrations of chloromethane (15,000 and 40,000 ppm) (von Oettingen et al. 1949, 1950). The dogs died within 4-6 hours. Cardiovascular effects have not been described in other species after acute, intermediate, or chronic exposure by inhalation.

Gastrointestinal Effects. Numerous case reports of humans exposed to chloromethane have described symptoms of nausea and vomiting (Baird 1954; Baker 1927; Battigelli and Perini 1955; Borovska et al. 1976; Hansen et al. 1953; Kegel et al. 1929; Mackie 1961; Jones 1942; Raalte and van Velzen 1945; Spevak et al. 1976; Verriere and Vachez 1949). In all cases, these symptoms were accompanied by central nervous system toxicity, which was usually severe. It is not clear, therefore, if the nausea and vomiting were secondary to the neurotoxic effects of chloromethane.

Histopathological examination of animals exposed to various concentrations of chloromethane for acute, intermediate, or chronic durations did not show evidence of gastrointestinal damage (CIIT 1981; McKenna et al. 1981a, 1981b).

Hematological Effects. No hematological effects were found in volunteers who participated in a study of neurological and neurobehavioral effects of acute inhalation exposure of up to 150 ppm chloromethane (Stewart et al. 1980). Case reports of human overexposure have also generally been negative for hematological effects.

No long-term effect on the hematological system from an acute exposure was reported by Gudmundsson (1977). Seventeen crew members (males) were exposed for 2 days in 1963 to chloromethane that leaked from a refrigerator under the crew sleeping quarters on board an Icelandic fishing trawler (no estimates of exposure levels were reported). Thirteen years later (i.e., in 1976) 10 of the 11 survivors were examined. All 10 were employed; 8 were employed at sea. The mean age of the 10 survivors examined was 38.3 years

(range 30-50 years). All 10 survivors had normal hemoglobin, white cell count, differential leukocyte count, erthrocyte sedimentation rate, and serum creatinine.

No studies were located regarding the hematological effects of chloromethane in humans following oral or dermal exposures.

The only hematological effects described in animals were spleen enlargement, suggestive of extramedullary hematopoiesis, and hemoglobinuria, suggestive of intravascular hemolysis, in mice exposed acutely to chloromethane by inhalation (Landry et al. 1985). It is not clear if similar hematological effects would occur in humans.

Musculoskeletd Effects. No studies were located regarding the musculoskeletal effects of chloromethane in humans or animals following inhalation, oral, or dermal exposures.

Hepatic Effects. Case reports of humans exposed to chloromethane vapors have described clinical jaundice and cirrhosis of the liver (Kegel et al. 1929; Mackie 1961; Weinstein 1937; Wood 1951), but exposure concentrations were not known.

Hepatic effects have also been observed in animals exposed by inhalation to chloromethane at concentrations > 1,000 ppm in acute, intermediate, and chronic duration experiments (Burek et al. 1981; Chellman et al. 1986a; CIIT 1981; Landry et al. 198.5; Mitchell et al. 1979; Morgan et al. 1982). Milder liver effects occurred in mice exposed acutely to an intermittent but relatively high concentration than to a low but continuous concentration (Landry et al. 1985). The greater susceptibility to continuous exposure may result from relatively greater metabolism to a toxic intermediate or from diurnal susceptibility. Hepatic effects were more severe in mice (necrosis and degeneration) than in rats (cloudy swelling, fatty infiltration, increased ALT and AST with no necrosis). Furthermore, no hepatic lesions were observed in rats over the course of 2 years of inhalation exposure to 1,000 ppm, while mice similarly exposed had necrotic lesions after 6 months (CIIT 1981). The greater susceptibility of mice to the hepatotoxic effects of chloromethane may be related to the greater ability of chloromethane to conjugate with hepatic glutathione in mice than in rats (Dodd et al. 1982; Kornbrust and Bus 1984). The reaction of chloromethane with glutathione appears to be a toxifying ratherthan a detoxication mechanism (Chellman et al. 1986b). While the exact mechanism for the hepatotoxic effects of chloromethane is unclear, chloromethane can elicit lipid peroxidation as a secondary consequence of depletion of glutathione (Kornbrust and Bus 1984). Comparison of lipid

peroxidation in the S-9 fraction from mouse and rat livers revealed much greater lipid peroxidation in mouse liver than in rat liver. The finding that mice exposed to 2,500 ppm chloromethane expired ethane to an extent comparable to that produced by 2 mL/kg carbon tetrachloride, and developed moderate to severe hepatocellular hydropic degeneration provide further evidence that the mechanism of hepatotoxicity may involve lipid peroxidation.

Endocrine Effects. No studies were located regarding the endocrine effects of chloromethane in humans following inhalation, oral, or dermal exposures.

Only one animal study reported fatty droplets in the epithelial cells of the zona fasciculata in the adrenals of Fischer 344 rats acutely exposed to 3,500 and 5,000 ppm chloromethane; the severity of the lesion increasing with dose (Morgan et al. 1982). Rats were exposed for 5 days, 6 hours/day with a break in exposure for 2 days, and then a further 4 days of exposure.

Renal Effects. Indicators of renal toxicity, such as albuminuria, increased serum creatinine and blood urea nitrogen, proteinuria, and anuria have been described in case reports of humans exposed to high levels of chloromethane vapors due to refrigerator leaks (Kegel et al. 1929; Mackie 1961; Spevak et al. 1976; Verriere and Vachez 1949).

Effects on the kidney have also been observed in animals exposed by inhalation for acute, intermediate, and chronic durations. In acute studies, rats developed more severe effects (evidence of renal failure) when 1,000 ppm chloromethane was administered continuously (Burek et al. 1981) than when a 2-fold higher concentration was administered intermittently (degeneration and necrosis of convoluted tubules) (Chellman et al. 1986a; Morgan et al. 1982). The greater susceptibility of mice to continuous exposure than to intermittent exposure for lethal and hepatotoxic effects (Landry et al. 1985), however, did not hold true for renal toxicity. Only the mice exposed intermittently to the highest concentration had degenerative and regenerative changes in the tubules. No explanation for this apparent contradiction was offered. Degeneration and regeneration of renal tubules were also found in other acute duration studies in mice (Jiang et al. 1985; Morgan et al. 1982), and hyperplasia and kidney tumors were found after 12 months of exposure and later in a 2-year study (CIIT 1981). The biological significance of the proliferative kidney lesions in mice is discussed more fully in the subsection on Cancer below.

The possible relationship between the degenerative effects in the kidneys of mice and granular layer lesions in the brain, which are also observed in mice, was discussed by Jiang et al. (1985). People who die of renal insufficiency (not due to chloromethane exposure) often have granular cell necrosis. Since the brain and kidney lesions in mice in this study were unrelated in severity, however, the brain lesions were probably not a direct consequence of chloromethane-induced kidney lesions. Although chloromethane depleted glutathione in the kidney, comparison of lipid peroxidation in the S-9 fractions revealed much less lipid peroxidation in kidney than in liver, suggesting that the mechanism for renal toxicity may not involve glutathione-related peroxidase activity (Kornbrust and Bus 1984).

Because some refrigerators more than 30 years old are still in use, leaks of chloromethane vapor at concentrations high enough to produce hepatic effects, renal effects, and neurotoxicity with consequent cardiovascular and gastrointestinal effects in humans are possible. It is not known whether exposure of humans to chloromethane outside or at hazardous waste sites could result in hepatic and renal effects.

Dermal Effects. No studies were located regarding the dermal effects of chloromethane in humans or animals following inhalation, oral, or dermal exposures.

Ocular Effects. No studies were located regarding the dermal effects of chloromethane in humans following inhalation, oral, or dermal exposures.

Ophthalmological examination of male cats and dogs exposed to 500 ppm continuously for 3 days (McKenna et al. 1981a), dogs exposed to 400 ppm for 90 days (McKenna et al. 1981b), or of rats and mice exposed to 1,000 ppm for up to 24 months (CIIT 1981) failed to reveal eye lesions. Mucopurulent conjunctivitis with total destruction of the eye in some cases was found in mice exposed to ≥375 ppm for 90 days (Mitchell et al. 1979). These lesions were attributed to exposure because no lesions were found in controls; however, the failure of longer-term studies to detect eye lesions at higher concentrations makes the findings of Mitchell et al. (1979) questionable. The effect was probably due to direct contact of the chloromethane vapor with the eye, rather than a consequence of inhalation.

Body Weight Effects. No studies were located regarding the body weight effects of chloromethane in humans or animals following inhalation, oral, or dermal exposure to chloromethane.

Metabolic Effects. No studies were located regarding the metabolic effects of chloromethane in humans or animals following inhalation, oral, or dermal exposures.

Immunological and Lymphoreticular Effects. No studies were located regarding immunological and/or lymphoreticular effects in humans after inhalation exposure to chloromethane.

The only effects that could possibly be considered immunological were lymphoid depletion of the spleen and splenic atrophy observed in mice exposed by inhalation for up to 2 years (CIIT 1981). Since more sensitive tests for immune function were not conducted, the biological significance of the splenic effects cannot be assessed. Furthermore, splenic alterations were not observed in rats in the same study. In another study, cats exposed continuously to chloromethane for 3 days had higher incidences of brain lesions than the control (McKenna et al. 1981a). The lesions were consistent with infection or post-vaccinal reaction (the cats were vaccinated for panleukopenia by the supplier). Exacerbation of viral-induced central nervous system disease, however, could not be ruled out. It is not known whether the exacerbation would represent an immunological effect.

Neurological Effects. Neurological effects have been described in numerous case reports of humans exposed to chloromethane vapors as a result of industrial leaks and leaks from defective home refrigerators (Baird 1954; Gudmundsson 1977; Hansen et al. 1953; Hartman et al. 1955; Kegel et al. 1929; MacDonald 1964; McNally 1946; Jones 1942; Raalte and van Velzen 1945; Spevak et al. 1976; Wood 1951). Depending on the extent of exposure and the availability of medical treatment, the signs and symptoms can range from staggering and blurred vision to coma, convulsions, and death. Such effects as abnormal gait, tremors, and personality changes may persist for several months or years (Gudmundsson 1977), but complete recovery may eventually occur. In cases in which exposure was quantitated, concentrations were generally >29,000 ppm (Battigelli and Perini 1955; Jones 1942). Symptoms of blurred vision, fatigue, vertigo, nausea, vomiting, tremor, and unsteadiness, however, developed in a man and a woman a few days after they stored insulated boards containing polystyrene foam in the basement of their house (Lanham 1982). The concentration of chloromethane in the house was found to be in excess of 200 ppm (exact levels not reported). It should be noted, however, that this exposure probably represented an unusual situation because the rate of air turnover in the couple's home was an order of magnitude lower than the typical rate. In addition, a small statistically nonsignificant decrement in performance in behavioral tests was found in volunteers exposed to 200 ppm (Putz-Anderson et al. 1981a).

Severe neurological signs (ataxia, tremors, limb paralysis, incoordination, convulsions) have been observed in rats, mice, rabbits, guinea pigs, dogs, cats, and monkeys exposed acutely by inhalation to high concentrations of chloromethane (Burek et al. 1981; Chellman et al. 1986a, 1986b; Landry et al. 1985; McKenna et al. 1981a; Morgan et al. 1982; Smith and von Oettingen 1947b). Signs of neurotoxicity developed after 6 and 12 months, and degeneration of the granular cell layer of the cerebellum was observed after 18 months in mice exposed by inhalation for 2 years (CIIT 1981). Cerebellar lesions have also been observed microscopically in guinea pigs and rats (Kolkmann and Volk 1975; Morgan et al. 1982). Mice were more susceptible than rats (CIIT 1981; Morgan et al. 1982), and dogs were more susceptible than cats to the neurological effects of chloromethane (McKenna et al. 1981a). Mice were more sensitive to neurological effects after continuous exposure to low concentrations than after intermittent exposure to higher concentrations of chloromethane (Landry et al. 1985). The greater sensitivity of mice to continuous exposure may be a consequence of metabolism of chloromethane to a toxic intermediate or diurnal susceptibility.

The mechanism by which chloromethane produces neurological effects is unclear. Pretreatment of mice with BSO to deplete glutathione protected mice from cerebellar damage due to inhalation exposure to chloromethane (Chellman et al. 1986b), suggesting that the reaction of chloromethane with glutathione to form S-methylglutathione is required for the degenerative changes in the brain to occur. In the metabolic scheme proposed by Kornbrust and Bus (1983), subsequent metabolism of S-methylglutathione produces methanethiol as an intermediate. Methanethiol produces signs and symptoms of neurotoxicity (tremors, convulsions, coma) similar to those seen in animals or humans acutely exposed to chloromethane (Chellman et al. 1986b). The possibility of a relationship between degenerative effects in mice was discussed by Jiang et al. (1985). Granular cell necrosis is often seen in people who die of renal insufficiency (not due to chloromethane exposure). Since the brain and kidney lesions in mice in this study were unrelated in severity, however, Jiang et al. (1985) concluded that the brain lesions were probably not a direct consequence of chloromethane-induced kidney lesions.

Because refrigerators more than 30 years old are still in use, leaks of chloromethane vapor at concentrations high enough to produce neurological effects in humans are possible. These exposures have generally occurred in rooms with poor ventilation. It is not known whether exposure of humans to chloromethane in the outside environment or at hazardous waste sites could result in neurological effects.

Reproductive Effects. No studies were located regarding reproductive effects in humans exposed to chloromethane by any route.

Acute-, intermediate-, and chronic-duration inhalation exposures of male rats to chloromethane have resulted in such reproductive effects as inflammation of the epididymis and 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, 1986b, 1987; CIIT 1981; Han-m et al. 1985; Morgan et al. 1982; Working and Bus 1986; Working et al. 1985a, 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 due to cytotoxicity of chloromethane to sperm in the testes at the time of exposure, rather than to a genotoxic effect on the sperm (Chellman et al. 1986a, 1986c, 1987; Working and Bus 1986; Working et al. 1985a, 1985b).

Although testicular effects were observed in mice in the CIIT (1981) study, the incidence was much lower and occurred much later in mice than it did in rats. The mechanism for testicular and epididymal effects has been studied only in rats. It is not known whether chloromethane could produce reproductive effects in humans.

Developmental Effects. No studies were located regarding developmental effects in humans exposed to chloromethane by any route.

Maternal toxicity, evidenced by decreased body weight gain and retarded development of 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 of metatarsals and phalanges of the anterior limbs, thoracic centra in the pubis of the pelvic girdle, and metatarsals of the hindlimbs. These researchers also reported increased incidences of heart malformations in the fetuses of mouse dams exposed by inhalation to 500 ppm chloromethane during Gd 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 Gd 11.5-12.5, 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. (1983) 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(l 985) 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.

The investigators also found increased incidences of heart malformations in the fetuses of mouse dams exposed by inhalation to 500 ppm chloromethane during Gd 6-17. Heart malformations, however, were not found in fetuses of mouse dams exposed to higher concentrations of chloromethane during Gd 11.5-12.5, which they considered to be the critical period for development of the embryonal heart (John-Greene et al. 1985). According to Wolkowski-Tyl(1985), however, the critical period of embryonal heart development 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.

Genotoxic Effects. Chloromethane has been tested for genotoxicity in a number of *in vitro* and *in vivo* systems (Tables 2-4 and 2-5). Chloromethane gave positive results for gene mutation, sister chromatid exchange, and transformation in cultured mammalian cells, including human lymphoblast cells (Fostel et al. 1985; Hatch et al. 1982, 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.

Although chloromethane was positive for unscheduled DNA synthesis in rat hepatocytes, spermatocytes, and tracheal epithelial cells *in vitro,* a marginally positive response was found only in hepatocytes of rats exposed to chloromethane *in vivo,* and only at very high concentrations (Working et al. 1986). Chloromethane exposure consistently produced dominant lethal mutations in the sperm of rats, as measured by postimplantation loss in females mated to the exposed males (Chellman et al. 1986c; Rushbrook 1984; Working et al. 1985a). Since concurrent exposure of male rats to chloromethane and BW755C, an anti-inflammatory agent, did not result in postimplantation loss, it was suggested that the dominant lethal

Table 2-4. Genotoxicity of Chloromethane *In Vivo*

Species (test system)	End point	Results	Reference
Rat (inhalation)	Dominant lethal	+	Working et al. 1985a
Rat (inhalation)	Dominant lethal	+	Chellman et al. 1986c
Rat (inhalation)	Dominant lethal	+	Rushbrook 1984
Rat (inhalation)			
hepatocytes	Unscheduled DNA synthesis	(+)	Working et al. 1986
spermatocytes	Unscheduled DNA synthesis	–	Working et al. 1986
tracheal epithelial cells	Unscheduled DNA synthesis	(+/–)	Working et al. 1986

– = negative results; + = positive results; (+) = marginally positive result; (+/–) = equivocal results.

Table 2-5. Genotoxicity of Chloromethane *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium (desiccator test for exposure to gases)	Gene mutation	+	+	Simmon et al. 1977
S. typhimurium TA1535 (gas exposure)	Gene mutation	+	+	Andrews et al. 1976
S. typhimurium (gas exposure)	Gene mutation			DuPont 1977
TA1535		+	+	
TA100		+	+	
TA1537		–	–	
TA18		–	–	
S. typhimurium TA677 (gas exposure)	Gene mutation	ND	+	Fostel et al. 1985
Mammalian cells:				
Human lymphoblasts	Gene muation	ND	+	Fostel et al. 1985
Human lymphoblasts	Sister-chromatid exchange	ND	+	Fostel et al. 1985
Human lymphoblasts	DNA strand breaks	ND	–	Fostel et al. 1985
Rat hepatocytes	Unscheduled DNA synthesis	NA	+	Working et al. 1986
Rat spermatocytes	Unscheduled DNA synthesis	ND	+	Working et al. 1986
Rat tracheal epithelial cells	Unscheduled DNA synthesis	ND	+	Working et al. 1986
Primary hamster embryo cells	DNA viral transformation	ND	+	Hatch et al. 1982, 1983

+ = positive result; – = negative result; NA = not applicable; ND = no data

mutation was probably due to chloromethane-induced epididymal inflammation, possibly by production by inflammatory cells of a superoxide capable of damaging DNA, rather than by a genotoxic effect of chloromethane itself (Chellman et al. 1986c). Since studies using ^{14}C-chloromethane indicated that the carbon atom from chloromethane becomes incorporated into normal macromolecules via the one-carbon pool rather than binding to macromolecules as an alkylating agent (Kornbrust et al. 1982; Peter et al. 1985), and since the dominant lethal effect may be secondary to inflammation, it is possible that *in vivo* genotoxicity and carcinogenicity (see Section 2.2.1.8) may be secondary to other toxic effects of chloromethane. Nevertheless, the *in vitro* studies demonstrate the direct genotoxicity of chloromethane.

Positive results have generally been found in the reverse mutation assay *in Salmonella typhimurium* with and without metabolic activation (Andrews et al. 1976; DuPont 1977; Simmon et al. 1977). In addition, a positive result was obtained in *S. typhimurium* for 8-azaguanine resistance (Fostel et al. 1985).

Cancer. The information regarding carcinogenicity in humans after exposure to chloromethane is limited. An epidemiology study on a cohort of 24 Icelandic fishermen reported a slight increase in excess mortality from all cancers, and more specifically, lung cancer (Rafnsson and Gudmundsson 1997). The study was conducted 32 years after an acute (i.e., 2 days) high level exposure to chloromethane from a leaking refrigerator. Confounding factors for lifestyle and smoking were not explicitly controlled in this study, but assumed to be similar based on controls for age, social class, and occupation. One epidemiology study of butyl rubber workers chronically exposed to chloromethane reported no statistically significant increase in the rate of death due to cancer (Holmes et al. 1986).

Chloromethane has been tested for carcinogenicity in animals only by the inhalation route. No evidence of a carcinogenic effect was found in rats or in female mice (CIIT 1981). In a 2-year inhalation study, a statistically significant increased incidence of kidney tumors developed in 1,000 ppm-exposed B6C3F$_1$ male mice. Renal hyperplasia was also observed after 12 months of exposure. In an acute study, Chellman et al. (1986b) found significant increases in cell proliferation in the kidneys of male B6C3F$_1$ mice, as measured by incorporation of tritiated thymidine into DNA of the kidneys. Such proliferation may be involved in the development of kidney tumors, a hypothesis supported by the evidence that chloromethane is probably not an alkylating agent, but acts by an epigenetic mechanism (Korubrust et al. 1982; Peter et al. 1985). Female B6C3F$_1$ mice exposed to 1,500 ppm chloromethane also had increased cell proliferation in the kidney (Chellman et al. 1986b), but did not develop kidney tumors in the CIIT (1981) study; however, the exposure concentrations in the CIIT (1981) study were lower than those in the study by Chellman et al. (1986b). In

addition, greater evidence of regeneration of renal tubular cells, presumably in response to cell death, was found in B6C3F$_1$ males than in females of the same strain exposed to 500 and 1,000 ppm chloromethane for 12 days (Morgan et al. 1982). In mice exposed to 2,000 ppm, however, there was no sex difference. It is possible, therefore, that at relatively low concentrations, female mice are less sensitive than male mice to the renal toxicity of chloromethane.

Since data that chloromethane exposure was associated with tumors were found in only one sex of one species in only one study, the evidence that chloromethane is a carcinogen is limited. It is not known whether cancer could develop in humans exposed to chloromethane by any route.

2.6 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate due to maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 5.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both pre-natal and post-natal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of

their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns and at various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults and sometimes unique enzymes may exist at particular developmental stages (Komori 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in the newborn who has a low glomerular filtration rate and has not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

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-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 (Chellman et al. 1986b; Jager et al. 1988; Kornbrust and Bus 1983, 1984; Nolan et al. 1985; Stewart et al. 1980; Warholm et al. 1995). It is anticipated that children would have a polymorphism similar to the adult population, although no specific data have been collected to test this hypothesis. If a polymorphism is present in children, then some children (i.e., those with higher levels of glutathione-S-transferase) would be more susceptible to the toxic effects of chloromethane.

Certain characteristics of the developing human may increase exposure or susceptibility while others may decrease susceptibility to the same chemical. For example, the fact that infants breathe more air per kilogram of body weight than adults may be somewhat counterbalanced by their alveoli being less developed, so there is a disproportionately smaller surface area for absorption (NRC 1993).

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. It is not known whether chloromethane affects the developing fetus or the development of young children.

There have also been no studies where young animals were exposed to chloromethane. With mid- to high levels of chloromethane administered to female adult rats and mice during pregnancy, the offspring were smaller than normal, with underdeveloped bones, and possibly abnormal hearts (although this latter effect remains uncertain and occoured only in mice).

It is not known whether chloromethane or methanethiol in the body can cross the placenta and enter into the developing young, or if either compound can enter into breast milk. We do know that chloromethane is broken down and eliminated from the body very quickly in adults (Nolan et al. 1985) and animals (Landry et al. 1983a; von Oettingen et al. 1949, 1950). Thus, it is unlikely that chloromethane would be stored in maternal tissues or be mobilized (i.e., released from stores) during pregnancy or lactation.

In adults, there appear to be two distinct populations with regard to metabolism and elimination of chloromethane. One population appears to have higher amounts of the metabolizing enzyme, glutathione-S-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 (Chelhnan et al. 1986b; Jager et al. 1988; Kornbrust and Bus 1983, 1984; Nolan et al. 1985; Stewart et al. 1980; Warholm et al. 1995). It is anticipated that children would have a polymorphism similar to the adult population, although no specific data have been collected to test this hypothesis. If a polymorphism is present in children, then some children (i.e., those with higher levels of glutathione-S-transferase) would be more susceptible to the toxic effects of chloromethane.

Although the breakdown and elimination of chloromethane is expected to be the same in children as in adults, 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 are no PBPK models for children, adults, or test animal models. There are no good biomarkers of exposure for children (or adults), although clinical symptoms of drunkenness or food poisoning, and a 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.

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 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 of metatarsals and phalanges of the anterior limbs, thoracic centra in the pubis of the pelvic girdle, and metatarsals of the hindlimbs.

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 Gd 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 Gd 11 S-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. (1983) 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 epididymis and 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, 1986b, 1987; CIIT 1981; Hamm et al. 1985; Morgan et al. 1982; Working and Bus 1986; Working et al. 1985a, 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 due to cytotoxicity of chloromethane to sperm in the testes at the time of exposure, rather than to a genotoxic effect on the sperm (Chellman et al. 1986a, 1986c, 1987; Working and Bus 1986; Working et al. 1985a, 1985b).

Chloromethane exposure consistently produced dominant lethal mutations in the sperm of rats, as measured by postimplantation 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 postimplantation 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 postimplantation loss, the dominant lethal mutations probably resulted secondary to the epididymal inflammatory response (Chellman et al. 1986c; Working and Chellman 1989). 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. 1979, 1981; Working et al. 1985a).

Chloromethane has been tested for genotoxicity in a number of *in vitro* and *in vivo* systems (see Tables 2-4 and 2-5). Chloromethane gave positive results for gene mutation, sister chromatid exchange, and transformation in cultured mammalian cells, including human lymphoblast cells (Fostel et al. 1985; Hatch et al. 1982, 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.

2.7 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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 or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic

compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to chloromethane are discussed in Section 2.7.1.

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 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 effects caused by chloromethane are discussed in Section 2.7.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organisms 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 2.9, Populations That Are Unusually Susceptible.

2.7.1 Biomarkers Used to Identify or Quantify Exposure to Chloromethane

Several 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 g-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) concluded that measurement of S-methylcysteine in urine is not a valid method for monitoring exposure to chloromethane.

In an evaluation of the use of blood and breath analysis of chloromethane to monitor exposure in volunteers exposed to up to 150 ppm chloromethane, breath levels immediately after exposure to 20 or 100 ppm correlated with exposure, but subsequent samples were difficult to interpret (Stewart et al. 1980). Exposure to 100 ppm could not be distinguished from exposure to 150 ppm. The excretion patterns following

prolonged exposure will differ from those observed in these experiments (Morgan et al. 1970), which followed single breath exposure (see Section 2.3.4.1); therefore, the data are not useful for monitoring occupational exposure. This conclusion probably applies to prolonged environmental exposure as well. Symptoms resembling drunkenness and food poisoning, along with a sweet odor of the breath, may alert physicians that a person has been exposed to chloromethane.

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

2.7.2 Biomarkers Used to Characterize Effects Caused by Chloromethane

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. 1977). Although volunteers exposed to 200 ppm chloromethane for 3 hours had a 4% decrement in their performance on behavioral tests, blood and alveolar air levels of chloromethane were too variable to be of practical use (Putz-Anderson et al. 1981a). The decrement in performance was also small and not statistically significant.

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990).

2.8 INTERACTIONS WITH OTHER CHEMICALS

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). Since both of these compounds are known to be central nervous system depressants, workers who are exposed to

chloromethane in industry or during cleanup of hazardous waste sites, or people who live near hazardous waste sites where chloromethane is present and are treated with diazepam or exposed to other central nervous system depressants, including alcohol, may have aggravated symptoms.

Minami et al. (1992) report on 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 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 acetycholinesterase activity.

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 administered to rats or mice exposed to chloromethane by inhalation to study the mechanism of chloromethane-induced toxicity (Chellman et al. 1986a, 1986b). These studies are discussed in Section 2.2. It is unlikely that these compounds would be found with chloromethane at hazardous waste sites.

2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to chloromethane than will most persons exposed to the same level of chloromethane in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters may result in reduced detoxification or excretion of chloromethane, or compromised function of target organs affected by chloromethane. Populations who are at greater risk due to their unusually high exposure to chloromethane are discussed in Section 5.7, Populations With Potentially High Exposure.

In general, people who have kidney or liver disease, anemia, or neurological deficits may be more susceptible to the toxic effects of chloromethane.

Two distinct populations of humans with differences in elimination of chloromethane have been identified. Some of the volunteers exposed by inhalation to chloromethane had distinctly higher chloromethane concentrations in alveolar breath samples than others (Stewart et al. 1980). In humans exposed to chloromethane by inhalation, the chloromethane was eliminated from the blood and expired air more slowly by the subjects who had higher venous blood and expired air concentrations than by those who had lower concentrations (Nolan et al. 1985). This finding was believed to be due to differences in metabolic rate. In six workers exposed to chloromethane occupationally, the excretion of S-methylcysteine showed wide variations, and there was little or no correlation between exposure levels and excretion (van Doom et al. 1980). In four of the workers, all concentrations of S-methylcysteine were higher than in controls, and appeared to increase during the course of the week. The other two workers had only small amounts of S-methylcysteine in the urine, but these workers had experienced the highest exposure concentrations. These results support the hypothesis that there are two distinct populations: fast eliminators, with lower body burdens and higher excretion; and slow eliminators, with higher body burdens and lower excretion. Because chloromethane is eliminated relatively rapidly, the observation of two distinct populations may have no toxicological significance (Nolan et al. 1985). Based on studies in mice, the reaction of chloromethane with glutathione, however, may lead to the formation of toxic compounds in humans that exert their action before they are eliminated. If slow eliminators have a deficiency of glutathione- S-transferase, the enzyme that catalyzes the conjugation of glutathione with chloromethane, or low levels of glutathione, they would be expected to be less susceptible to the toxic effects of chloromethane. The extent to which chloromethane reacts with glutathione in humans, however, is not known.

As discussed in Section 2.8, workers treated with diazepam and exposed to chloromethane had an additive impairment in performing behavioral tests (Putz-Anderson et al. 1981a). These results imply that people who are occupationally exposed to chloromethane and treated with diazepam, or perhaps other drugs that depress the central nervous system, may have aggravated symptoms.

2.10 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to chloromethane. However, because some of the treatments discussed may be experimental and

unproven, this section should not be used as a guide for treatment of exposures to chloromethane. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to chloromethane:

Goldfrank LR, Flomenbaum NE, Lewin NA, et al. 1994. *Goldfrank's Toxicologic Emergencies.* Fifth edition. Norwalk. CT: Appleton & Lange, 1231-1244.

Ellenhorn MJ, Barceloux DG. 1988. *Medical Toxicology: Diagnosis and Treatment of Human Poisoning.* New York, NY. Elsevier, 982-983.

ATSDR. 1994. Agency for Toxic Substances and Disease Registry. *Medical Management Guidelines for Acute Chemical Exposures: Formaldehyde.* Atlanta, GA.

2.10.1 Reducing Peak Absorption Following Exposure

Acute inhalation exposure to high levels of chloromethane primarily causes neurological effects with signs and symptoms that can range from staggering and blurred vision to coma, convulsions, and death. Such effects as abnormal gait, tremors, and personality changes may persist for several months or more, but complete recovery may also occur eventually. Because chloromethane is so rapidly absorbed, metabolized, and distributed; treatment to reduce absorption would have to be administered promptly. No treatments, however, were located in the literature except the general indication of supportive treatment. This usually consists of ensuring open airways, adequate supply of fresh air, and establishing and monitoring proper cardiovascular function.

2.10.2 Reducing Body Burden

No information was located on reducing body burdens of absorbed chloromethane.

2.10.3 Interfering with the Mechanism of Action for Toxic Effects

The mechanism(s) of chloromethane toxicity remains unclear, and thus it is uncertain whether depletion or protection of glutathione pools would be appropriate for any given exposure or target organ.

Methanethiol and formaldehyde formation, and increased lipid peroxidation due to glutathione depletion have been suggested as the toxic intermediates and mechanism responsible for the toxicity of chloromethane (Dekant et al. 1995; Jager et al. 1988; Kombrust and Bus 1983, 1984; Ristau et al. 1989, 1990).

Dodd et al. (1982) also proposed possible mechanisms for the toxicity of chloromethane related to glutathione depletion including enhancement of the toxicity of chemicals that are detoxified via conjugation with GSH; prevention of GSH from acting as a cellular reducing agent, thereby interfering with a variety of physiological functions; or an increase in chloromethane-glutathione conjugates that are then further metabolized to putative toxic metabolite (e.g., formaldehyde or methanethiol).

Chellman et al. (1986b), however, concluded that the depletion of GSH protected mice from cerebellar damage due to exposure to chloromethane. The mechanism may involve conjugation of chloromethane with glutathione in the liver, followed by biliary excretion and enterohepatic circulation of the glutathione conjugate or possibly a cysteine conjugate and further metabolism by kidney and/or gut flora beta-lyase to methanethiol. Methanethiol produces similar central nervous system symptoms (tremors, convulsion, coma) as seen in animals or humans acutely intoxicated with chloromethane (Chellman et al. 1986b).

There is only a limited amount of information available from animal studies on interfering with putative mechanism of chloromethane-induced toxicity. Interference with specific toxic events has been demonstrated for BW755C, an anti-inflammatory agent, and for BSO, a depleter of glutathione, when administered to rats or mice that have been exposed to chloromethane by inhalation (Chellman et al. 1986a, 1986b). BW755C protected rats from chloromethane-induced epididymal or testicular lesions, but did not alter chloromethane metabolism, tissue distribution, or excretion of ^{14}C-chloromethane, or decrease hepatic glutathione content. An alternate mechanism for BW755C's protective effects against testicular damage could be an inhibition of leukotriene and prostaglandin synthesis.

2.11 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of chloromethane is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of chloromethane.

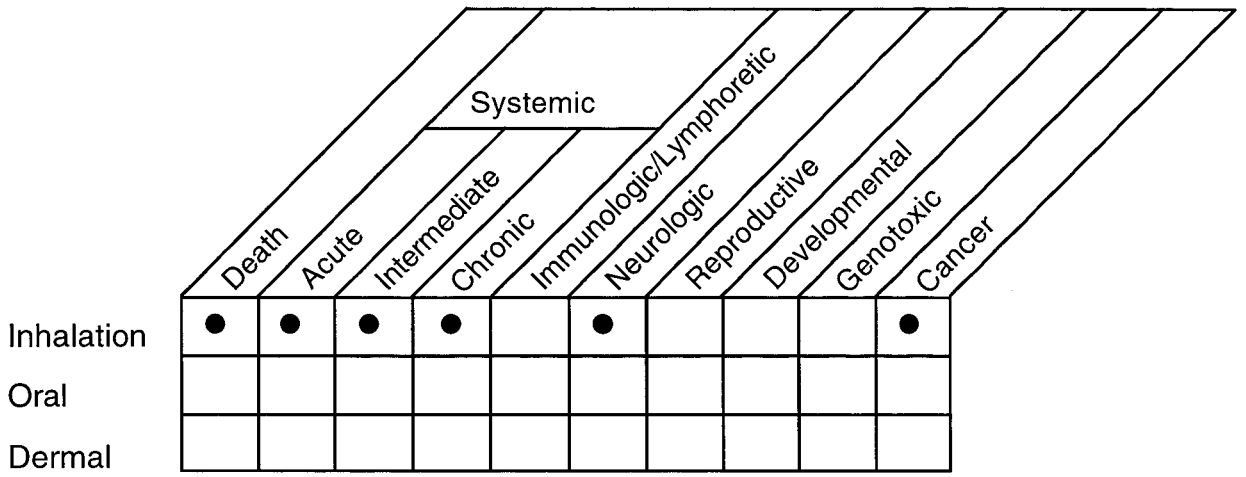
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.11.1 Existing Information on Health Effects of Chloromethane

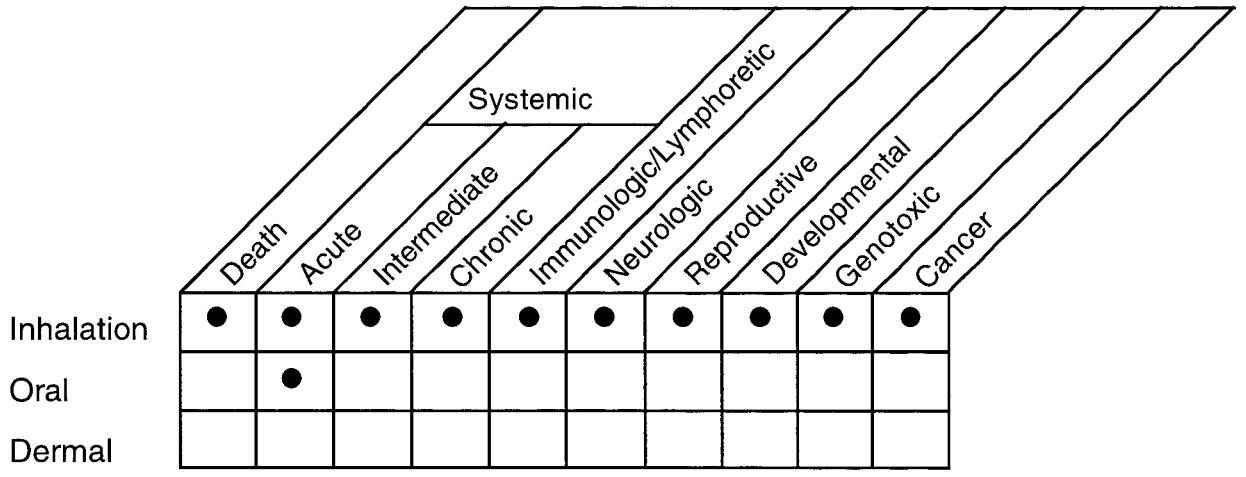
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to chloromethane are summarized in Figure 2-5. The purpose of this figure is to illustrate the existing information concerning the health effects of chloromethane. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As shown in Figure 2-5, information on the health effects in humans exposed to chloromethane is available only for inhalation or occupational exposures. Accidental leaks of chloromethane from refrigeration units or from occupational sources involves dermal as well as inhalation exposure; however, the primary exposure route during an accidental spill or leak is inhalation exposure. The organs or systems adversely affected in humans after exposure to chloromethane include the liver, kidney, neurological system (including behavioral alterations), and the cardiovascular and gastrointestinal systems (possibly secondary to the neurological effects). Death may occur at sufficiently high doses. Information on the adverse health effects of chloromethane has been presented for occupational exposures of acute, intermediate, and chronic duration. One epidemiological study found no association between exposure to chloromethane and cancer at any site. One epidemiological study found a slight excess of mortality from all cancers, and more specifically, from lung cancers, 32 years following an acute high level exposure to inhaled chloromethane. No information was available regarding immunological, developmental, reproductive, or genotoxic effects in humans exposed to chloromethane by any route.

Figure 2-5. Existing Information on Health Effects of Chloromethane



Human



Animal

● Existing Studies

There have been no studies to determine if children are more or less susceptible than adults to adverse health effects from a given amount or duration of exposure to chloromethane, or if chloromethane affects the developing fetus or the development of young children. There is no information on the potential movement of chloromethane or its metabolites across the placenta and into the developing young. We also do not know if chloromethane or its metabolites can migrate into breast milk.

A number of studies have evaluated the health effects of chloromethane exposure in animals for the inhalation route, although only a single comprehensive chronic study in rats and mice has been performed. Health effects of acute, intermediate, and chronic inhalation exposure in animals include increased mortality, liver damage, kidney damage and tumors, neurological damage; and adverse reproductive, genotoxic and possibly developmental effects. In the only oral study in animals, an attempt was made to compare the hepatotoxicity of chloromethane with that of carbon tetrachloride and chloroform. The administered dose of chloromethane, however, was too low to produce hepatic effects, and the use of a higher dose was precluded due to neurotoxicity.

2.11.2 Identification of Data Needs

Chloromethane is highly volatile, and chloromethane in water or soil will likely evaporate to the air (Chapter 5). Given the volatility of chloromethane, inhalation exposures and toxicity are of primary concern and have been the most studied. The oral and dermal routes of exposure are also of concern because chloromethane is ubiquitous in the environment; yet, with the exception of a single-dose oral study (Reynolds and Yee 1967) and ocular effects from a presumptive dermal exposure in whole-body inhalation chambers (CIIT 1981; McKenna et al. 1981a, 1981b; Mitchell et al. 1979), no information was located regarding the health effects of chloromethane in humans or animals after oral or dermal exposure. It is not possible to predict whether effects following oral or dermal exposure to chloromethane would be similar to those following inhalation exposure, partially because the pharmacokinetic disposition of chloromethane has not been compared for the three routes of exposure. Differences in absorption, distribution, and metabolic pathways could lead to differences in toxic response and different target organs following the three routes of exposure. Therefore, additional studies using oral and dermal routes of exposure are also needed.

Acute-Duration Exposure. Case reports of humans exposed acutely to high concentrations of chloromethane have described severe neurological effects, sometimes followed by death (Baird 1954; Battigelli and Perini 1955; Borovska et al. 1976; Gudmundsson 1977; Jones 1942; Kegel et al. 1929;

Lanham 1982; McNally 1946; Spevak et al. 1976; Thordarson et al. 1965). Effects on the cardiovascular system, liver, and kidney have also been described in case reports of humans exposed for brief periods, or occupationally for more prolonged periods (Gummert 1961; Hansen et al. 1953; Kegel et al. 1929; McNally 1946; Rafnsson and Gudmundsson 1997; Schamweber et al. 1974; Spevak et al. 1976; Verriere and Vachez 1949). Only one epidemiology study addressed cancer following an acute exposure (Rafnsson and Gudmundsson 1997). The results indicate a slight elevation in death from all cancers, and a clear increase in deaths due to cardiovascular disease, but the usefulness of the study conclusions are limited due to assumptions about similar lifestyle factors between the exposed population and the reference group, including smoking and drinking habits.

Acute inhalation exposure levels of chloromethane causing death in animals are available for rats and mice (Burek et al. 1981; Chellman et al. 1986a, 1986b, 1987; Jiang et al. 1985; Landry et al. 1985; Morgan et al. 1982; Smith and von Oettingen 1947a, 1947b; von Oettingen et al. 1949, 1950; Wolkowski-Tyl et al. 1983a, 1983b). Numerous acute inhalation studies have identified the liver and kidney as target organs in rats and mice (Burek et al. 1981; Chapin et al. 1984; Chelhnan et al. 1986a; Jiang et al. 1985; Landry et al. 1985; Morgan et al. 1982); the spleen as a target organ in mice (Landry et al. 1985); the central nervous system as a target system in rats, mice, and dogs (Chellman et al. 1986a, 1986b; Jiang et al. 1985; McKenna et al. 1981a; Smith and von Oettingen 1947a, 1947b); and the testes and epididymides as target organs in rats (Chapin et al. 1984; Chellman et al. 1987; Morgan et al. 1982; Working et al. 1985b). The respiratory and cardiovascular systems may be targets in dogs (Dunn and Smith 1947; Smith 1947; Smith and von Oettingen 1947a, 1947b; von Oettingen et al. 1949, 1950). These studies have shown that species differ in susceptibility, and that lower levels are needed when administered continuously to produce toxicity compared with the higher levels needed in intermittent exposures. Some information on the mechanism of hepatic, renal, neurological, and reproductive effects in mice is available, but more is needed.

The data for acute effects in animals were sufficient to derive an acute inhalation MRL for chloromethane based on a NOAEL for neurological effects in mice.

Only one acute oral study was reported, and this was not sufficient to derive an MRL. In this study, rats were dosed orally with chloromethane, and livers were examined for pathology (Reynolds and Yee 1967). The administered dose was too low to cause hepatic effects, and higher doses were not administered because of the neurotoxic effects of chloromethane.

No studies were located regarding effects in humans or animals after dermal exposure to chloromethane.

Pharmacolcinetic data are insufficient to identify target organs of chloromethane after oral and dermal exposure and more studies are needed. As discussed above, the potential for humans to be exposed to chloromethane is greater for the inhalation route than for the oral and dermal routes, however, chloromethane is ubiquitous in the environment. Therefore, acute studies in animals exposed by oral or dermal routes are needed to identify target organs and dose-response relationships for these routes.

Intermediate-Duration Exposure. Information regarding effects in humans after intermediate-duration exposure to chloromethane is limited to findings of neurological symptoms in humans occupationally exposed. Inhalation studies conducted in rats, mice, and dogs have identified the liver as a target organ in rats and mice (CIIT 1981; Mitchell et al. 1979; Smith and von Oettingen 1947a); the testes as a target organ in rats (CIIT 1981; Hamm et al. 198.5); and the kidney, spleen, and central nervous system as targets in mice (CIIT 1981). The data were sufficient to derive an intermediate-duration inhalation MRL. No studies were located regarding effects in humans or animals after intermediate-duration oral or dermal exposure, and pharmacokinetic data are insufficient to identify or predict target organs of chloromethane for these routes of exposure. As discussed above, although the potential for humans to be exposed to chloromethane is greater for the inhalation route than for the oral and dermal routes, chloromethane is ubiquitous in the environment. Intermediate-duration studies in animals exposed by oral or dermal routes are needed to identify target organs and dose-response relationships for these routes.

Chronic Duration Exposure and Cancer. Only one study was located regarding effects of chloromethane in humans after chronic inhalation exposure. No studies were located for other routes.

A 2-year inhalation study in animals has been conducted in which both sexes of rats and mice were exposed to several concentrations of chloromethane (CIIT 1981). The liver, kidney, spleen, and brain were identified as target organs in mice, and the testes were identified as target organs in rats and mice. Data were sufficient to derive a chronic inhalation MRL. No studies were located regarding effects in animals after chronic oral or dermal exposure to chloromethane. Pharmacokinetic data are insufficient to identify or predict target organs of chloromethane for these routes of exposure. Although the potential for humans to be exposed to chloromethane is greater for the inhalation route than for the oral and dermal routes, chloromethane is ubiquitous in the environment. Therefore, chronic-duration studies in animals exposed by oral or dermal routes are needed to identify target organs and dose-response relationships for these routes.

The carcinogenic effects of chloromethane were observed in male, but not female mice nor in rats of either sex. Male mice had increased incidences of kidney tumors at the highest exposure level. The rats and mice were exposed to the same concentrations, but differences in ventilation rate, the ability to conjugate chloromethane with glutathione, the further metabolism of the glutathione conjugate, and body weight effects make it probable that mice received a higher internal dose than rats. It is possible, therefore, that the exposure concentration was not sufficient in rats to produce kidney tumors. Additional chronic inhalation studies are needed to provide more information on differences in species susceptibility and to further evaluate the potential for and the mechanisms of chronic and carcinogenic effects of chloromethane in humans.

Genotoxicity. Chloromethane has been shown to be genotoxic (Chellman et al. 1986c; Ristau et al. 1990; Rushbrook 1984; Working et al. 1985a). DNA strand breaks have been evaluated in human lymphoblasts (Fostel et al. 1985). Genotoxic effects have also been evaluated for mutations in *S. typhimurium* (Andrews et al. 1976; DuPont 1977; Simmon et al. 1977), sister-chromatid exchange (Fostel et al. 1985) unscheduled DNA synthesis in rat hepatocytes (Working et al. 1986), effects on spermatocytes and tracheal epithelial cells (Working et al. 1986), and DNA viral transformation in primary hamster embryo cells (Hatch et al. 1982, 1983). Studies of the mechanism of dominant lethal mutations in rat sperm resulting from inhalation exposure of male rats to chloromethane suggest that the dominant lethal effects may be secondary to inflammation of the epididymis (Chellman et al. 1986c). There remains, however, some controversy about chloromethane's alkylating and genotoxic potential, and additional studies are needed to evaluate the genotoxic risks to humans.

Reproductive Toxicity. No information was available regarding reproductive effects of chloromethane in humans.

Several inhalation studies, however, have demonstrated that chloromethane is a reproductive toxicant in male rats (Burek et al. 1981; Chapin et al. 1984; Chellman et al. 1986a, 1986b, 1987; CIIT 1981; Hamm et al. 1985; Morgan et al. 1982; Working and Bus 1986; Working et al. 1985a, 1985b). The mechanism of this reproductive toxicity has been studied extensively only in rats because testicular lesions in mice occurred at lower incidences and later time periods than in rats in the 2-year inhalation study by CIIT (1981). Testicular effects were not observed in male dogs and cats exposed to chloromethane by inhalation (McKenna et al. 1981a), but the exposure concentrations may not have been high enough. Species differences in sensitivity exist for other end points as well. No studies were located regarding the

reproductive effects of chloromethane in animals after oral or dermal exposure, and pharmacokinetic data are insufficient to support the potential for reproductive effects across routes of exposure. Therefore, additional inhalation, oral, and dermal studies for reproductive effects in other species at higher exposure levels are needed to further evaluate the potential adverse reproductive effects in humans from exposure to chloromethane.

Developmental Toxicity. No information was located regarding developmental effects in humans after exposure to chloromethane by any route.

The teratogenicity of inhalation exposure to chloromethane has been studied in rats and mice (Wolkowski-Tyl et al. 1983a). In rats, delayed fetal development was found at a concentration that also resulted in maternal toxicity. Positive results in mice have been reported (Wolkowski-Tyl 1985); however there is some controversy related to conflicting results reported from other laboratories (John-Greene et al. 1985). Additional studies are needed to further evaluate the pharmacokinetics and the potential teratogenic effects of exposure to chloromethane.

No studies were located regarding the developmental effects of chloromethane in animals after oral and dermal exposure, and the pharmacokinetic data are insufficient to extrapolate to these routes of exposure. Additional studies in mice and other species are needed to evaluate the potential developmental risks to humans from these routes of exposure.

Immunotoxicity. No information was located regarding immunotoxic effects in humans after exposure to chloromethane by any route.

The immunotoxic effects reported in the literature from exposure to chloromethane were lymphoid depletion of the spleen and splenic atrophy observed in mice exposed by inhalation to chloromethane for 2 years (CIIT 1981). Cats exposed continuously to chloromethane for 3 days had higher incidences of brain lesions than the control (McKenna et al. 1981a), but the lesions were consistent with infection or post-vaccinal reaction (the cats were vaccinated for panleukopenia by the supplier). Exacerbation of viral-induced central nervous system disease could not be ruled out. Additional studies are needed to further evaluate the potential immunotoxicity of chloromethane to humans.

Neurotoxicity. The neurotoxic effects in humans from inhalation exposure to chloromethane are described in numerous case studies (Baird 1954; Battigelli and Perini 1955; Gudmundsson 1977; Hansen et al. 1953; Hartman et al. 1955; Jones 1942; Kegel et al. 1929; Lanham 1982; MacDonald 1964; McNally 1946; Raalte and van Velzen 1945; Spevak et al. 1976; Wood 1951), but the mechanism is unclear. S-methylcysteine appears to be a metabolite in humans (Kornbrust and Bus 1983), and mechanisms involving conjugation with glutathione are likely to be relevant to human toxicity. Methanethiol produces similar central nervous system effects as seen in humans and animals exposed to chloromethane (Jager et al. 1988; Kornbrust and Bus 1983, 1984).

The neurotoxic effects of inhalation exposure to chloromethane are also well defined in animals (Burek et al. 1981; Chelhnan et al. 1986a, 1986b; CIIT 1981; Kolkmann and Volk 1975; Landry et al. 1985; McKenna et al. 1981a; Morgan et al. 1982; Smith and von Oettingen 1947b). The mechanism for the induction of cerebellar lesions in mice exposed by inhalation may involve conjugation of chloromethane with glutathione, with further metabolism leading to production of methanethiol (Chellman et al. 1986b). The relative importance of conjugation with glutathione in other species has not been determined.

Monkeys provide a better animal model compared with rodents when evaluating neurobehavioral effects in humans. Neurobehavioral studies in monkeys and additional mechanistic studies in rodents are needed to further evaluate the mechanism and dose-response relationships of chloroform-induced neurotoxicity in humans.

No studies were located regarding the neurotoxic effects of chloromethane in animals after oral and dermal exposure, and pharmacokinetic data are insufficient to extrapolate to other routes of exposure.

Epidemiological and Human Dosimetry Studies. A retrospective epidemiological study was conducted in workers exposed to chloromethane in a butyl rubber manufacturing facility (Holmes et al. 1986). No association was found between chloromethane exposure and death due to cardiovascular disease or cancer at any site. In a study of workers from fabricating plants, occupational exposure to chloromethane below 100 ppm produced subtle, quantifiable behavioral effects, but the threshold for changes in functional capacity could not be determined precisely (Repko et al. 1977). An experimental study by Stewart et al. (1980) found no effects on pulmonary function, cardiac function or ECG, and no hematological, neurological, or behavioral effects in human volunteers exposed by inhalation to chloromethane, but the protocol was too confusing to clearly define the exposures. A slight decrement in

performance of behavioral tasks was found in human volunteers exposed to 200 ppm for 3 hours (Putz-Anderson et al. 1981a). An epidemiology study on a cohort of 24 Icelandic fishermen reported a slight increase in excess mortality from all cancers (more specifically, lung cancer) and a clear increase in death from cardiovascular disease (Rafnsson and Gudmundsson 1997). The study was conducted 32 years after an acute (i.e., 2 days) high level exposure to chloromethane from a leaking refrigerator (although no estimates of exposure levels were reported). The usefulness of these results are limited because confounding factors for lifestyle and smoking were not explicitly controlled, but assumed to be similar based on controls for age, social class, and occupation. Exposure levels were also not quantified. Additional epidemiology and dosimetry studies are therefore needed to further evaluate the occupational and environmental health risk from exposure to chloromethane.

Biomarkers of Exposure and Effect.

Exposure. A number of studies have unsuccessfully tried to relate blood and alveolar air levels of chloromethane and urinary levels of S-methylcysteine with exposure (DeKok and Antheunius 1981; Nolan et al. 1985; Stewart et al. 1980; Van Doorn et al. 1980). The blood and alveolar air levels of chloromethane and the urinary levels of S-methylcysteine are highly variable. Symptoms resembling drunkenness and food poisoning, along with a sweet odor on the breath, may alert a physician that a person has been exposed to chloromethane, but such symptoms could easily be mistaken for the conditions they resemble.

Although Xu et al. (1990) reported low chloromethane reactivity with hemoglobin, protein adducts may still hold promise as potential biomarkers for chloromethane exposure. In view of chloromethane's genotoxicity in short-term assays, an assay for a DNA adduct or indicator of oxidative damage to DNA from chloromethane exposure might also be pursued. Further studies are, therefore, needed to identify a metabolite or biomarker that can be used to monitor chloromethane exposure.

Effect. Attempts to correlate blood levels and expired air concentrations of chloromethane with health effects of occupational and experimental inhalation exposures of humans have also been unsuccessful (Putz-Anderson et al. 1981a; Repko et al. 1977). Blood and alveolar levels are highly variable and are not sensitive indicators of neurological function or behavior. Further studies are needed to identify a metabolite or biomarker that can be correlated with the known toxic end point and that would lead to early detection and possibly treatment.

Absorption, Distribution, Metabolism, and Excretion. Experimental inhalation studies in animals and humans indicate that chloromethane is rapidly taken up from the lungs into the blood, widely distributed throughout the body and extensively metabolized, incorporated into macromolecules, and excreted as CO_2 or other metabolites in the urine (Dekant et al. 1995; Dodd et al. 1982; Heck et al. 1982; Jager et al. 1988; Kornbrust and Bus 1983, 1984; Kornbrust et al. 1982; Landry et al. 1983a, 1983b; Putz-Anderson et al. 1981a, 1981b; Redford-Ellis and Gowenlock 1971a, 1971b; Van Doorn et al. 1980; von Oettingen et al. 1949, 1950). Differences in the rate and extent of absorption, metabolic pathways, and disposition will have a profound effect on the toxicity of chloromethane. Oral and dermal routes of exposure may be of particular concern because chloromethane is ubiquitous in the environment. Additional pharmacokinetic studies are needed to evaluate the potential for delivery of toxic levels of chloromethane to human target tissues from different routes of exposure and durations of exposure.

Comparative Toxicokinetics. Studies on the pharmacokinetics of chloromethane following inhalation exposure have been conducted in rats, mice, dogs, and humans (Dekant et al. 1995; Dodd et al. 1982; Heck et al. 1982; Jager et al. 1988; Kombrust and Bus 1983, 1984; Kombrust et al. 1982; Landry et al. 1983a, 1983b; Putz-Anderson et al. 1981a, 1981b; Redford-Ellis and Gowenlock 1971a, 1971b; Van Doorn et al. 1980; von Oettingen et al. 1949, 1950). The kinetics of chloromethane in humans were similar to those in rats and dogs, with data for each species consistent with a 2-compartment model. Some species differences can be explained by differences in respiratory minute volumes and basal metabolic rates (rat > dog > human). Additional pharmacokinetic studies in different species and with different routes of exposure are needed to further evaluate the target tissues and the differences in potential toxic metabolites. Additional studies are especially needed to resolve the relative importance of glutathione conjugation and P-450 oxidation to the toxicity of chloromethane. These studies should be performed in different tissues, species, and sexes to resolve potential differences. Additional studies are needed to evaluate the importance of varying levels of human endogenous erythrocyte, glutathione transferase (as has been recently shown to exist) to the toxicity of chloromethane and to the identification of potentially susceptible populations.

Methods for Reducing Toxic Effects. Additional studies are needed to further define the mechanism of chloromethane's toxicity. Especially important are studies to determine whether depletion or protection of glutathione pools is needed to protect against toxicity for any given exposure route or target organ. The mechanisms and the beneficial or detrimental contribution of glutathione may be different for different end points or target tissues.

Children's Susceptibility. There have been no studies on whether children are more or less susceptible than adults to adverse health effects from a given amount or duration of exposure to chloromethane, or if chloromethane affects the developing fetus or the development of young children. There have also been no studies in which young animals were exposed to chloromethane.

Only limited information is available from rat and mouse studies on potential effects in the developing young (see above in Data Needs for Developmental Toxicity). In one rat study (Wolkowski-Tyl et al. 1983a), at levels that also produced maternal toxicity, fetal effects consisted of reduced fetal body weight and crownrump length and reduced ossification of metatarsals and phalanges of the anterior limbs, thoracic centra in the pubis of the pelvic girdle, and metatarsals of the hindlimbs. Wolkowski-Tyl et al. (1983a) also found increased incidences of heart malformations in the fetuses of mouse dams exposed to 500 ppm chloromethane during Gd 6-17; however, heart malformation were not found in fetuses of mouse dams exposed to higher concentrations of chloromethane during Gd 11.5-12.5 (John-Greene et al. 1985). The developmental toxicity of chloromethane in mice is, therefore, controversial, and further studies are needed to determine potential adverse effects on development from maternal and fetal exposure to chloromethane.

There is no information on the movement of chloromethane or its metabolites across the placenta or into the developing young. There is no information on the movement of chloromethane or its metabolites into a nursing women's milk. Chloromethane is broken down and eliminated from the body very quickly in adults (Nolan et al. 1985) and animals (Landry et al. 1983a; von Oettingen et al. 1949, 1950). Thus, it is unlikely that chloromethane would be stored in maternal tissues or be mobilized (i.e., released from stores) during pregnancy or lactation. However, further studies are needed to answer these questions.

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-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 (Chellman et al. 1986b; Jager et al. 1988; Kombmst and Bus 1983, 1984; Nolan et al. 1985; Stewart et al. 1980; Warholm et al. 1995). It is anticipated that children would have a polymorphism similar to the adult population, although no specific data have been collected to test this hypothesis. If a polymorphism is present in children, then some children (i.e., those with higher levels of glutathione-S-transferase) would be more susceptible to the toxic effects of chloromethane. Moreover, cytochrome P-450 dependent metabolism of methanethiol may yield formaldehyde and formic acid whose carbon atoms can then enter the one-carbon

pool for incorporation into macromolecules or formation of CO_2 (Heck et al. 1982; Jager et al. 1988; Kombrust and Bus 1983). Guengerich and Shimada (1991) suggest that the human cytochrome P-450 enzyme 2El is a major catalyst in the oxidation of chloromethane. Formaldehyde may also be a direct product of chloromethane via oxidative dechlorination. Studies are therefore needed to evaluate the differences among and between children and adults for P-450 and transferase levels and isoforms, and for differences in chloroform metabolism.

There are no PBPK models for children, adults, or test animal models. There are no good biomarkers of exposure for children (or adults), although clinical symptoms of drunkenness or food poisoning, and a 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. Further studies are needed to evaluate the toxicokinetics of chloromethane and its metabolites in children and to develop better biomarkers of exposure and effects.

Child health data needs relating to exposure are discussed in Section 5.8.1, Data Needs: Exposures of Children.

2.11.3 Ongoing Studies

No ongoing studies were found that address the health effects of chloromethane.

The National Science Foundation is sponsoring a study to analyze the degradation products of a methane oxidizing bacteria (methanotrophic degradation) for selected contaminants including chloromethane to demonstrate that no toxic products are formed. A laboratory scale treatment column will also be used to optimize conditions for the removal of chlorinated aliphatics from contaminated waters. The principal researcher is Samuel Fogel, Cambridge Analytical Associates, Inc., Boston, Massachusetts.