CHAPTER 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 on 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. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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

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 by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to chloromethane, but may not be inclusive of the entire body of literature. A systematic review of the scientific evidence of the health effects associated with exposure to chloromethane was also conducted; the results of this review are presented in Appendix C.

Summaries of the human observational studies are presented in Table 2-1. Animal inhalation studies are presented in Table 2-2 and Figure 2-2, and animal oral studies are presented in Table 2-3 and Figure 2-3; no dermal data were identified for chloromethane.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. 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 (SLOAELs) 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 endpoint 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 endpoints. 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. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of chloromethane are indicated in Table 2-2 and Figure 2-2.

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

The health effects of chloromethane have been evaluated in epidemiological, human controlled trial and experimental animal studies. As illustrated in Figure 2-1, the vast majority of the health effects data come from inhalation exposure studies in animals. Animal data from inhalation studies are available for each health effect category and exposure duration category. Much of the data for chloromethane comes from toxicity studies which evaluated a suite of endpoints. The most reported effects on systems from the literature include reproductive, neurological, renal, and hepatic effects of chloromethane. Case reports and cohort studies also evaluated or summarized the impact chloromethane had on the nervous and cardiovascular systems and potential association with various cancers. Only a single oral exposure study in animals was identified, which evaluated potential hepatic effects following acute-duration exposure.

As outlined in Chapter 1, the neurological, hepatic, cardiovascular, developmental, and male reproductive systems appear to be sensitive targets of toxicity following inhalation exposure to chloromethane; the neurological endpoints appear to be the most sensitive (see Figure 1-2). A systematic review was conducted on the available human and animal inhalation studies for these endpoints. The information in these studies indicate the following on the potential targets of chloromethane toxicity:

- **Cardiovascular Endpoints.** Data are inadequate to conclude whether cardiovascular effects are associated with chloromethane exposure. Case reports and data from a cohort of accidentally exposed individuals suggest that chloromethane exposure may increase risk of death from cardiovascular disease or result in other cardiac abnormalities such as tachycardia, increased pulse rate, and sustained changes in blood pressure. One study in dogs reports elevated blood pressure followed by a precipitous decrease in blood pressure and heart rate prior to death following inhalation exposure to very high chloromethane levels; findings may be secondary to CNS depression. While a few studies report elevated heart weight following intermediate- or chronic-duration exposure, no exposure-related changes in heart histology were observed following inhalation exposure in experimental animal studies.

- **Hepatic Endpoints.** Hepatic effects are a presumed health effect for humans exposed to chloromethane via inhalation based on a high level of evidence in rodents following acute-, intermediate-, and chronic-duration inhalation exposure. Hepatic lesions and elevated liver weights have been observed in rodents following acute-, intermediate-, or chronic-duration inhalation exposure. Hepatic enzyme changes have also been observed in some studies. Mice appear to be more sensitive to hepatic effects than rats or guinea pigs.

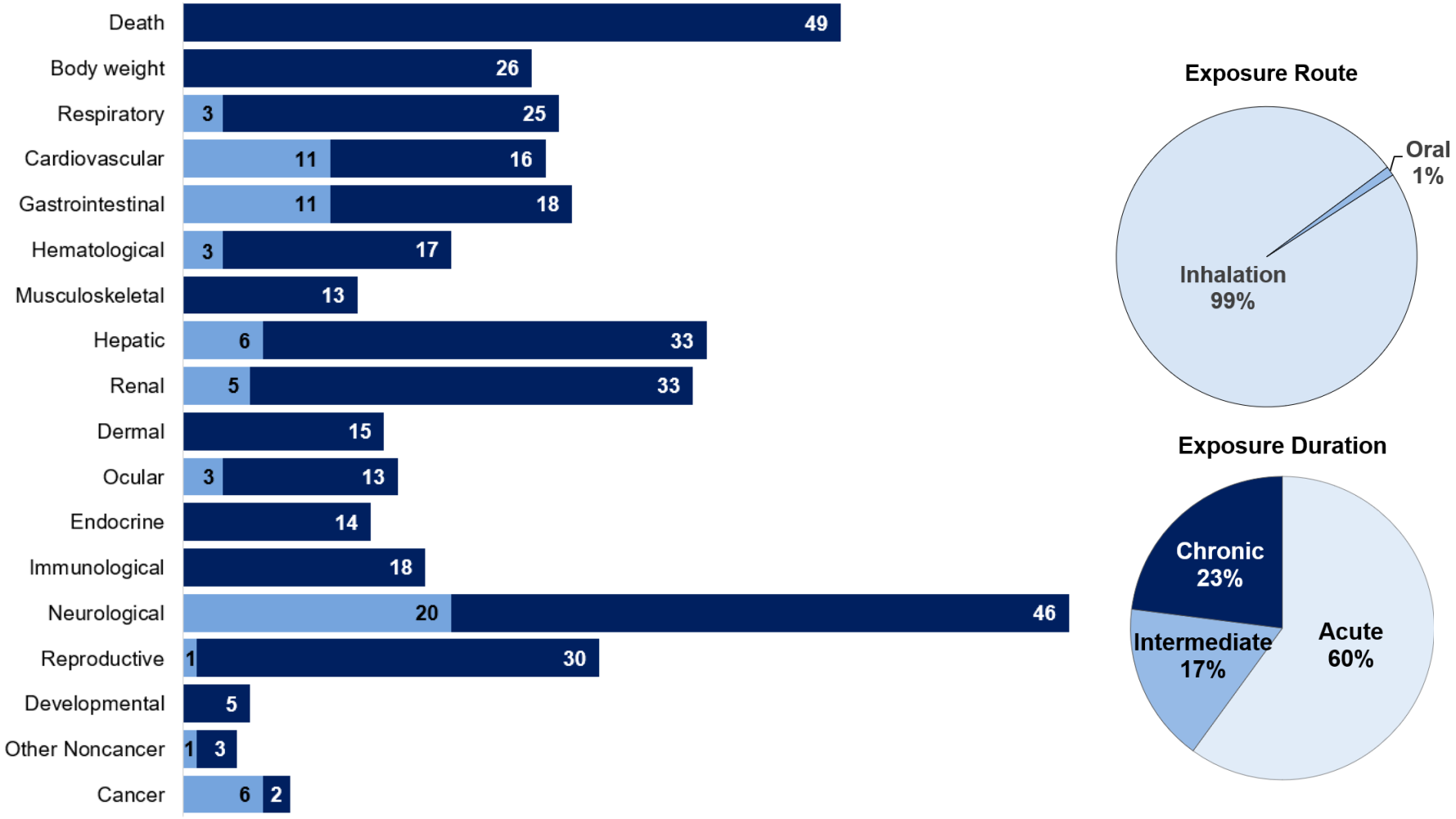
- **Neurological Endpoints.** Neurological effects are a presumed health effect associated with chloromethane exposure via inhalation based on a low level of evidence in humans and a high level of evidence in animals. Case reports clearly indicate neurological effects associated with chloromethane exposure. Epidemiological studies provide limited evidence in humans, while animal inhalation studies consistently report effects including impaired performance on sensorimotor tests, mild-to-severe clinical signs of toxicity (e.g., incoordination, ataxia, paralysis), and histopathological lesions on the cerebellum and spinal cord.

- **Male Reproductive Endpoints.** Male reproductive effects are a presumed health effect associated with chloromethane exposure via inhalation based on a high level of evidence from rodent studies. Decreased fertility attributable to sperm effects and testicular lesions in male rats has been observed following acute- and intermediate-duration exposures. Additional studies report testicular damage in rats and mice following acute-, intermediate-, and chronic-duration exposure to chloromethane.

- **Developmental Endpoints.** Developmental effects are not a classifiable health effect for humans based on results of animal studies. Experimental animal studies provide low evidence of an association between chloromethane exposure via inhalation and adverse developmental outcomes based on interspecies differences. Reduced growth and delayed skeletal development were observed in rats, heart defects were observed in mice, and no developmental effects were noted in rabbits. The toxicological significance of the heart defects in mice has been questioned, and the defects may have been misdiagnosed and/or artifacts of the fixation and sectioning methods used.

Figure 2-1. Overview of the Number of Studies Examining Chloromethane Health Effects*

Most studies examined the potential neurological, hepatic, and renal effects of chloromethane
Fewer studies evaluated health effects in humans than animals (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 96 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Table 2-1. Health Effects Evaluated in Humans Exposed to Chloromethane—Epidemiological Studies

Reference, study type, and population	Exposure	Outcome evaluated	Result
Barry et al. 2011 Case-control; 518 women with NHL (classified by subtype) and 597 controls (Connecticut)	Job-exposure matrix used to estimate subject's probability and intensity of occupational exposure to chloromethane	Total NHL	↔ (ever versus never exposed)
		CYP2E1 polymorphism (TT)	↑ (ever versus never exposed)
		CYP2E1 polymorphism (TA/AA)	↔ (ever versus never exposed)
		Diffuse large B-cell lymphoma	↔ (ever versus never exposed)
		CYP2E1 polymorphism (TT)	↔ (ever versus never exposed)
		CYP2E1 polymorphism (TA/AA)	↔ (ever versus never exposed)
		Follicular lymphoma	↑ (ever versus never exposed)
		CYP2E1 polymorphism (TT)	↑ (ever versus never exposed)
		CYP2E1 polymorphism (TA/AA)	↔ (ever versus never exposed)
Delfino et al. 2003 Panel study; 22 Hispanic children (10–16 years old) with asthma living in Los Angeles community with high traffic density (California)	Daily mean (SD) in ppb: 0.58 (0.14)	Asthma symptoms	Association with daily air levels
		Bothersome	↔
		Severe (interfere with daily activities)	↔
Dosemeci et al. 1999 Case-control study; 438 white subjects (273 men, 165 women) diagnosed with renal cell carcinoma and 687 controls (462 men, 225 women) (Minnesota)	Job-exposure matrix used to estimate subject's probability of occupational exposure to chloromethane	Renal cell carcinoma	
		All	↔ (ever versus never exposed)
		Men	↔ (ever versus never exposed)
		Women	↔ (ever versus never exposed)
Holmes et al. 1986 Occupational cohort; 852 males employed ≥1 month at a synthetic rubber manufacturing plant; cohort mortality compared to U.S. averages (Louisiana)	Job-exposure matrix used to estimate subject's probability and intensity of occupational exposure to chloromethane	Standard mortality ratio	
		All causes	↓ (observed versus expected)
		All malignant neoplasms	↔ (observed versus expected)
		Digestive	↔ (observed versus expected)
		Respiratory	↔ (observed versus expected)
		Lymphatic	↔ (observed versus expected)
		Unspecified	↔ (observed versus expected)
		Circulatory system diseases	↔ (observed versus expected)

Table 2-1. Health Effects Evaluated in Humans Exposed to Chloromethane—Epidemiological Studies

Reference, study type, and population	Exposure	Outcome evaluated	Result
Kernan et al. 1999 Case-control; 63,097 patients that died from pancreatic cancer and 252,386 controls that died from causes other than cancer (United States)	Job-exposure matrix used to estimate subject's probability and intensity of occupational exposure to chloromethane	Pancreatic cancer All Men Women Black men	 ↔ (exposed versus unexposed) ↔ (exposed versus unexposed) ↔ (exposed versus unexposed) ↑ (high probability of exposure versus no exposure)
NIOSH 1976 Cross-sectional; 122 workers exposed to chloromethane (144 male, 8 female) and 49 unexposed workers (46 male, 3 female) for seven different locations of the same company (United States)	Mean (range) [range from facility means] air levels in ppm: 33.57 (1.8–70.0) [8.46–58.72] Mean (range) [range of facility means] worker breath levels in ppm: 13.32 (0.4–79.5) [10.81–24.19]	Neurobehavioral and neurofunctional tasks	↔ (exposed versus unexposed)
		EEG	↔ (exposed versus unexposed)
Rafnsson and Gudmundsson 1997 Occupational cohort (32-year follow-up); 24 male crew members from an Icelandic fishing boat that experienced accidental exposure; 120 referent Icelandic fishermen (Iceland)	Estimates not available; acute-duration exposure occurred due to leaking refrigerant on fishing vessel.	Death (all causes) Cancer Cardiovascular diseases	↑ (exposed versus referent) ↔ (exposed versus referent) ↑ (exposed versus referent)
Rafnsson and Kristbjornsdottir 2014 Occupational cohort (47-year follow-up); 27 male crew members from an Icelandic fishing boat that experienced accidental exposure; 135 referent Icelandic fishermen (Iceland)	Estimates not available; acute-duration exposure occurred due to leaking refrigerant on fishing vessel.	Death (all causes) All cancers Kidney cancer All cardiovascular disease Acute coronary heart disease Cerebrovascular disease Suicide	↑ (exposed versus referent) ↔ (exposed versus referent) ↑ (exposed versus referent) ↑ (exposed versus referent) ↑ (exposed versus referent) ↑ (exposed versus referent) ↑ (exposed versus referent)

↑ = association with increase; ↓ = association with decrease; ↔ = no association; CYP2E1 = cytochrome P450 2E1; EEG = electroencephalogram; NHL = non-Hodgkin's lymphoma; SD = standard deviation

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Putz-Anderson et al. 1981a									
1	Human 4–12 B	3 hours	0, 100, 200	NX	Neuro	200			
Putz-Anderson et al. 1981b									
2	Human 12 B	3 hours	0, 199	NX	Neuro	199			
Stewart et al. 1980									
3	Human 4 M, 4 F	2–5 days; 1, 3, or 7.5 hours/day	0, 20, 100, 150, 50+100+150 average 100	CS, BC, HE, UR, NX	Resp	150			
					Cardio	150			
					Hemato	150			
					Neuro	150			
Smith and von Oettingen 1947a, 1947b									
4	Monkey (NS) 2–5 NS	2 weeks 6 days/week 6 hours/day	300, 500, 2,000	LE, CS	Death			2,000	5/5 died
					Neuro	500		2,000	Motor impairments, incoordination, seizures, loss of consciousness
Burek et al. 1981									
5	Rat (Sprague-Dawley) 20 M, 20 F	72 hours continuous	0, 198, 504, 976, 1,950	CS, BW, HE, BC, UR, GN, OW, HP	Death			976	6/10 males and 8/10 females died; 100% mortality at 1,950 ppm
					Bd wt	504 F 198 M	504 M	976	LOAEL: 15% decrease in body weight in males
									Serious LOAEL: 29–30% decrease in body weight
					Resp		1,950		Congestion and edema of the lungs in animals that died
					Hemato	504	976		Increased red blood cell count, hemoglobin, and hematocrit (secondary to dehydration)

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic		198		Males: Decreased absolute and relative liver weight and altered tinctorial appearance of hepatocytes (slight) Females: Increased lipid accumulation and slight extramedullary hematopoiesis
					Renal	198 M 504 F	504 M	976	LOAEL: multifocal renal tubules in males Serious LOAEL: renal failure and histopathological changes in the kidney in both sexes, alterations in urinalysis in both sexes, increased BUN in females
					Neuro	504	976		Lethargy
					Repro	198 M	504 M		Sperm granulomas, decreased sperm in the tubule lumen, interstitial edema, coagulated proteinaceous obstruction of lumen, inflammation, sperm granuloma formation, testicular atrophy secondary to alterations
Burek et al. 1981									
6	Rat (Sprague-Dawley) 20 M, 20 F	48 hours continuous	0, 196, 501, 972, 1,968	LE, CS, BW, HE, BC, UR, GN, OW, HP	Death			972 F 1,968 M	1/20 females at 972 ppm and 14/20 males and 10/20 females at 1,958 ppm died
					Bd wt	972 F	1,968 F		18% decrease in body weight
						501 M		972 M	20% decrease in body weight
					Resp	1,968			
					Hemato	972	1,968		Increased red blood cell count, hemoglobin, and hematocrit (secondary to dehydration)
					Hepatic	972 F	1,968 F		Dark, congested, or mottled liver

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
							196 M		Decreased liver weight
					Renal	501		972	Renal tubular necrosis, increased renal tubular cytoplasmic homogeneity, and increased lipid accumulation in renal tubular cells; alterations in urinalysis
					Neuro	501	972		Lethargy
					Repro	196 M	501 M		Sperm granulomas, decreased sperm in the tubule lumen, interstitial edema, coagulated proteinaceous obstruction of lumen
Chapin et al. 1984									
7	Rat (Fischer-344) 2–8 M	12 days 4-5 days/week 6 hours/day	0, 3,500	BC, HP	Repro		3,500		Decreased serum testosterone, delayed spermiation, seminiferous epithelium vacuolation, and bilateral epididymal granulomas
Chellman et al. 1986a									
8	Rat (Fischer-344) 5–12 M	2 days 6 hours/day	0, 7,500	LE, CS, BW, OW, HP	Death			7,500	8/12 died
					Repro		7,500		Bilateral epididymal granulomas
Chellman et al. 1986a									
9	Rat (Fischer-344) 5 M	5 days 6 hours/day	0, 5,004	LE, CS, BW, OW, HP	Death			5,004	1/5 died
					Bd wt			5,004	20% decreased body weight
					Hepatic		5,004		Hepatocellular degeneration - cloudy swelling of hepatocytes, obliteration of sinusoids
					Renal			5,004	Necrosis of proximal convoluted tubules
					Endocr		5,004		Vacuolation of cell cytoplasm in the adrenal cortex

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro			5,004	Severe cerebellar degeneration (granule layer), tremors, ataxia, and limb paralysis
					Repro			5,004	Severe epididymis granulomas, pachytene spermatocytes and early stage spermatids in the tubular lumen, slight separation of early stage spermatids, and formation of multinucleated giant cells
Chellman et al. 1986c									
10	Rat (Fischer-344) 20-40 M	5 days 6 hours/day prior to breeding	0, 3009	HP, RX	Repro			3,009	Increased pre- and post-implantation loss in mated females, and increased infiltration of neutrophils and macrophages into interstitium of cauda epididymis
Chellman et al. 1987									
11	Rat (Fischer-344) 18 M	5 days 6 hours/day	0, 3056	BW, OW, HP	Bd wt	3,056			
					Repro			3,056	Decreased testes weight, delayed spermiation, decreased sperm production, and sperm motility and an increase in abnormal sperm
Dunn and Smith 1947; Smith and von Oettingen 1947a, 1947b									
12	Rat (NS) 10–59 NS	2 weeks 6 days/week 6 hours/day	0, 300, 500, 1,000, 2,000, 3,000, 4,000	LE, CS, HP	Death			2,000	50% mortality; 100% mortality at ≥3,000 ppm
					Resp	1,000		2,000	Lung congestion and slight edema
					Hepatic	1,000		2,000	Fat accumulation, centrilobular necrosis
					Renal	1,000		2,000	Renal tubule necrosis
					Neuro	1,000	2,000		Agitation, hunched posture

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Morgan et al. 1982									
13	Rat (Fischer-344) 10 M, 10 F	9 days 6 hours/day	0, 2,000, 3,500, 5,000	LE, CS, HP	Death			3,500 F 5,000 M	2/10 females at 3,500 ppm and 6/10 males and 5/10 females at 5,000 ppm sacrificed moribund
					Gastro	2,000	3,500		Diarrhea
					Hepatic	2,000 M	2,000 F 3,500 M		Minimal hepatocyte degeneration
					Renal	2,000 F		3,500 F 2,000 M	Degeneration and necrosis of proximal convoluted tubules
					Endocr	2,000	3,500		Clear droplets in endothelial cytoplasm assumed to be fatty degeneration of adrenals
					Neuro	3,500		5,000	Hindlimb paralysis, forelimb incoordination, minimal cerebellar degeneration (granule layer)
					Repro			2,000 M	Reduction in spermatids and sperm, separation of spermatocytes and early stage spermatids with sloughing of cells into the lumen and fusion into giant cells
Wolkowski-Tyl et al. 1981a, 1983a									
14	Rat (Fischer-344) 25 F	13 days 6 hours/day GDs 7–19	0, 102, 479, 1,492	LE, BW, FI, WI, RX, DX	Bd wt	102		479	21% reduction in body weight gain from GD 7 to 15
					Repro	1,492			
					Develop	479	1,492		Delayed skeletal development (reduced ossification and fewer caudal bones); 10% decrease in fetal body weight in both sexes, and 4% decrease in crown-rump length in females

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Working and Bus 1986									
15	Rat (Fischer-344) 10–30 M	5 days prior to mating, 6 hours/day	0, 1,000, 3,000	CS, RX	Repro	1,000		3,000	≥16% decrease in fertilization rate
Working et al. 1985a									
16	Rat (Fischer-344) 40 M	5 days prior to mating, 6 hours/day	0, 1,000, 3,000	CS, BW, RX	Bd wt	1,000	3,000		16% decrease in body weight
					Repro	1,000		3,000	Postimplantation loss in female rats mated with exposed males, and persistent decreased fertility
Working et al. 1985b									
17	Rat (Fischer-344) 40 M	5 days prior to mating, 6 hours/day	0, 1,000, 3,000	CS, GN, OW, HP, RX	Repro	1,000		3,000	Decreased number of live and total implants, increased post-implantation loss, reversible disruption of spermatogenesis, transient reduction in testes weights
Chellman et al. 1986b									
18	Mouse (B6C3F1) 5–15 M	6 hours	500, 1,000, 1,500, 2,000, 2,500	LE, CS	Death			2,200	LC_{50}
					Neuro			2,500	Tremors, ataxia, and forelimb/hindlimb paralysis
Chellman et al. 1986b									
19	Mouse (B6C3F1) 6 M	6 hours	0, 1,500	LE, CS, BC	Hepatic			1,500	Increased serum ALT and hepatocellular necrosis and cytoplasmic vacuolization
Chellman et al. 1986b									
20	Mouse B6C3F1 36-45 M	2 weeks 5 days/week 6 hours/day	0, 1,500	LE, CS, BC, BI, UR, HP, OF	Death			1,500	5/45 died
					Renal	1,500			
					Neuro			1,500	Multiple degenerative and necrotic foci in cerebellar granular cell layer

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Chellman et al. 1986b									
21	Mouse (B6C3F1) NS B	2 weeks 5 days/week 6 hours/day	0, 1,500	OF	Renal		1,500		Increased renal cell regeneration (3-fold increased thymidine incorporation)
Dunn and Smith 1947; Smith 1947; Smith and von Oettingen 1947a, 1947b									
22	Mouse (Swiss, Strain A, C3H) 20–61 NS	2 weeks 6 days/week 6 hours/day	0, 300, 500, 1,000, 2,000, 3,000	LE, CS, UR, HP	Death			1,000	50% mortality; 100% mortality at ≥2,000 ppm
					Resp			2,000	Lung congestion
					Hepatic			2,000	Centrilobular necrosis, fatty metamorphosis
					Renal	500	1,000	2,000	LOAEL: Hemoglobinuria Serious LOAEL: Renal necrosis, fatty metamorphosis, hemoglobin globules and casts
					Neuro	300		500	Neuromuscular abnormalities, impaired gait, hindlimb drag
[Histopathology assessed at 2,000 ppm only]									
Jiang et al. 1985									
23	Mouse (C57BL/6) 10 F	2 weeks 5 days/week 6 hours/day	0, 1,500	LE, CS, GN, HP	Death			1,500	2/10 died
					Renal		1,500		Slight degeneration of proximal tubules
					Neuro			1,500	Motor incoordination, severe cerebellar degeneration (granule layer)
Landry et al. 1985									
24	Mouse (C57BL/6) 12 F	11 days 5.5 hours/day	0, 150, 2,400	LE, CS, BW, GN, OW, HP, NX	Death			2,400	All sacrificed moribund after 8 or 9 days
					Bd wt	150	2,400		16% decrease in body weight on day 8
					Hemato	150	2,400		Enlarged spleen and hemoglobinuria (suggestive of extramedullary hematopoiesis)

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic	150	2,400		Decreased hepatocyte size; glycogen depletion
					Renal	150	2,400		Slight multifocal degeneration and regeneration of tubules
					Immuno	150	2,400		Thymus atrophy; decrease in absolute and relative thymus weight
					Neuro	150		2,400	Sedation, hindlimb rigidity, impaired motor coordination on day 8, slight cerebellar degeneration (granular layer)
					Other noncancer	150		2,400	Inanition (exhaustion caused by lack of nourishment), decreased food consumption
Landry et al. 1985									
25	Mouse (C57BL/6) 12 F	11 days 5.5 hours/day	0, 400, 800, 1,600	LE, CS, BW, GN, OW, HP, NX	Bd wt	1,600			
					Hepatic		400		Decreased hepatocyte size; glycogen depletion
					Renal	1,600			
					Immuno	800	1,600		Thymus atrophy; decreased absolute and relative thymus weight
					Neuro		400	1,600	LOAEL: slight cerebellar degeneration (granular layer) SLOAEL: sedation, hindlimb rigidity

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Landry et al. 1985									
26	Mouse (C57BL/6) 12 F	11 days 22 hours/day	0, 15, 50, 150	LE, CS, BW, GN, OW, HP, NX	Death			150	All sacrificed moribund after 10.5 days
					Bd wt	50	150		12% decrease in body weight
					Hepatic	50	150		Decreased hepatocyte size; glycogen depletion
					Renal	150			
					Immuno	50	150		Decreased absolute and relative thymus weight
					Neuro	50[b]	150		Moderate cerebellar degeneration (granular layer); impaired motor coordination
					Other noncancer	50		150	Inanition (exhaustion caused by lack of nourishment), decreased food consumption
Landry et al. 1985									
27	Mouse (C57BL/6) 12 F	11 days 22 hours/day	0, 100, 200, 400	LE, CS, BW, GN, OW, HP, NX	Death			200	100% mortality by day 5
					Bd wt	100		200	32% decrease in body weight by day 4
					Hepatic	50	100		Decreased hepatocyte size; glycogen depletion
					Renal	100			
					Immuno	100			
					Neuro		100	200	LOAEL: slight cerebellar degeneration (granular layer) Serious LOAEL: ataxia, prostration, inability to perform motor assessment
					Other noncancer	100		200	Inanition (exhaustion caused by lack of nourishment), decreased food consumption, decreased feces amount

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Morgan et al. 1982									
28	Mouse (B6C3F1) 5 M, 5 F	12 days 6 hours/day	0, 500, 1,000, 2,000	LE, CS, HP	Death			2,000	100% mortality/moribundity
					Hepatic	1,000	2,000 F	2,000 M	Minimal-to-moderate hepatocellular degeneration in both sexes; hepatocellular necrosis in males
					Renal	500	1,000	2,000	LOAEL: minimal-to-moderate basophilic renal tubules in both sexes and hematuria in females Serious LOAEL: minimal-to-severe degeneration and necrosis of renal proximal convoluted tubules
					Neuro	1,000		2,000	Ataxia in both sexes; minimal cerebellar degeneration (granular layer) in females
Morgan et al. 1982									
29	Mouse (C57Bl/6) 5 M, 5 F	12 days 6 hours/day	0, 500, 1,000, 2,000	LE, CS, HP	Death			2,000	100% mortality
					Hepatic		500	2,000 M	LOAEL: minimal hepatocellular degeneration Serious LOAEL: severe hepatocellular degeneration and necrosis
					Renal	500	1,000	2,000	LOAEL: minimal basophilic renal tubules in males and hematuria in females Serious LOAEL: moderate degeneration and necrosis of renal proximal convoluted tubules
					Neuro	500	1,000 M	1,000 F	Cerebellar degeneration (granular layer; minimal in males, moderate-to-severe in females)

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Morgan et al. 1982									
30	Mouse (C3H) 5 M, 5 F	12 days 6 hours/day	0, 500, 1,000, 2,000	LE, CS, HP	Death			2,000 F	100% mortality
					Hepatic	2,000 F	500 M		Minimal hepatocellular degeneration
					Renal	500	1,000	2,000	LOAEL: minimal-to-moderate basophilic renal tubules in both sexes; hematuria in females Serious LOAEL: severe degeneration and necrosis of renal proximal convoluted tubules in both sexes; hematuria in males
					Neuro	1,000		2,000	Ataxia
von Oettingen et al. 1949, 1950									
31	Mouse (White) 20 NS	7 hours	2,900, 3,100, 3,400, 3,750, 5,100	LE, CS	Death			3,080	LC_{50}
					Neuro			2,900	Convulsions, decreased activity
Wolkowski-Tyl et al. 1981a, 1983a									
32	Mouse (C57BL/6); fetus (B6C3F1) 33 F	12 days 6 hours/day GDs 6–17	0, 102, 479, 1,492	LE, BW, FI, WI, RX, DX	Death			1,492	100% mortality/moribundity
					Neuro	479		1,492	Tremors, piloerection, difficulty righting, focal granule cell necrosis in cerebellum
					Repro	479			
					Develop	102		479	Increased heart defects (reduction or absence of valves and muscles)

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Wolkowski-Tyl et al. 1981b, 1983b									
33	Mouse (C57BL/6); fetus (B6C3F1) 74–77 F	12 days 6 hours/day GDs 6–17	0, 251, 502, 749	LE, BW, OW, RX, DX	Death			749	6/75 died, 1/75 moribund
					Bd wt	251		749	41% decrease in maternal body weight gain during gestation
					Hepatic	749			
					Neuro	251		502	Ataxia; tremors, convulsions, increased then reduced activity, hypersensitivity to touch and sound at 749 ppm
					Repro	502			
					Develop	251		502	Increased heart defects (reduction or absence of valves and muscles)
Dunn and Smith 1947; Smith and von Oettingen 1947a, 1947b									
34	Guinea Pig (NS) 22–62 NS	6 days 6 hours/day	0, 300, 500, 1,000, 2,000, 3,000	LE, CS, HP	Death			1,000	50% mortality by day 4; 100% mortality at ≥2,000 ppm
					Resp	1,000		2,000	Marked lung congestion and edema
					Hepatic	500	1,000		Fatty metamorphosis
					Renal	500	1,000		Fatty metamorphosis
					Neuro	500		1,000	Convulsions, lost righting reflex, backward arching of the head, neck, and spine
McKenna et al. 1981a									
35	Dog (Beagle) 3 M	3 days 23.5 hours/ day	0, 197, 496	CS, BC, HE, OP, GN, OW, HP, NX	Bd wt	496			
					Resp	496			
					Cardio	496			
					Gastro	496			
					Hemato	496			
					Hepatic	496			
					Renal	496			

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Dermal	496			
					Ocular	496			
					Endocr	496			
					Neuro	197		496	Clinical signs of neurotoxicity (incoordination, impaired gait, limb paresis and stiffness, tremors, ataxia); Slight, multifocal lesions in brain and spinal cord; vacuolization, swollen axons, and loss of axons
					Repro	496			
Smith and von Oettingen 1947a, 1947b									
36	Dog (NS) 6–12 NS	2 weeks 6 days/week 6 hours/day	0, 300, 500, 1,000, 2,000, 3,000	LE, CS	Death			1,000	5/10 died; 100% mortality at 3,000 ppm
					Resp	500		1,000	Dyspnea (prior to death)
					Neuro	300		500	Severe clinical signs of neurotoxicity (e.g., tremors, spasticity, impaired gait)
McKenna et al. 1981a									
37	Cat (NS) 3 M	3 days 23.5 hours/ day	0, 192, 501	CS, BW, HE, BC, OP, GN, OW, HP	Bd wt	501			
					Resp	501			
					Cardio	501			
					Gastro	501			
					Hemato	501			
					Hepatic	501			
					Renal	501			
					Dermal	501			
					Ocular	501			
					Endocr	501			
					Neuro	501			
					Repro	501			

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Smith and von Oettingen 1947a, 1947b									
38	Cat (NS) 4 NS	6 days 6 hours/day	2,000	LE, CS	Neuro			2,000	Weakness, ataxia, loss of righting reflex
Smith and von Oettingen 1947a, 1947b									
39	Rabbit (NS) 4–12 NS	2 weeks 6 days/week 6 hours/day	0, 300, 500, 1,000, 2,000, 3,000, 4,000	LE, CS	Death			2,000	Decreased survival; 100% mortality at 4,000 ppm
					Neuro			2,000	Neuromuscular dysfunction of hindlegs; spastic adduction
INTERMEDIATE EXPOSURE									
Smith and von Oettingen 1947a, 1947b									
40	Monkey (NS) 2 NS	120 days 6 days/week 6 hours/day	300, 500	LE, CS	Death			500	2/2 died
					Neuro	300		500	Progressive debility, prostration, loss of consciousness
CIIT 1981									
41	Rat (Fischer-344) 10 M, 10 F	6 months 5 days/week 6 hours/day	0, 51, 224, 997	LE, CS, BW, HE, BC, BI, UR, OP, OW, GN, HP	Bd wt	224	997		10–11% decreased body weight
					Resp	224 F 997 M		997 F	Interstitial pneumonia with peribronchiolitis and perivasculitis, alveolar hyperplasia, alveolar luminal infiltrates, subacute tracheitis
					Cardio	997			
					Gastro	997			
					Musc/skel	997			
					Hepatic	997			
					Renal	997			
					Dermal	997			
					Ocular	997			
					Endocr	997			
					Immuno	997			
					Neuro	997			

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	224 M 997 F		997 M	Degeneration and atrophy of seminiferous tubules; sperm granulomas
Dunn and Smith 1947; Smith and von Oettingen 1947a, 1947b									
42	Rat (NS) 18–59 NS	175 days 6 days/week 6 hours/day	0, 300, 500, 1,000	LE, CS, HP	Death			1,000	100% mortality
					Resp	1,000			
					Hepatic	1,000			
					Renal	1,000			
					Neuro	1,000			
Hamm et al. 1985									
43	Rat (Fischer-344) 40 M, 80 F	2-generation study 12–19 weeks per generation 5–7 days/week 6 hours/day	0, 151, 472, 1,502	CS, BW, GN, OW, HP, RX, DX	Bd wt	472	1,502		10–20% decrease in F0 body weight gain
					Repro	151 M	472 M	1,502 M	LOAEL: decreased number of fertile F0 males, decreased number of litters per copulation plug in F0 rats Serious LOAEL: 100% F0 male sterility, atrophy of the seminiferous tubules, epididymal granulomas
					Develop	472			
McKenna et al. 1981b									
44	Rat (Sprague-Dawley) 10 M, 10 F	93 days 5 days/week 6 hours/day	0, 51, 149, 399	LE, BW, OW, GN, HP, BC, CS, UR, HE, NX	Bd wt	399			
					Resp	399			
					Cardio	399			
					Gastro	399			
					Hemato	399			
					Musc/skel	399			
					Hepatic	399 F 149 M	399 M		Increased relative liver weight
					Renal	399			

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Dermal	399			
					Immuno	399			
					Neuro	51[c]	149		Impaired sensorimotor function
					Repro	399 M			
Mitchell et al. 1979									
45	Rat (Fischer-344) 10 M, 10 F	90 days 5 days/week 6 hours/day	0, 368, 741, 1,473	BW, OW, FI, HP, BC, CS, UR, HE, OP	Bd wt	741 F 368 M	1,473 F 741 M	1,473 M	LOAEL: 10–11% decrease in body weight Serious LOAEL: 22% decrease in male body weight
					Resp	1,473			
					Cardio	1,473			
					Hemato	1,473			
					Musc/skel	1,473			
					Hepatic	1,473			
					Renal	1,473			
					Dermal	1,473			
					Ocular	1,473			
					Endocr	1,473			
					Immuno	1,473			
					Neuro	1,473			
					Repro	1,473			
CIIT 1981									
46	Mouse (B6C3F1) 9 M, 11 F	6 months 5 days/week 6 hours/day	0, 51, 224, 997	LE, BW, BI, OW, GN, HP, BC, CS, UR, HE, OP	Bd wt	224 F 997 M	997 F		16% decrease in body weight
					Resp	997			
					Cardio	997			
					Hemato	997			
					Musc/skel	997			

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic	224	997		Hepatocellular degeneration (males: diffuse that was midzonal; females: diffuse or multifocal centrilobular)
					Renal	997 F 224 M	997 M		Decreased absolute and relative kidney weight
					Dermal	997			
					Ocular	997			
					Endocr	997			
					Immuno	224	997		Lymphoid depletion of spleen in males and females; thymic lymphoid necrosis in females
					Repro	997			
					Neuro	997			
McKenna et al. 1981b									
47	Mouse (CD-1) 10 M, 10 F	94 days 5 days/week 6 hours/day	0, 51, 149, 399	LE, BW, OW, GN, HP, CS, NX	Resp	399			
					Cardio	399 F 149 M	399 M		Increased relative heart weight
					Gastro	399			
					Hemato	399			
					Musc/skel	399			
					Hepatic	399 M 149 F	399 F		Increased relative liver weight
					Renal	399			
					Dermal	399			
					Immuno	399			
					Neuro	399			
					Repro	399 M			

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Mitchell et al. 1979									
48	Mouse (B6C3F1) 10 M, 10 F	90 days 5 days/week 6 hours/day	0, 368, 741, 1,473	BW, OW, FI, HP, BC, CS, UR, HE, OP	Bd wt	1,473			
					Resp	1,473			
					Cardio	1,473			
					Gastro	1,473			
					Hemato	1,473			
					Musc/skel	1,473			
					Hepatic	368	741		Increased relative liver weight
					Renal	1,473			
					Dermal	1,473			
					Ocular	1,473			
					Endocr	1,473			
					Immuno	1,473			
					Neuro	1,473			
					Repro	1,473			
Smith and von Oettingen 1947a, 1947b									
49	Mouse (Swiss, Strain A, C3H) 22–34 NS	266 days 6 days/week 6 hours/day	0, 300, 500, 1,000	LE, CS	Death			500	82% mortality in adults, 27% mortality in “young” animals; 100% mortality at 1,000 ppm
					Neuro	300		500	Persistent neuromuscular abnormalities, impaired gait, hindlimb drag
Smith and von Oettingen 1947a, 1947b									
50	Guinea Pig (NS) 22–36 NS	266 days 6 days/week 6 hours/day	0, 300, 500, 1,000	LE, CS	Death			500	84% mortality in adult mice; 53% mortality in “young” mice; 100% mortality at 1,000 ppm
					Neuro	500		1,000	Progressive weakness, inability to walk, convulsions, loss of righting reflex

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
McKenna et al. 1981b									
51	Dog (Beagle) 4 M	93 days 5 days/week 6 hours/day	0, 51, 149, 399	LE, BW, OW, GN, HP, CS, UR, HE, BC	Resp	399			
					Cardio	399			
					Gastro	399			
					Hemato	399			
					Musc/skel	399			
					Hepatic	399			
					Renal	399			
					Dermal	399			
					Ocular	399			
					Immuno	399			
					Neuro	399			
					Repro	399			
Smith and von Oettingen 1947a, 1947b									
52	Dog (NS) 6–12 NS	211 days 6 days/week 6 hours/day	0, 300, 500, 1,000	LE, CS	Death			500	4/6 dogs died; 100% mortality at 1,000 ppm
					Resp	500		1,000	Dyspnea (prior to death)
					Neuro	300		500	Severe clinical signs of neurotoxicity (e.g., tremors, spasticity, impaired gait)
Dunn and Smith 1947; Smith and von Oettingen 1947a, 1947b									
53	Cat (NS) 4 NS	32 days 6 days/week 6 hours/day	2,000	LE, CS, HP	Death			2,000	4/4 died
					Resp			2,000	Gasping, pulmonary congestion
					Neuro			2,000	Inability to walk, extensor spasms, heightened reflexes
Smith and von Oettingen 1947a, 1947b									
54	Rabbit (NS) 4–12 NS	266 days 6 days/week 6 hours/day	0, 300, 500, 1,000, 2,000	LE, CS	Death			500	50% mortality; 100% mortality at 1,000 ppm

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Theuns-van Vliet 2016									
55	Rabbit (New Zealand) 22 F	23 days 6 hours/day GDs 6–28	0, 265, 511, 1012	CS, BW, FI, GN, OW, DX, RX	Bd wt	1,012			
					Repro	1,012			
					Develop	1,012			
CHRONIC EXPOSURE									
CIIT 1981									
56	Rat (Fischer-344) 10 M, 10 F	12 months 5 days/week 6 hours/day	0, 51, 224, 997	LE, BW, BI, OW, GN, HP, BC, CS, UR, HE, OP	Bd wt	997 M 224 F	997 F		10% decrease in body weight
					Resp	997			
					Cardio	997 F 224 M	997 M		Increased absolute and relative heart weight
					Gastro	997			
					Hemato	997			
					Musc/skel	997			
					Hepatic	997 F 224 M	997 M		Increased serum ALT levels
					Renal	997			
					Dermal	997			
					Endocr	997			
					Immuno	997			
					Neuro	997			
					Repro	224 M 997 F		997 M	Degeneration and atrophy of seminiferous tubules
CIIT 1981									
57	Rat (Fischer-344) 20 M, 20 F	18 months 5 days/week 6 hours/day	0, 51, 224, 997	LE, BW, BI, OW, GN, HP, BC, CS, UR, HE, OP, NX	Bd wt	997 F 224 M	997 M		12% decrease in body weight
					Resp	997			
					Cardio	997			
					Gastro	997			

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hemato	997			
					Musc/skel	997			
					Hepatic	997			
					Renal	997			
					Dermal	997			
					Endocr	997			
					Immuno	997			
					Neuro	997			
					Repro	224 M 997 F		997 M	Degeneration and atrophy of seminiferous tubules; sperm granulomas
CIIT 1981									
58	Rat (Fischer-344) 65–68 M; 57–61 F	21–24 months 5 days/week 6 hours/day	0, 51, 224, 997	LE, BW, BI, OW, GN, HP, BC, CS, UR, HE, OP, NX	Bd wt	224 F 997 M	997 F		10% decrease in body weight
					Resp	997			
					Cardio	997 F 224 M	997 M		Increased relative heart weight
					Gastro	997			
					Hemato	997			
					Musc/skel	997			
					Hepatic	997			
					Renal	997			
					Dermal	997			
					Ocular	997			
					Endocr	997			
					Immuno	997			
					Neuro	997			
					Repro	224 M 997 F		997 M	Degeneration and atrophy of seminiferous tubules; sperm granulomas

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
CIIT 1981									
59	Mouse (B6C3F1) 10 M, 10 F	12 months 5 days/week 6 hours/day	0, 51, 224, 997	LE, BW, BI, OW, GN, HP, BC, CS, UR, HE, OP	Death			997 F	10% decrease in survival
					Bd wt	224	997		15–18% decrease in body weight
					Resp	997			
					Cardio	224 F 997 M	997 F		Increased absolute and relative heart weight
					Hemato	997			
					Musc/skel	997			
					Hepatic	224	997		Increased absolute and relative liver weight in females; Increased serum ALT, necrosis, cytomegaly, karyomegaly, and polykaryocytes in males
					Renal	997 F 224 M	997 M		Renal tubule hyperplasia
					Dermal	997			
					Ocular	997			
					Endocr	997			
					Immuno	997			
					Neuro	997			
					Repro	997			

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
CIIT 1981									
60	Mouse (B6C3F1) 7 M; 8–10 F	18 months 5 days/week 6 hours/day	0, 51, 224, 997	LE, BW, BI, OW, GN, HP, BC, CS, UR, HE, OP, NX	Death			997 F	17% decrease in survival
					Bd wt	224		997	20–25% decrease in body weight
					Resp	997			
					Cardio	997			
					Hemato	997			
					Musc/skel	997			
					Hepatic	224	997 F	997 M	LOAEL: increased absolute and relative liver weight Serious LOAEL: increased serum ALT, centrilobular degeneration, karyomegaly, and cytomegaly
					Renal	997 F 224 M	997 M		Renal tubule hyperplasia
					Dermal	997			
					Ocular	997			
					Endocr	997			
					Immuno	224	997		Diffuse splenic atrophy in mice that died
					Neuro		51[d]	997	LOAEL: swelling and degeneration of axons in spinal cord Serious LOAEL: tremor, paralysis, altered neurofunction (abnormal gait and reflexes), minimal-to-mild degeneration of cerebellar granule cell neurons
					Repro	224 M 997 F		997 M	Testicular seminiferous tubule degeneration and atrophy

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
CIIT 1981									
61	Mouse (B6C3F1) 20–32 M; 57–68 F	21–24 months 5 days/week 6 hours/day	0, 51, 224, 997	LE, BW, BI, OW, GN, HP, BC, CS, UR, HE, OP, NX	Death			997	100% mortality
					Bd wt	224		997	Body weight decreased by 31% in males and 36% in females in animals sacrificed moribund at 21–22 months
					Resp	997			
					Cardio	51 F 997 M	224 F		Increased relative heart weight
					Hemato	997			
					Musc/skel	997			
					Hepatic	224		997	Necrosis, cytomegaly, karyomegaly, and polykaryocytes (males sacrificed at 21 months, females at 22 months)
					Renal	224	997		Renal tubule hyperplasia
					Dermal	997			
					Ocular	997			
					Endocr	997			
					Immuno	224 F 997 M	997 F		Splenic atrophy and mild-to-moderate lymphoid depletion of the spleen and thymus
					Neuro		51[d]	997	LOAEL: swelling and degeneration of axons in spinal cord Serious LOAEL: tremor, paralysis, altered neurofunction (abnormal gait and reflexes), minimal-to-mild degeneration of cerebellar granule cell neurons
					Repro	224 M 997 F		997 M	Testicular degeneration and atrophy

Table 2-2. Levels of Significant Exposure to Chloromethane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Cancer			997 M	CEL: renal cortex adenocarcinomas, metastatic fibrosarcoma in the lung

Green shading indicates critical study selected for MRL derivation.

[a]The number corresponds to the entries in Figure 2-2.
[b]This value was used to derive the acute-duration inhalation MRL. The NOAEL of 50 was converted to a $NOAEL_{HEC}$ of 46 ppm and then divided by a total uncertainty factor of 100 resulting in a MRL of 0.5 ppm. See Appendix A for more detailed information regarding the MRL.
[c]This value was used to derive the intermediate-duration inhalation MRL. The NOAEL of 31 was converted into a $NOAEL_{HEC}$ of 9 ppm and then divided by a total uncertainty factor of 30 resulting in a MRL of 0.3 ppm. See Appendix A for more detailed information regarding the MRL.
[d]This value was used to derive the chronic-duration inhalation MRL. The LOAEL of 51 was converted to a $LOAEL_{HEC}$ of 9 ppm and then divided by a total uncertainty factor of 300 resulting in a MRL of 0.03 ppm. See Appendix A for more detailed information regarding the MRL.

ALT = alanine aminotransferase; B = both males and females; BC = serum (blood) chemistry; BI = biochemical changes; BUN = blood urea nitrogen; BW or Bd wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); FI = food intake; Gastro = gastrointestinal; GD = gestational day; GN = gross necropsy; HE or Hemato = hematological; HEC = human equivalent concentration; HP = histopathology; Immuno = immunological; LC_{50} = median lethal concentration; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OF = organ function; OP = ophthalmology; OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive function; UR = urinalysis; WI = water intake

Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Acute (≤14 days)

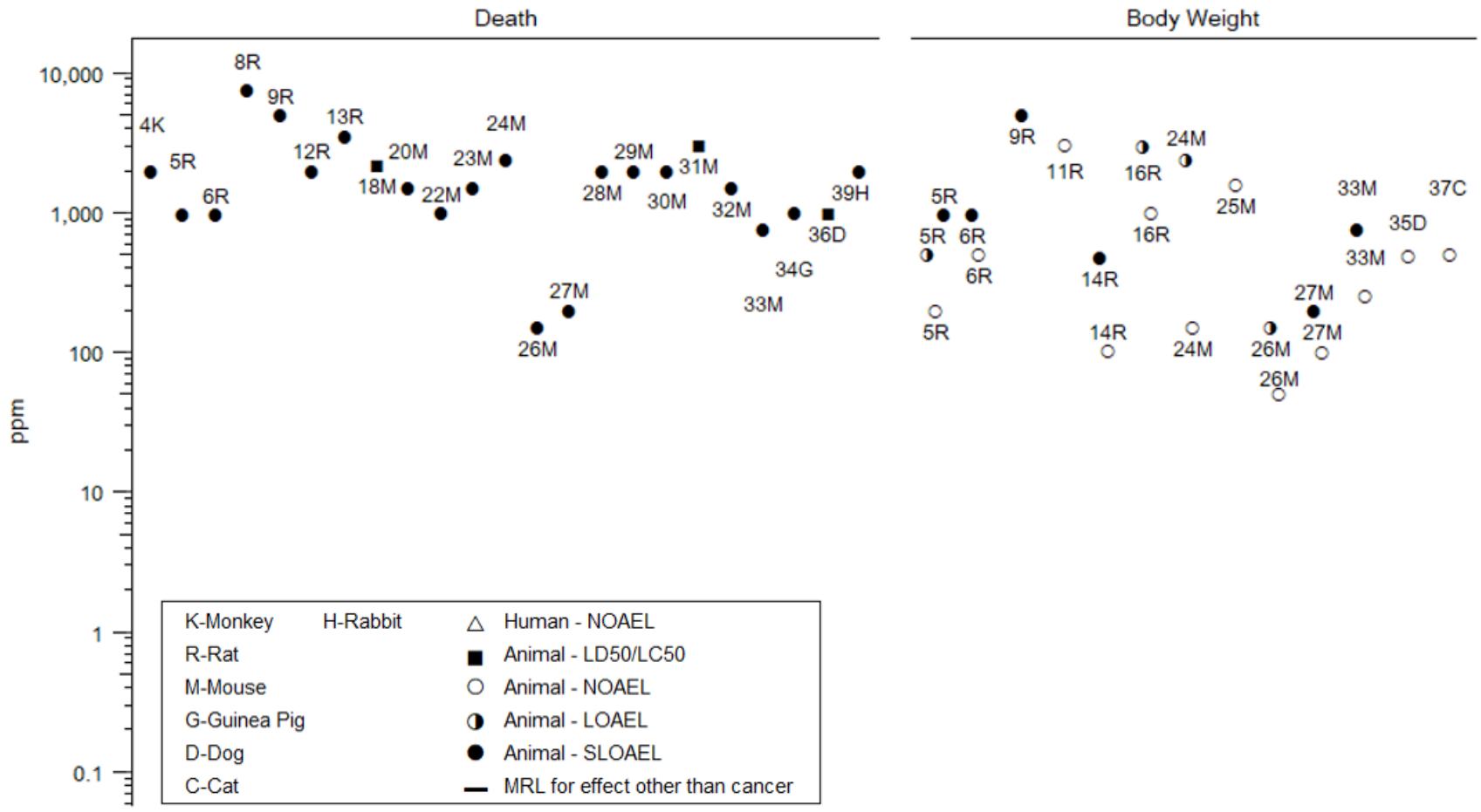


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Acute (≤14 days)

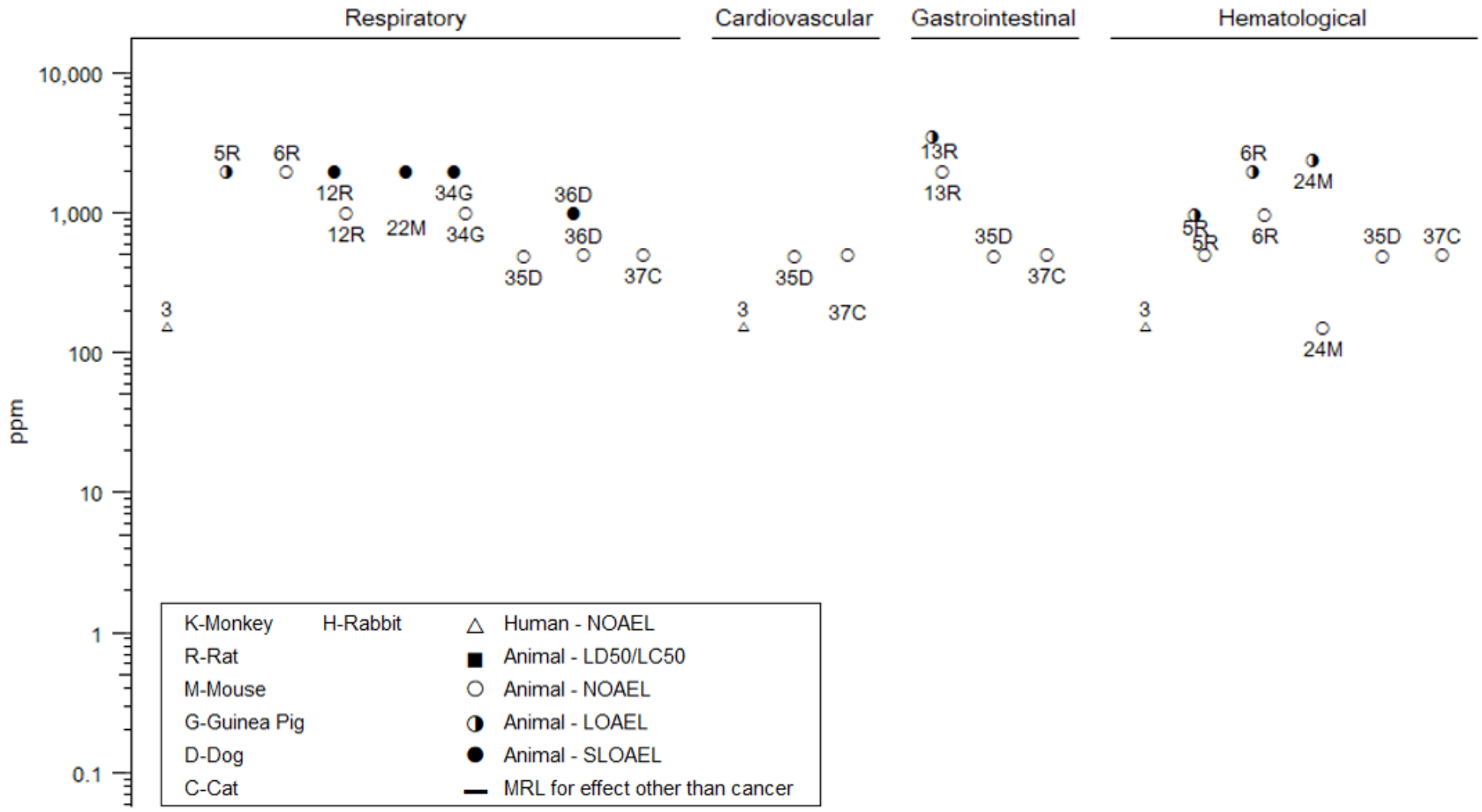


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Acute (≤14 days)

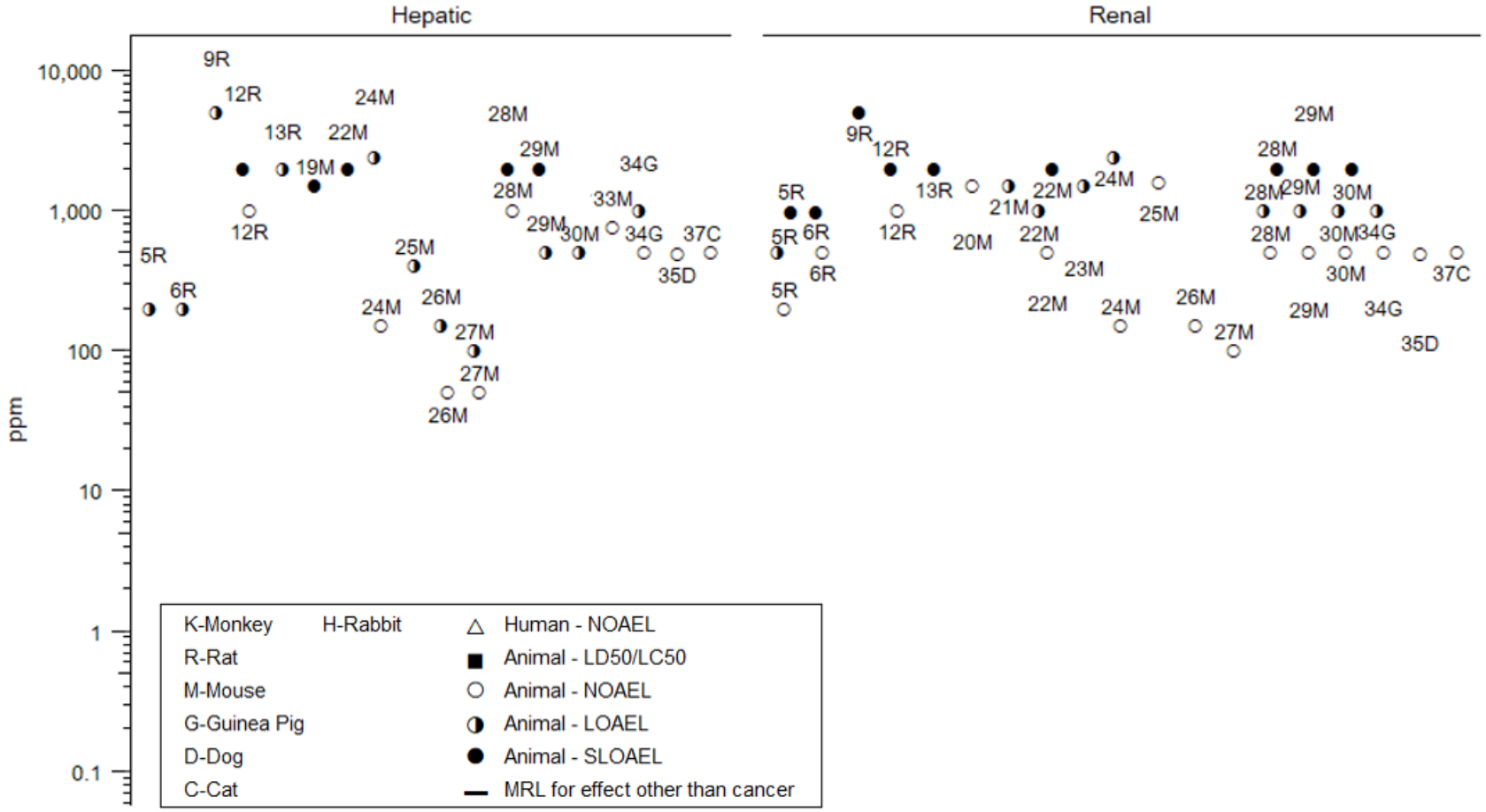


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Acute (≤14 days)

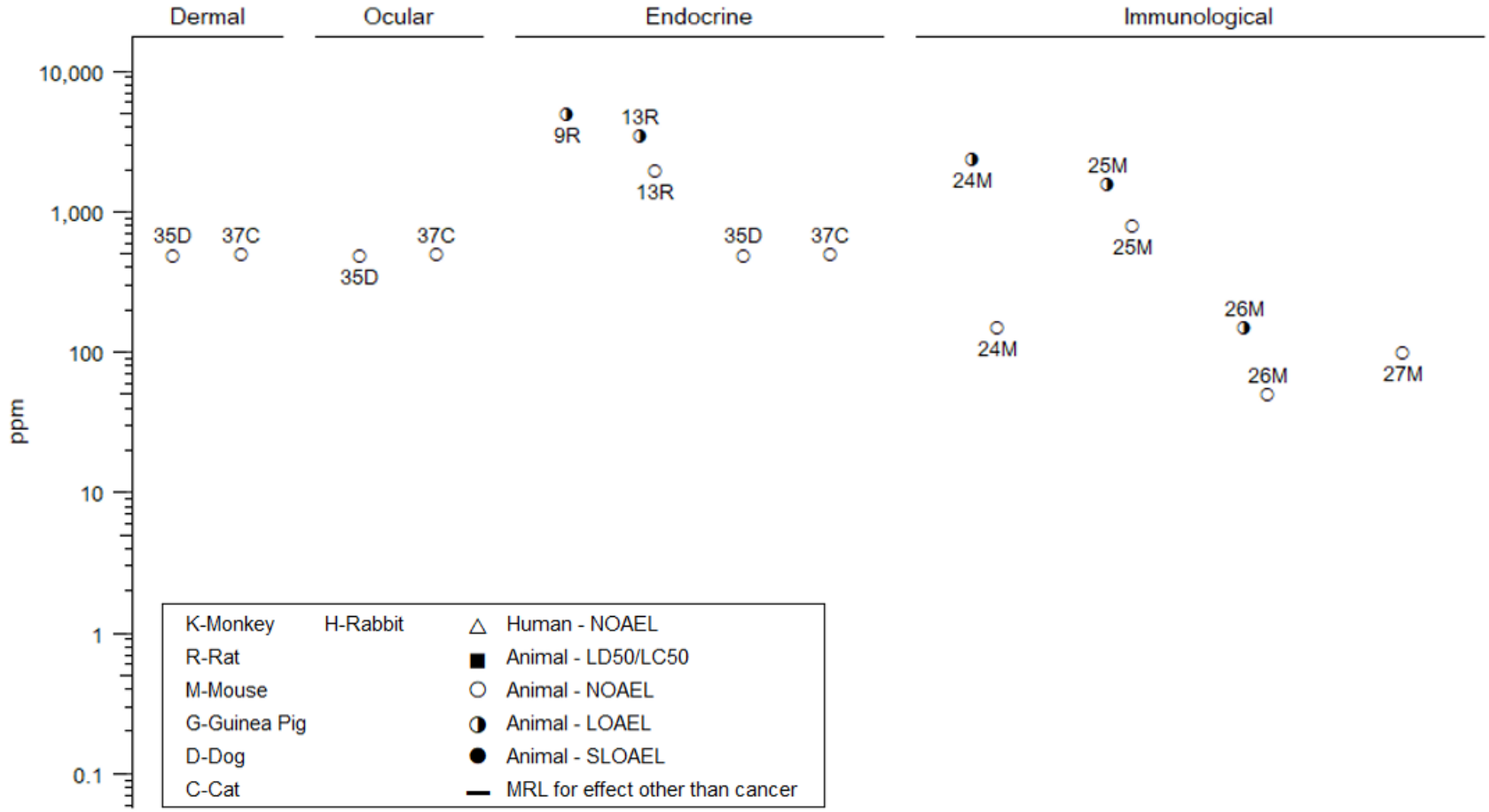


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Acute (≤14 days)

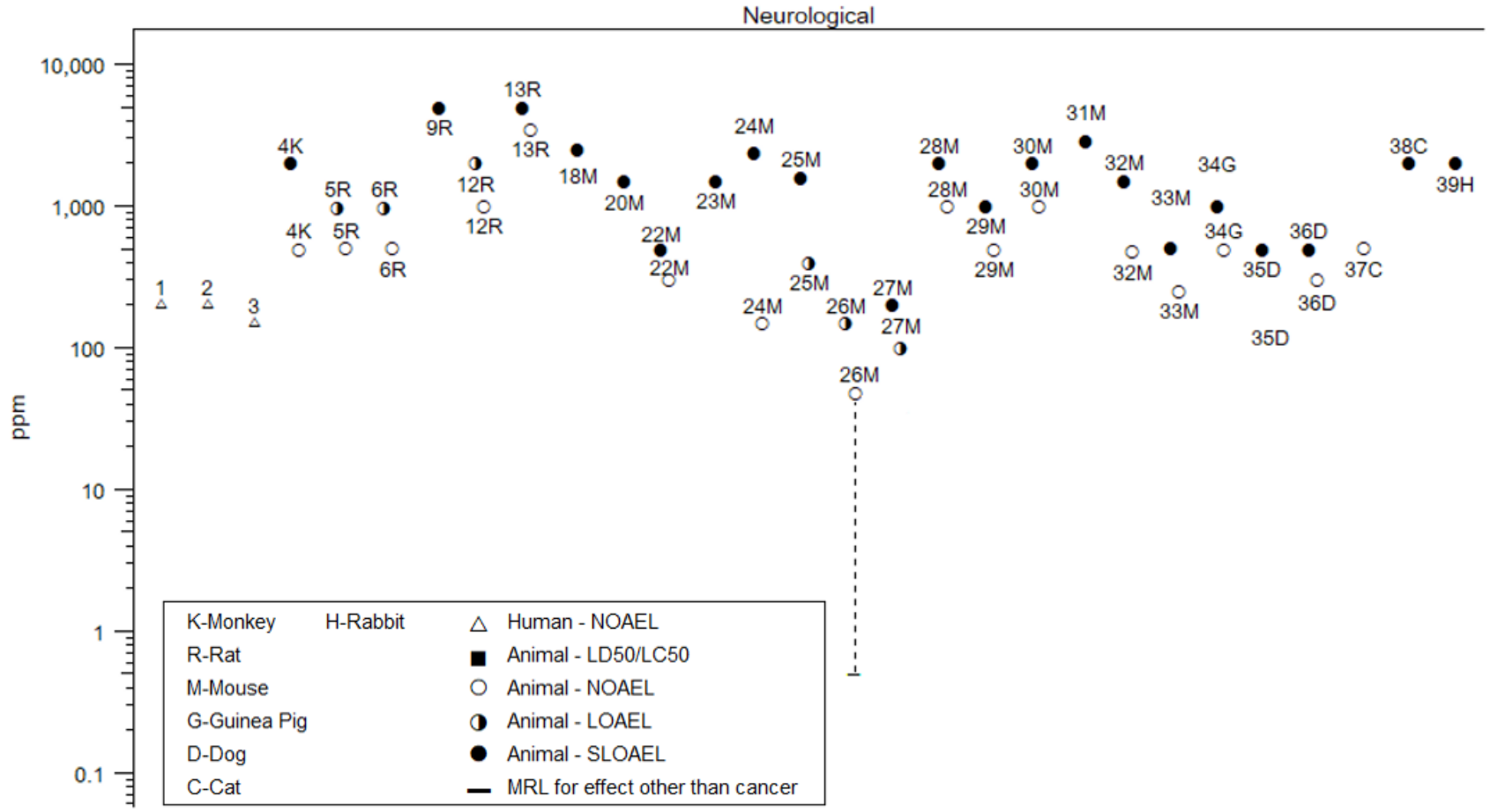


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Acute (≤14 days)

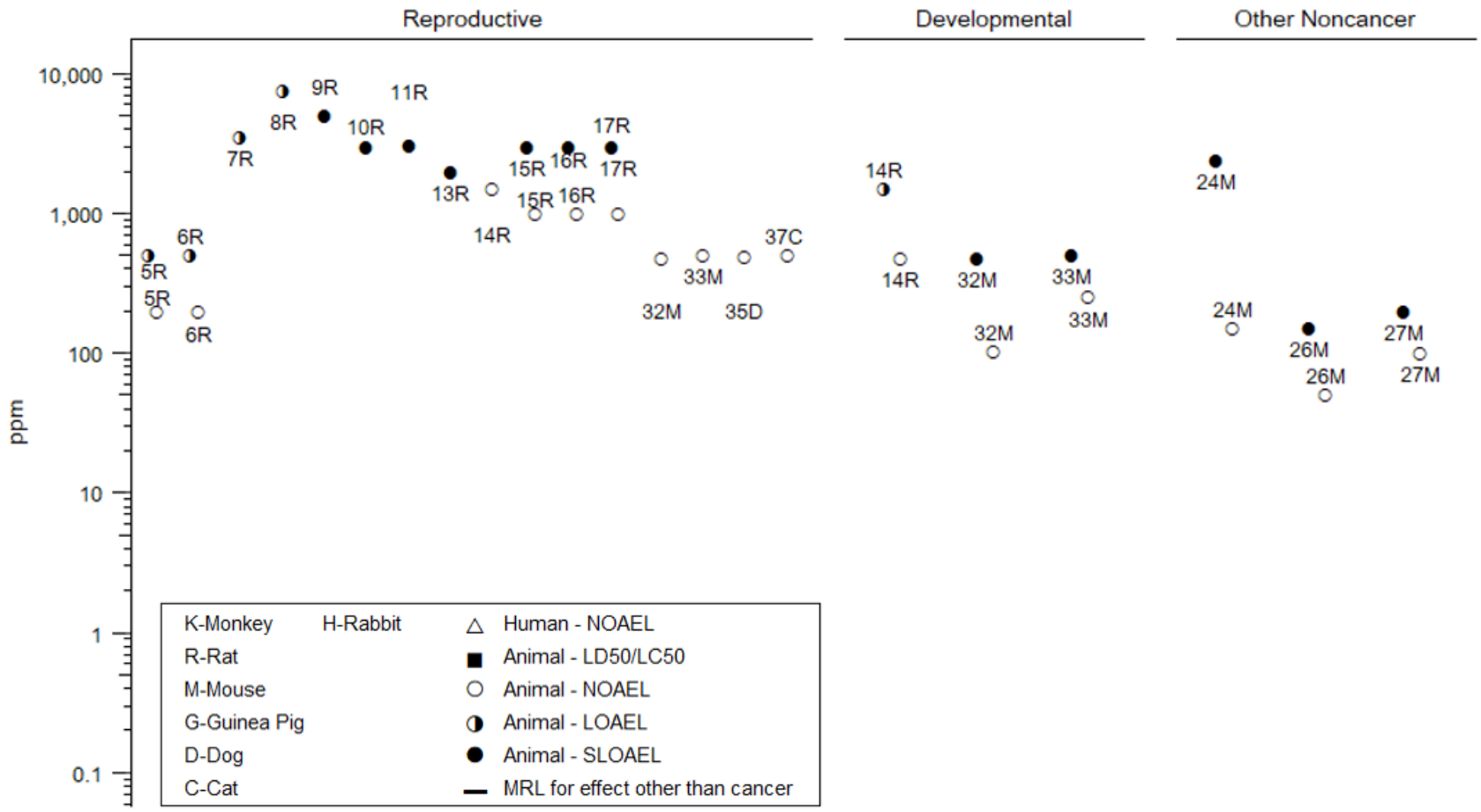


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Intermediate (15-364 days)

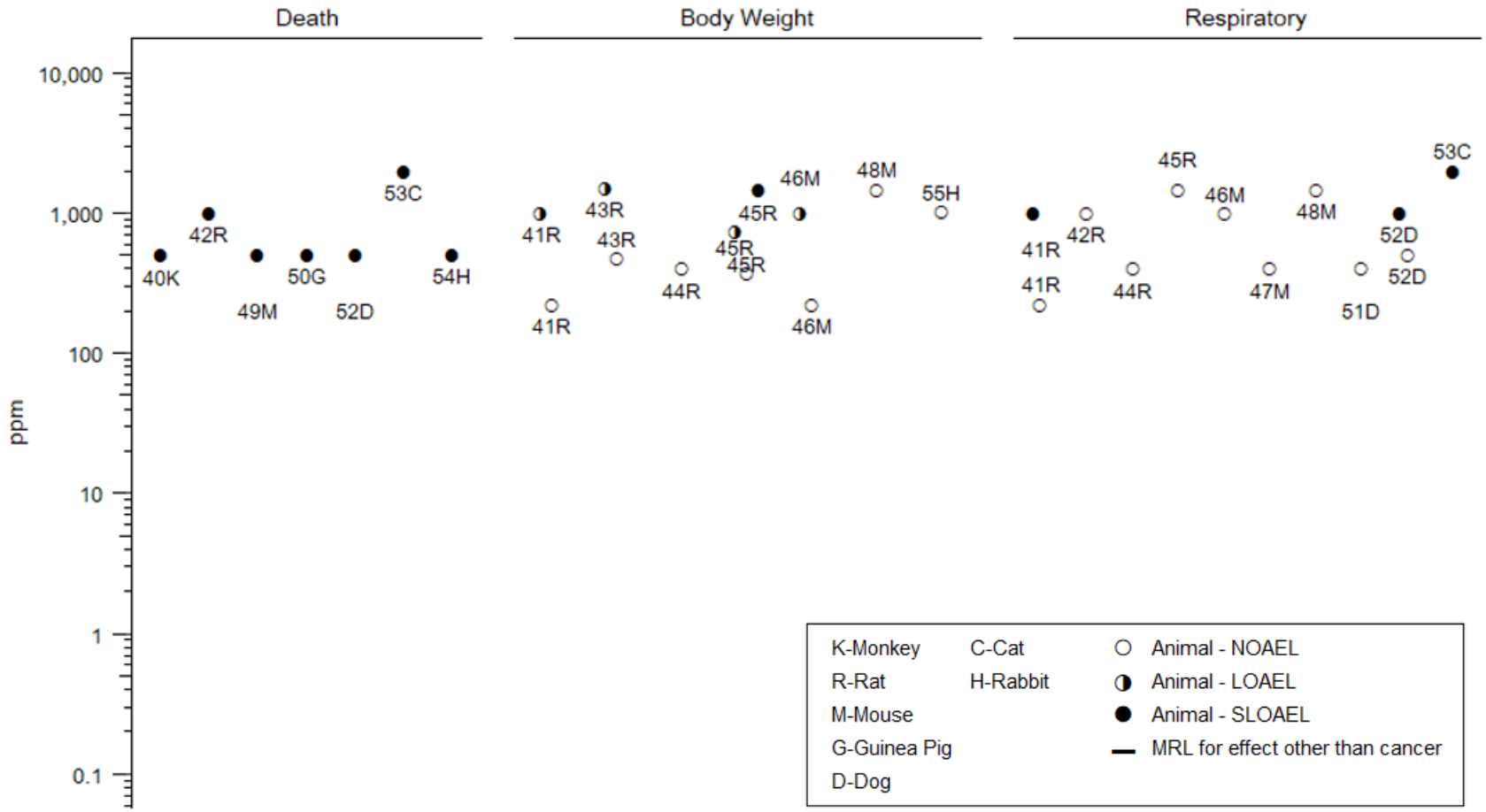


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Intermediate (15-364 days)

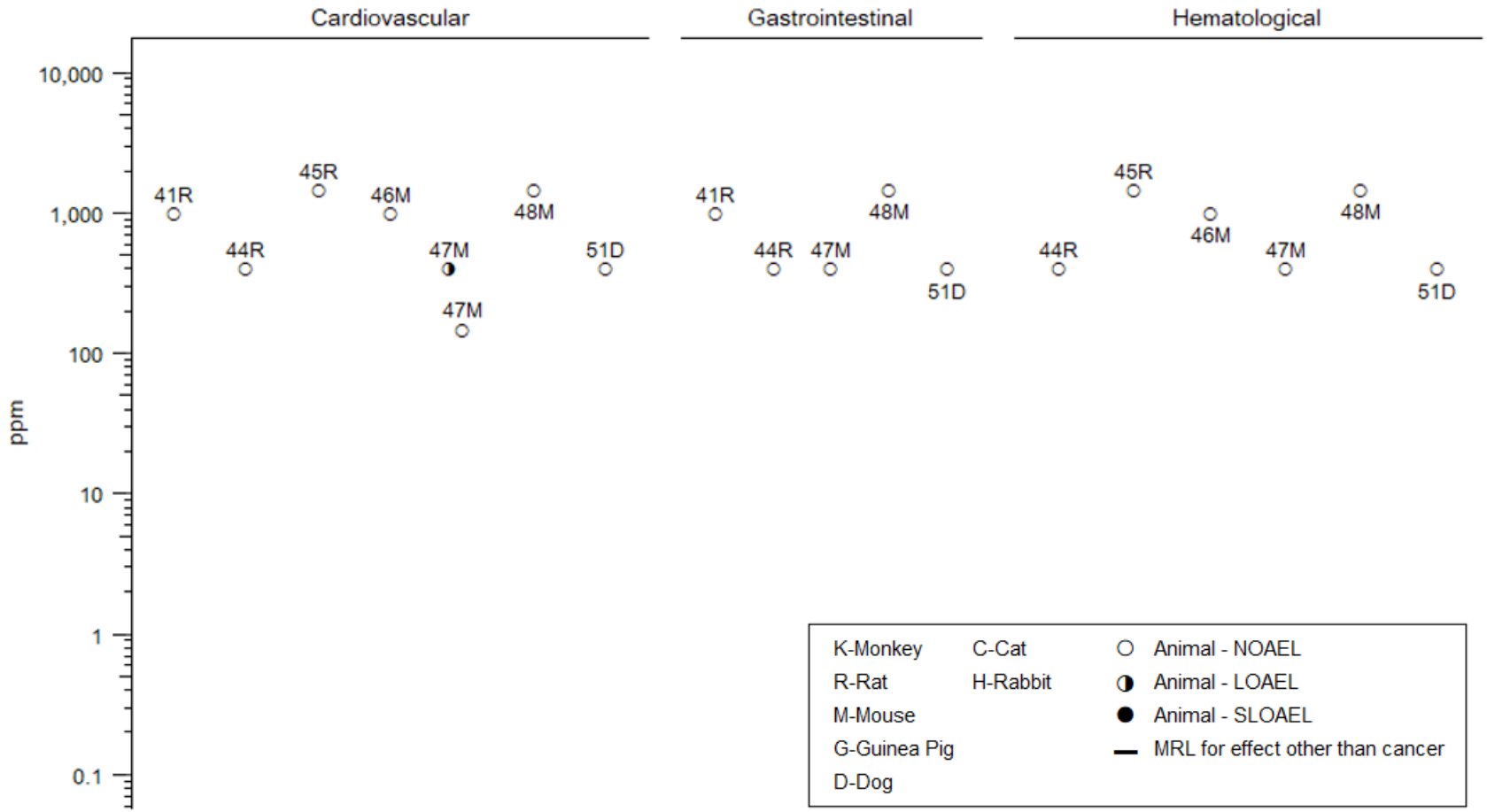


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Intermediate (15-364 days)

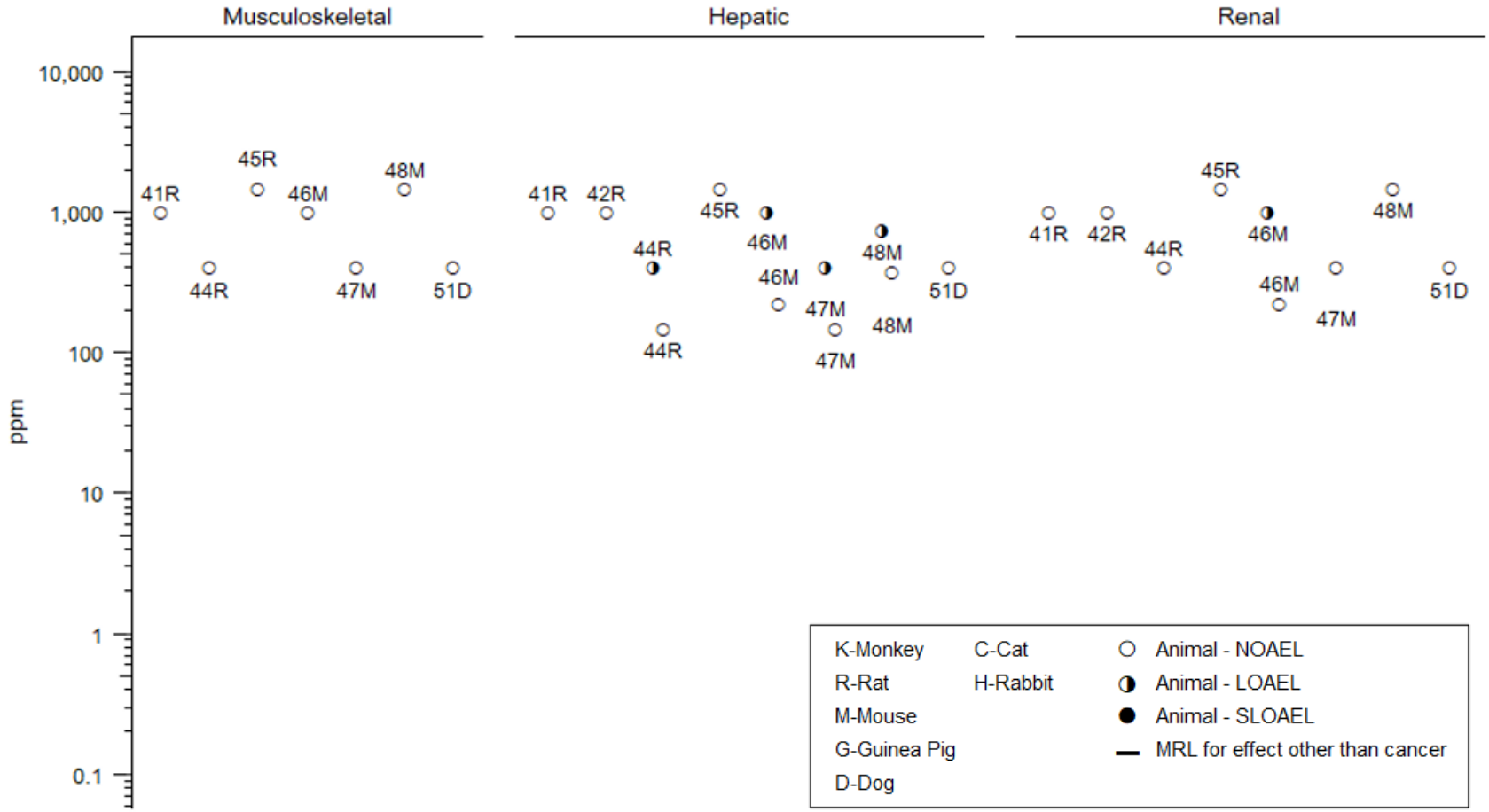


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Intermediate (15-364 days)

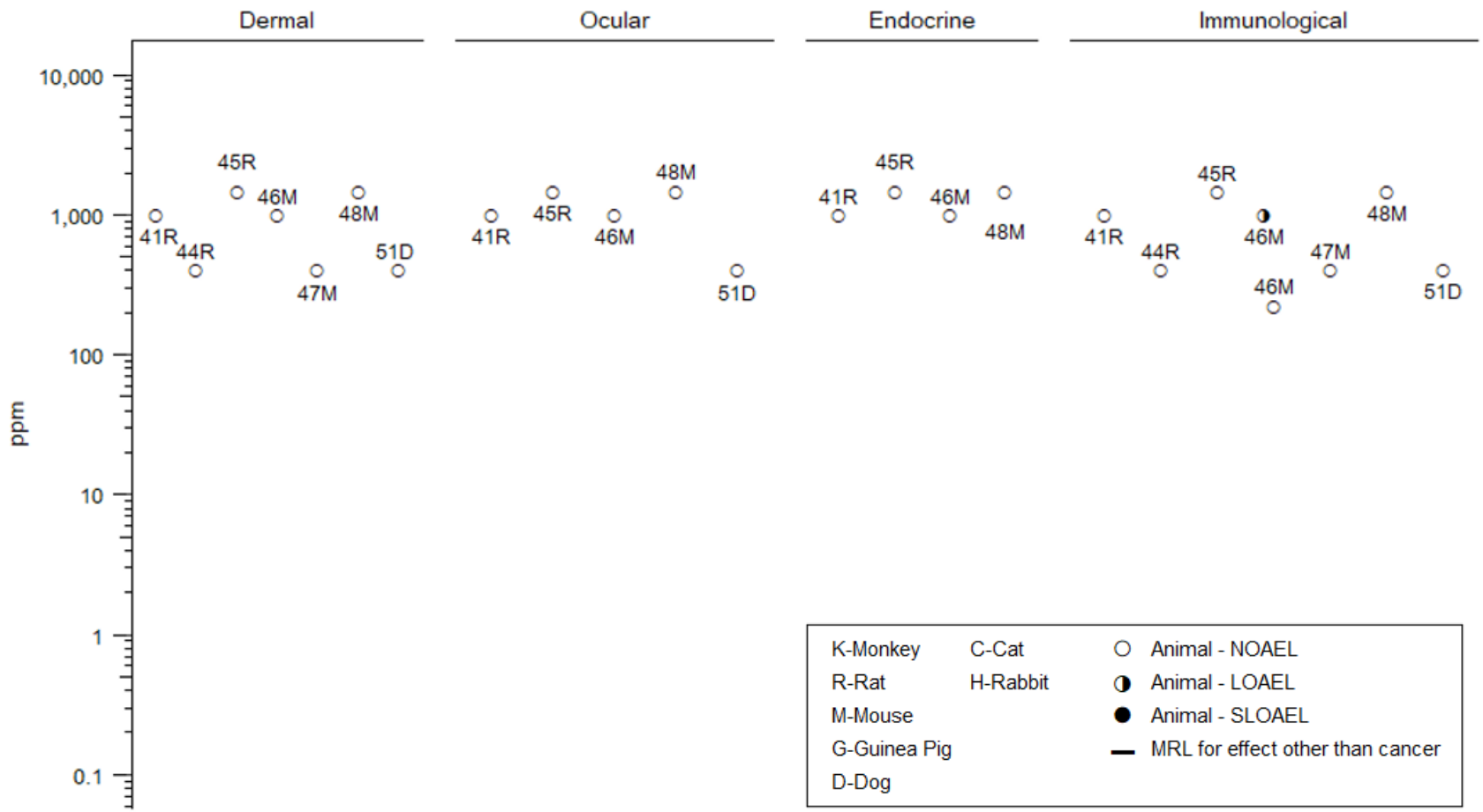


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Intermediate (15-364 days)

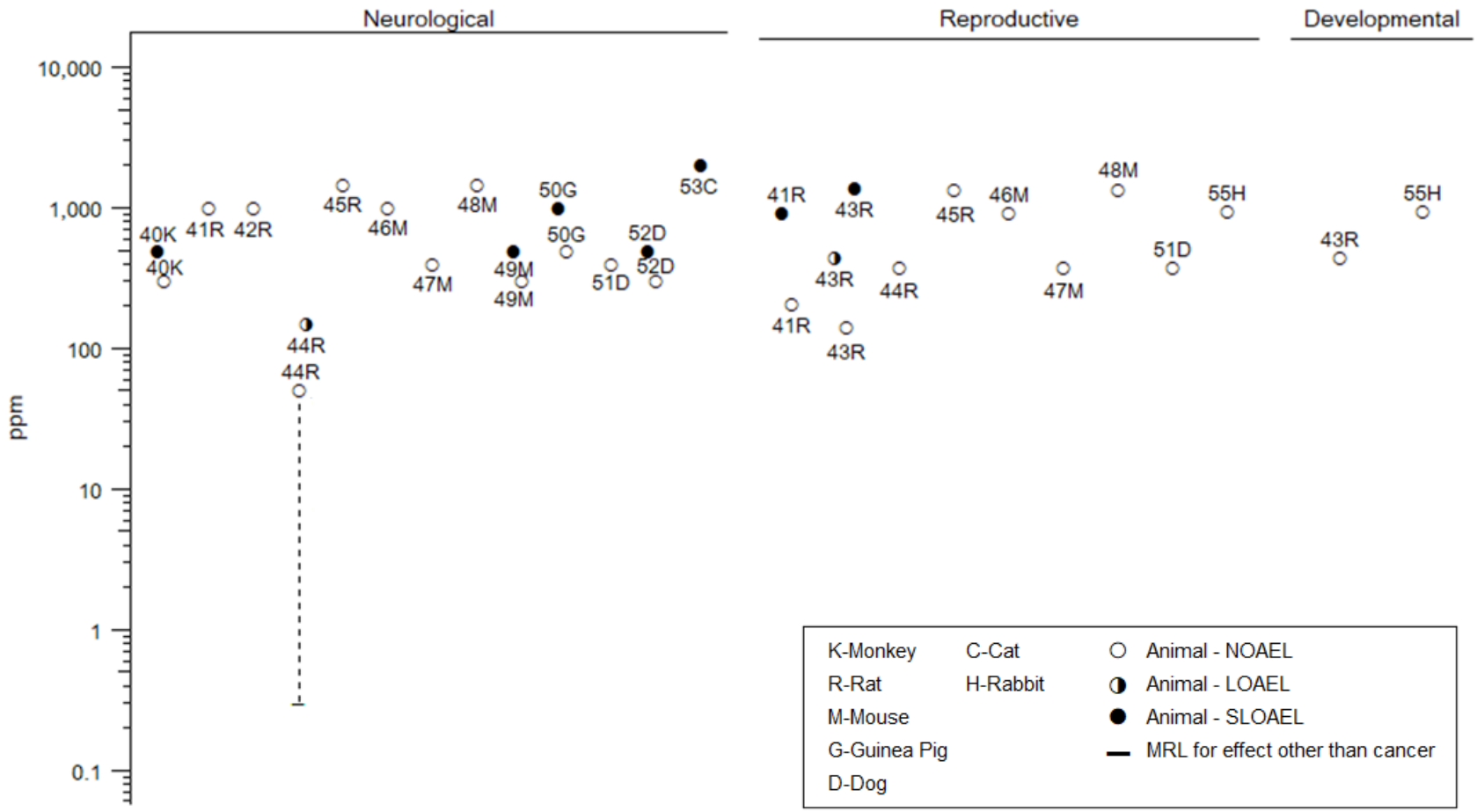


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Chronic (≥365 days)

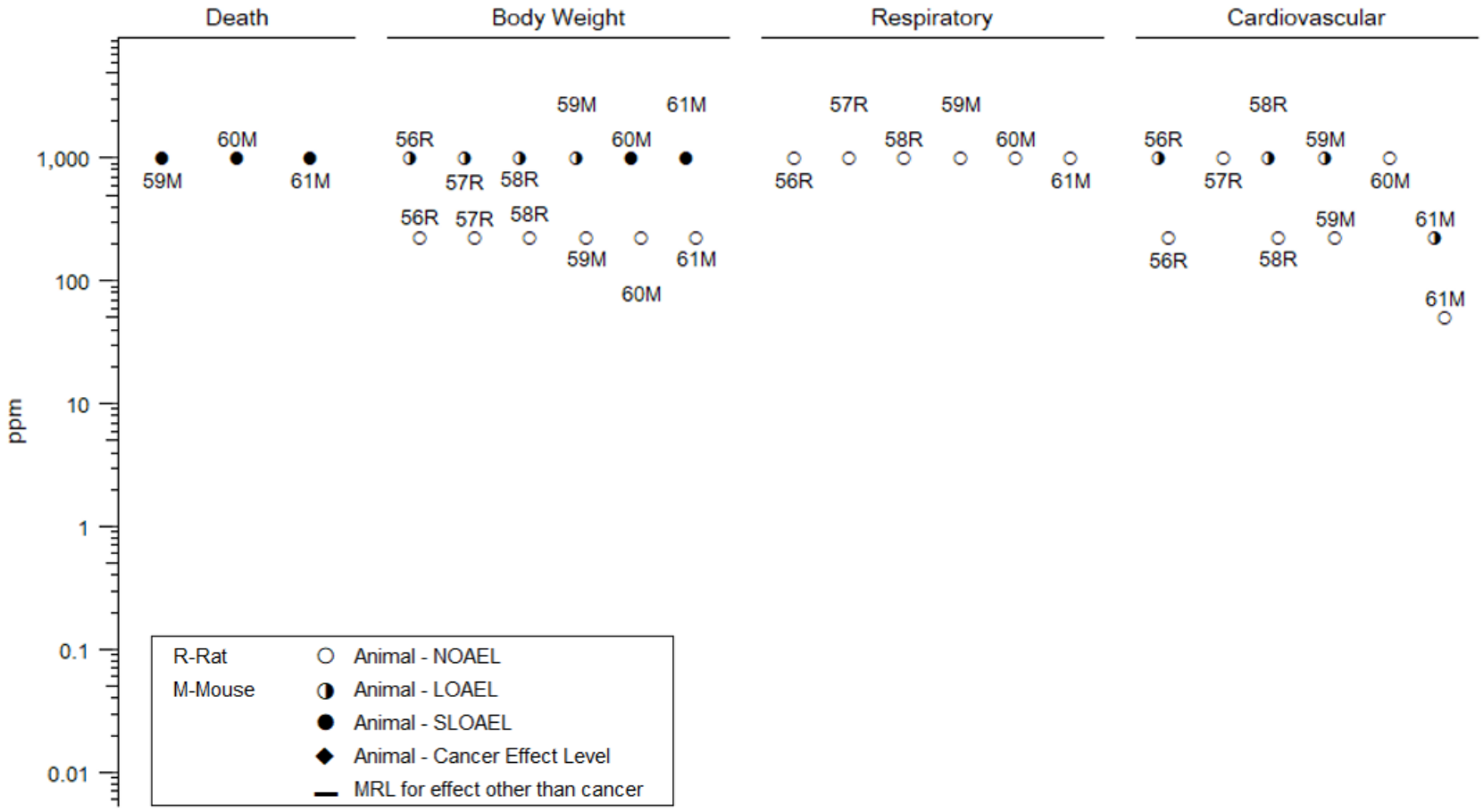


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Chronic (≥365 days)

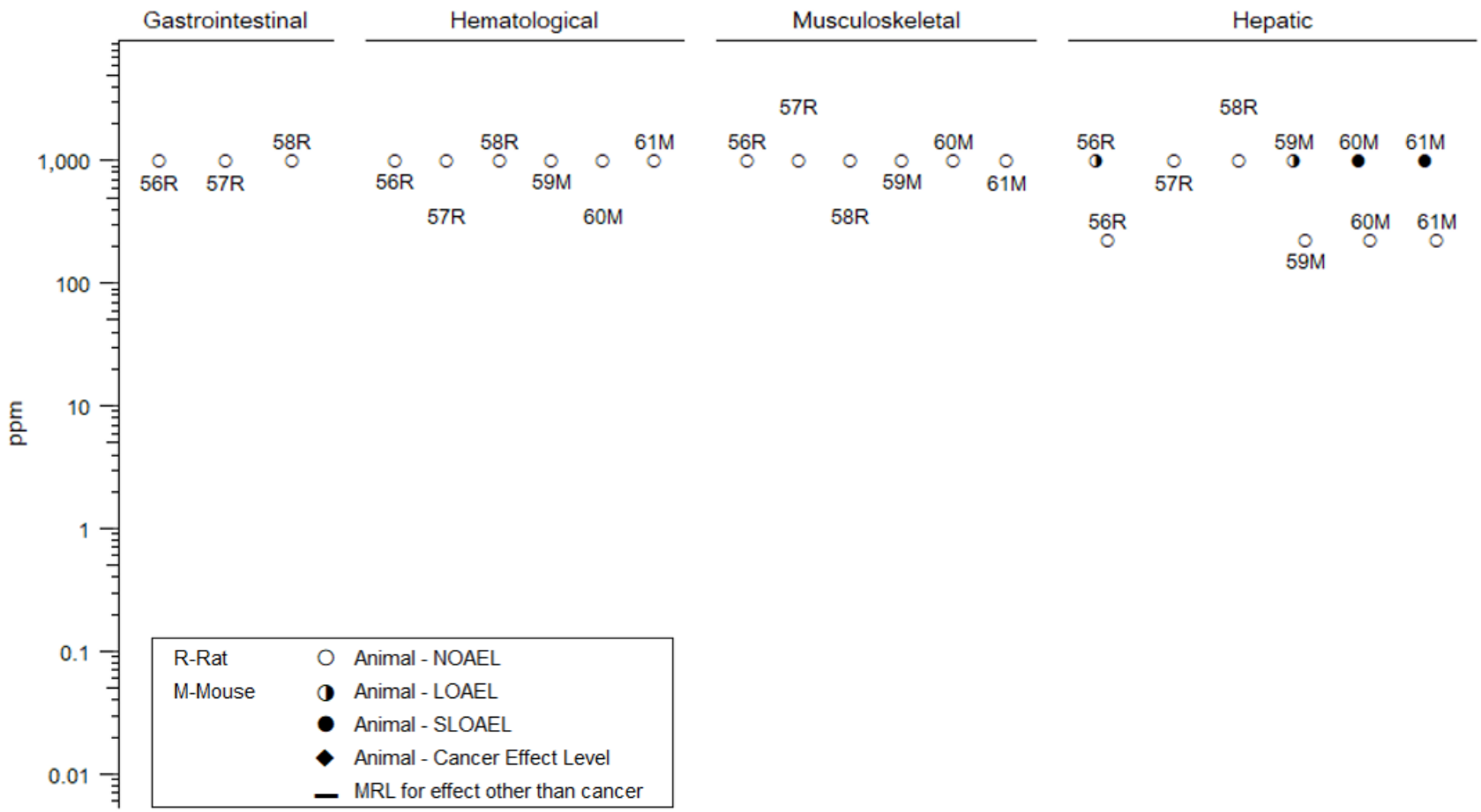


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Chronic (≥365 days)

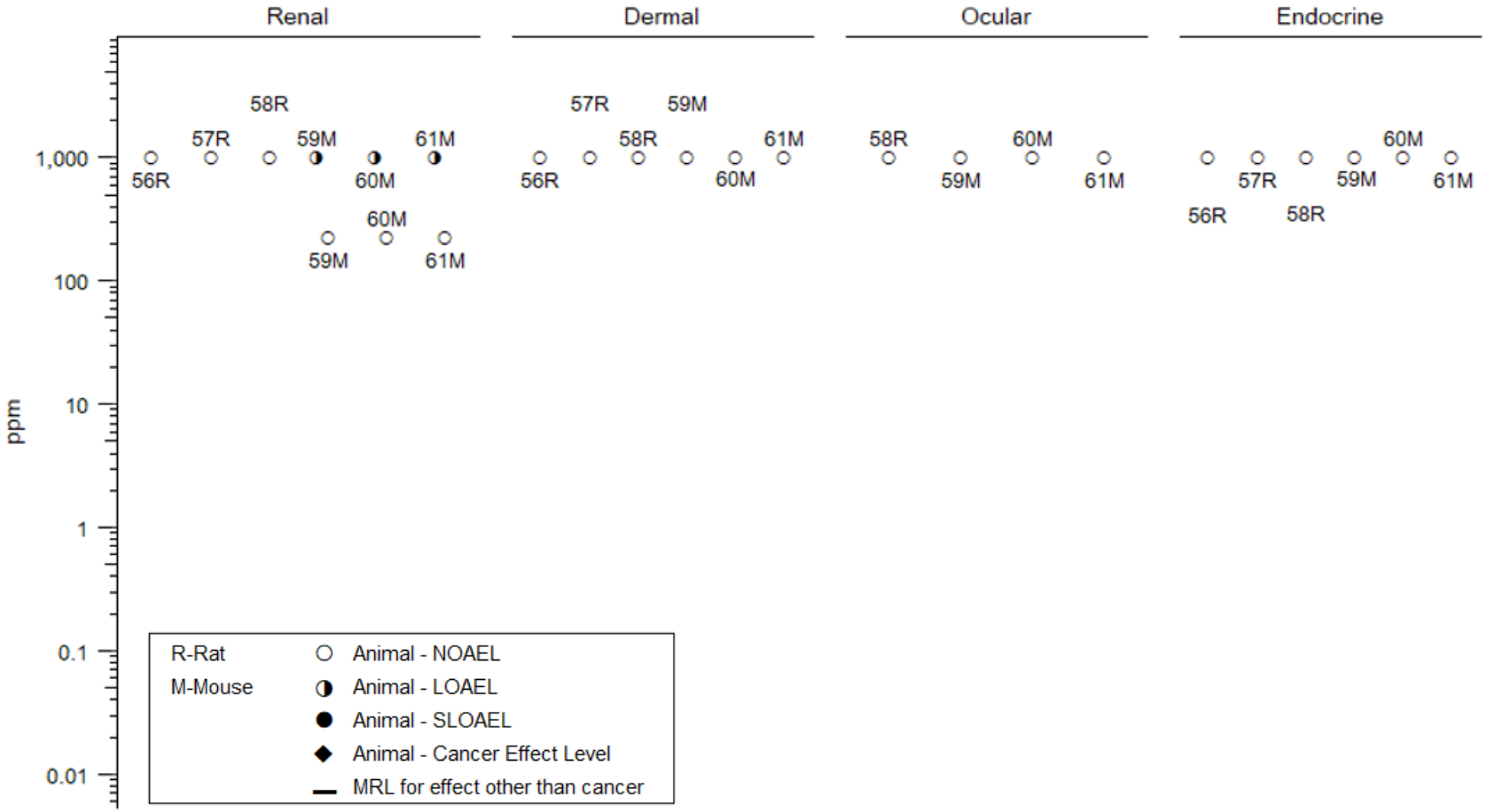


Figure 2-2. Levels of Significant Exposure to Chloromethane – Inhalation
Chronic (≥365 days)

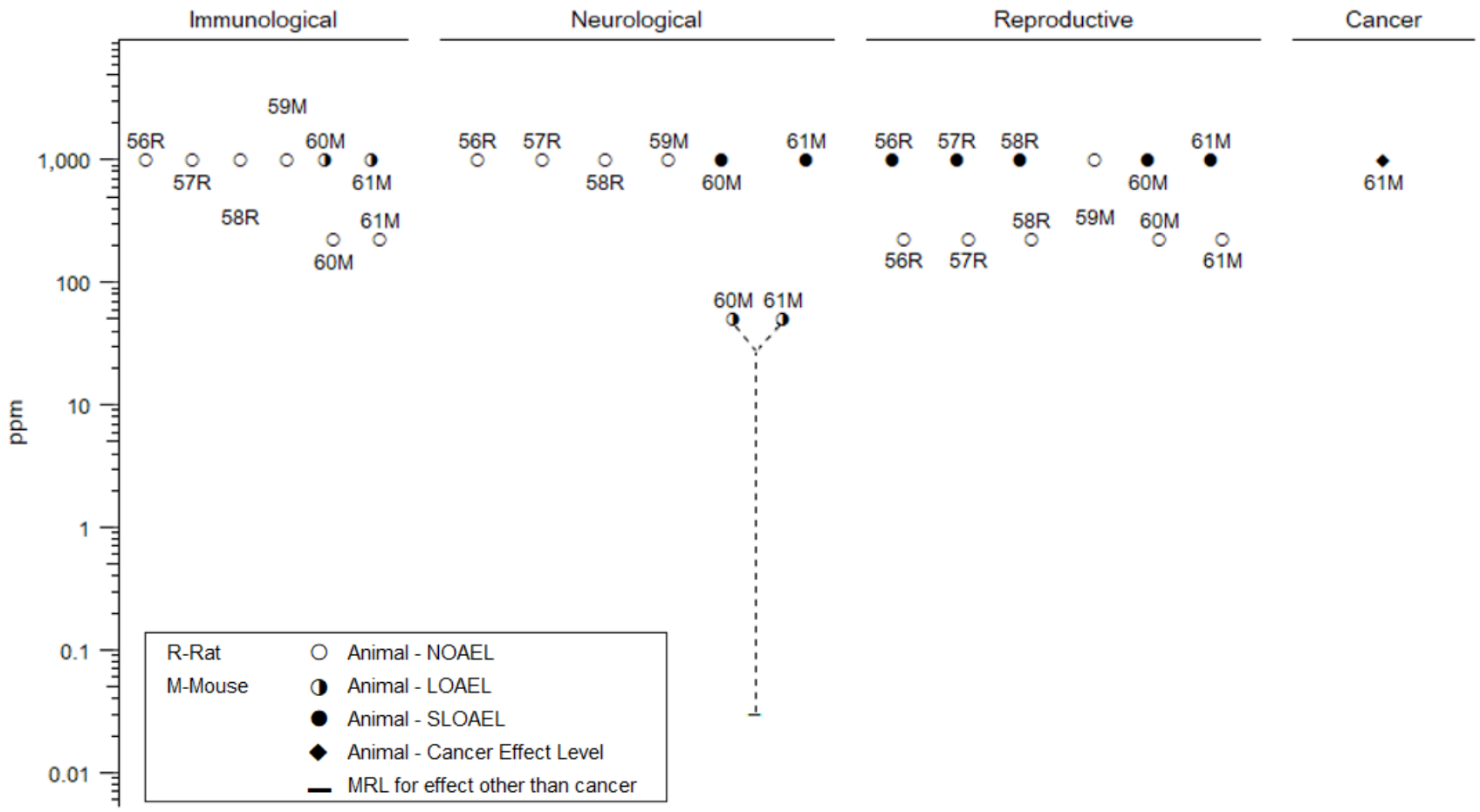


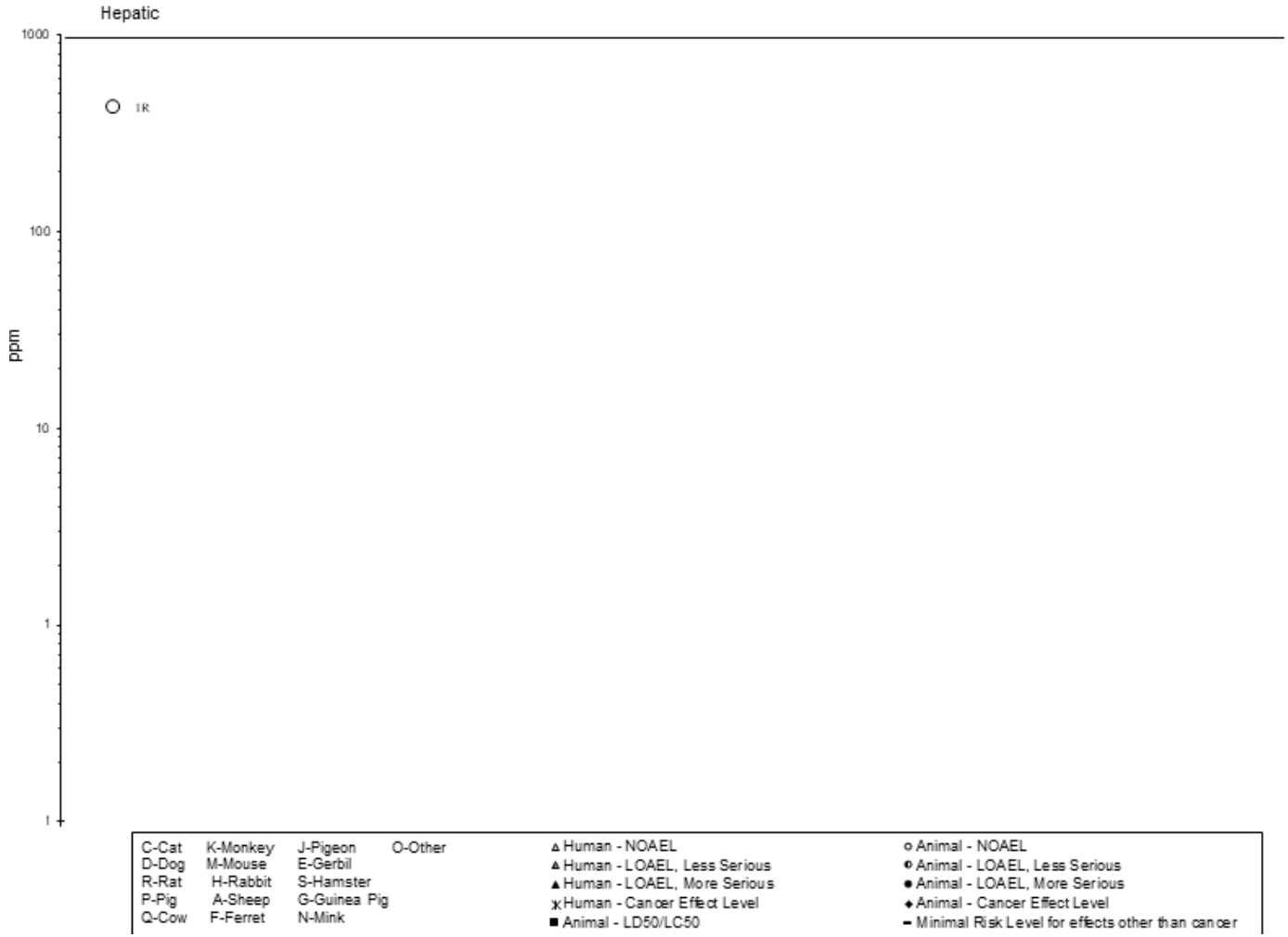
Table 2-3. Levels of Significant Exposure of Animals to Chloromethane – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Reynolds and Yee 1967									
1	Rat (Charles River) NS M	Once (GO)	0, 420	HP	Hepatic	420			

[a]The number corresponds to the entries in Figure 2-3.

HP = histopathology; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified

Figure 2-3. Level of Significant Exposure of Animals to Chloromethane – Oral
Acute (≤14 days)



2.2 DEATH

Exposure-related deaths have been reported in human and laboratory animals following inhalation exposure to high concentrations of chloromethane. No studies were located regarding death in humans or animals after oral or dermal exposure to chloromethane. Studies that examine the potential association of chloromethane exposure with death specifically from cancer are reviewed in Section 2.19.

In the late 1920s chloromethane began being used as a refrigerant (UNEP, 1999). Subsequently, there were several case reports of human deaths resulting 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). Numerous neurological symptoms were reported prior to death in these cases, including headache, dizziness, nausea and vomiting, anorexia, visual disturbances, slurred speech, unstable gait, weakness, fatigue, tremors, and/or convulsions.

In 1963, an Icelandic fisherman died within 24 hours of an accidental exposure to high (unspecified) concentrations of chloromethane due to a refrigerator leak (Gudmundsson 1977). Follow-up studies of the remaining 27 exposed fishermen through 2010 showed an increased risk of death, compared to unexposed Icelandic fishermen, specifically deaths associated with kidney cancer, cardiovascular diseases, and suicide (Table 2-1) (Rafnsson and Gudmundsson 1997; Rafnsson and Kristbjornsdottir 2014). The increase in mortality was greater in the deckhands (n=20), who were estimated to have received the greatest exposure due to the location of their living quarters, compared to officers (n=7). While the reference and exposure group had similar occupations and thus likely similar socioeconomic status, the study authors did not directly control for lifestyle factors, such as smoking habits, intensity of work demands, or diet. Due to the small number of individuals in the exposure group (n=27) and the assumption that the exposed and referent groups had similar lifestyle factors, generalization of these results to the general population must be done with caution.

In another occupational cohort study, all-cause mortality was decreased in synthetic rubber workers exposed to chloromethane; which may reflect the healthy worker effect (Holmes et al. 1986). Specific analysis did not find increased risk of death from cancer or circulatory system diseases (Table 2-1). While no exposure estimates are available, exposure to rubber workers is likely lower than the acute-duration exposure experienced in the Icelandic fisherman cohort and case reports associated with refrigerant leaks.

In animals, reported acute inhalation LC_{50} values in mice were 2,220 ppm following a 6-hour exposure (Chellman et al. 1986b) and 3,080 ppm following a 7-hour exposure (von Oettingen et al. 1949, 1950). Several additional acute-duration exposure studies in experimental animals observed increased mortality or instances in which researchers "killed animals *in extremis*" (at the point of death). In the majority of cases, this occurred at chloromethane concentrations ≥972 ppm in rats and ≥150 ppm in mice via continuous exposure (22–24 hours/day) (Burek et al. 1981; Landry et al. 1985), or at concentrations ≥1,500 ppm in both rats and mice via intermittent exposure (5.5–6 hours/day) (Chellman et al. 1986a, 1986b; Jiang et al. 1985; Landry et al. 1985; Morgan et al. 1982; Wolkowski-Tyl et al. 1983b; Wolkowski-Tyl et al. 1983a). The study authors attributed death to kidney (Burek et al. 1981; Morgan et al. 1982) or liver toxicity (Morgan et al. 1982). In dogs exposed to extremely high air levels of chloromethane, the average survival time was 5.9 hours at 14,661 ppm and 4 hours at 40,560 ppm; death was preceded by CNS depression and a precipitous drop in blood pressure and respiratory rate (von Oettingen et al. 1949, 1950).

Smith and von Oettingen (1947a, 1947b) exposed a variety of species including monkeys, rats, mice, guinea pigs, rabbits, dogs, and cats to concentrations ranging from 300 to 4,000 ppm for 6 hours/day, 6 days/week for up to 64 weeks. Exposure continued until animals died, allowing study authors to determine mean survival time and time until 50% of animals died (LT_{50}). Findings showed differences in susceptibility between different species, and different ages within the same species. The lowest concentrations associated with 50% lethality following acute-duration exposure were 1,000 ppm in guinea pigs (LT_{50} of 4 days), dogs (LT_{50} of 6 days), and mice (LT_{50} of 10.5 days); 2,000 ppm in monkeys (LT_{50} of 10 days); 3,000 ppm in rats (LT_{50} of 5 days); and 4,000 ppm in rabbits (LT_{50} of 13 days). All four cats survived acute-duration exposure to 2,000 ppm (only concentration evaluated in cats). For intermediate-duration exposure, the lowest concentrations associated with 50% lethality were 500 ppm in dogs (LT_{50} of 23 days), guinea pigs (LT_{50} of 71 days), monkeys (LT_{50} of 115 days), mice (LT_{50} of 143 days), and rabbits (LT_{50} of 192 days); and 2,000 ppm in rats (LT_{50} of 15 days) and cats (LT_{50} of 23 days). No exposure-related changes in survival were observed in monkeys, rats, mice, guinea pigs, dogs, or rabbits exposed to 300 ppm for 64 weeks. In species that evaluated both adult and "young" animals (rat, mouse, guinea pig, dog, rabbit), adult animals were generally more susceptible compared to younger animals. Across all species, severe clinical signs of neurotoxicity were commonly observed prior to death (see Section 2.15 for more details). Dogs and cats also displayed dyspnea and gasping, respectively, prior to death. At necropsy, deaths were associated with lung congestion and liver and kidney toxicity in rats, mice, and guinea pigs (Dunn and Smith 1947).

In longer-duration studies, no exposure-related increases in mortality were observed in rats following exposure to concentrations up to 997 ppm for 24 months (CIIT 1981). However, increased mortality was observed in similarly exposed mice (CIIT 1981). In females, overall survival was significantly decreased at 997 ppm, compared to control. Decreased survival was first noted at 10 months, with a dramatic decrease at 20 months. In males exposed to 997 ppm, increased mortality was observed at 17 months with a precipitous drop in survival at 19 months; however, survival was not statistically different from control. Findings in male mice were confounded during the first year by several deaths attributed to dominance fighting across all exposure groups, predominantly in the first 6 months, resulting in decreased survival of the control group during the first year of the study, compared to exposure groups. The lack of a statistically significant, exposure-related effect on male mouse survival, despite a dramatic decrease in survival at the end of the exposure period, is attributed to an unusually low survival rate in male control mice. Due to high mortality, remaining mice from the 997-ppm group were terminated at 21 months (2 males) and 22 months (18 females) (CIIT 1981).

2.3 BODY WEIGHT

No studies were located regarding body weight effects in humans exposed to chloromethane. A consistent systemic effect of chloromethane exposure in rodents is reduced body weight and/or body weight gain, which was observed in rats and mice exposed to chloromethane via inhalation for acute-, intermediate-, and chronic-duration exposures. No studies were located regarding body weight in animals after oral or dermal exposure to chloromethane.

In acute-duration studies, continuous exposure (22–24 hours/day) was associated with decreased body weights in rats at ≥504 ppm (Burek et al. 1981) and in mice at ≥150 ppm (Landry et al. 1985). With intermittent exposure over an acute duration, decreased body weights were observed in rats at ≥3,000 ppm (Chellman et al. 1986a; Working et al. 1985a) and in mice at 2,400 ppm (Landry et al. 1985). Some of the observed body weight effects may be secondary to decreased food consumption and water intake associated with overall poor health of the animals at high exposure concentrations (Landry et a. 1985). In maternal rats and mice, decreased body weights following gestational exposure were observed at ≥479 ppm (Wolkowski-Tyl et al. 1981a, 1981b, 1983a, 1983b). In intermediate- and chronic-duration studies, reduced body weights were consistently observed in rats at concentrations ≥741 ppm (CIIT 1981; Hamm et al. 1985; Mitchell et al. 1979). In one study, the study authors attributed body weight effects to transient reductions in body weight gain during weeks 3–8 of a 13-week study; however, despite body

weight gains comparable to control from weeks 9 to 13, final body weights were still reduced by >10% in males at ≥741 ppm and females at 1,473 ppm (Mitchell et al. 1979). In mice, no body weight effects were noted after exposure to concentrations up to 1,473 ppm for 90 days (Mitchell et al. 1979). However, decreased body weights were observed at 997 ppm in males exposed for ≥6 months and in both sexes exposed for ≥12 months (CIIT 1981).

No effect on body weight was observed in dogs and cats exposed for 72 hours to 500 ppm chloromethane (McKenna et al. 1981a). Additionally, no impact on body weight was observed in New Zealand white rabbits exposed to chloromethane at concentrations up to 1,012 ppm 6 hours/day on gestation days (GDs) 6–28 (Theuns-van Vliet 2016). These findings may be due to species difference in response to exposure to chloromethane.

2.4 RESPIRATORY

Available human studies are too limited to determine if inhalation exposure to chloromethane affects respiratory health or function. Based on inhalation studies in animals, there is limited evidence that exposure to high concentrations of chloromethane may cause adverse respiratory effects. No studies were located regarding respiratory effects in humans or animals after oral or dermal exposure to chloromethane.

Case reports generally have described limited respiratory effects in humans exposed to chloromethane. In a case study of individuals who were exposed to chloromethane from refrigeration leaks in a refrigerator manufacturing plant or in kitchenette apartments in Chicago in 1928 and 1929, several survivors presented with increased respiration and an autopsy of one case showed diffuse dilation of the alveolar space. Many presented cases were noted as having breath that smelled musty and sweetish, and the odor of acetone surrounded them (Kegel et al. 1929); or the work area where exposure occurred smelled sweet like methyl alcohol (Baird 1954). In a neurological study with volunteers, no effects on pulmonary function were observed following acute-duration inhalation exposure of up to 150 ppm chloromethane (Stewart et al. 1980). This study, however, had several limitations such as small sample size and subjects lost to attrition.

One epidemiological paper evaluated how subjects' respiratory outcomes changed with changes in air pollutants, including chloromethane. No association between self-reported bothersome or more severe asthma symptoms (i.e., symptoms that were anticipated to interfere with daily activities) and daily

chloromethane air levels was seen in a cohort of Hispanic children from an East Los Angeles community with high traffic density (Table 2-1) (Delfino et al. 2003). However, given the very low levels of exposure (mean 0.58 ppb) and small subject number (n=22), this study is limited in its evaluation of chloromethane-associated respiratory effects.

As discussed in Section 2.2, lethal exposure to chloromethane at acute-duration concentrations ≥1,000 ppm is associated with lung congestion and/or edema in several species, including rats, mice, guinea pigs, and cats (Dunn and Smith 1947; Smith and von Oettingen 1947a). Burek et al. (1981) also reported lung congestion and edema in rats that died following exposure to 1,950 ppm for up to 72 hours (Burek et al. 1981). In dogs exposed to extremely high concentrations for 4–6 hours (14,661 or 40,560 ppm), reduced respiration rates were observed prior to death (von Oettingen et al. 1949, 1950). Additionally, dyspnea was observed in dogs prior to death following acute-duration exposure to ≥1,000 ppm or intermediate-duration exposure to 500 ppm (Smith and von Oettingen 1947a, 1947b). Effects observed in dogs may be secondary to CNS depression rather than a direct effect on the respiratory system.

Acute-duration studies evaluating nonlethal concentrations failed to find any exposure-related respiratory effects. No exposure-related histopathological lesions or clinical signs of respiratory distress were noted in the lungs of dogs and cats exposed continuously (23.5 hours/day) to concentrations up to 496 and 501 ppm, respectively (McKenna et al. 1981a) or rats exposed continuously for 48 hours to concentrations up to 1,968 ppm (Burek et al. 1981). Similar to the acute-duration studies, intermediate-duration exposure studies did not find any association between chloromethane and histopathologic lesions in the lungs in dogs at concentrations up to 399 ppm or in rats and mice at concentrations up to 1,473 ppm (McKenna et al. 1981b; Mitchell et al. 1979). CIIT (1981) reported a significant increase in absolute and/or relative lung weight at ≥51 ppm in male rats following exposure for 6 months; however, this was not associated with exposure-related histopathological lesions. In females, respiratory findings at 997 ppm included minimal-to-moderate interstitial pneumonia with lymphocytic peribronchiolitis and perivasculitis; alveolar cell hyperplasia; mild alveolar luminal infiltrates consisting of large macrophages, lymphocytes, and in some areas, a few neutrophils; and areas of minimal subacute tracheitis. However, at 12, 18, or 24 months after the initial exposure, no chloromethane-related lung effects were observed in rats at concentrations up to 997 ppm. No effects on lungs were observed at any time point in similarly exposed mice. These respiratory effects observed in this study were considered transient and unrelated to exposure by the study authors (CIIT 1981).

2.5 CARDIOVASCULAR

A systematic review of the literature (Appendix C) determined that chloromethane is not classifiable as it relates to cardiovascular outcomes based on inadequate evidence from inhalation studies in humans and laboratory animals. No studies were located regarding cardiovascular effects in humans or animals after oral or dermal exposure to chloromethane.

Several case reports of humans exposed to high acute levels of chloromethane associated with refrigerator leaks have reported cardiovascular effects. The effects of these exposures vary by case and include electrocardiogram abnormalities, tachycardia, increased pulse rate, elevated body temperature, and both hypertension and decreased blood pressure (Hansen et al. 1953; Kegel et al. 1929; McNally 1946; Scharnweber et al. 1974; Spevak et al. 1976; Verriere and Vachez 1949). The concentrations and durations of exposure in these studies are not known. Kegel et al. (1929) reported that body temperatures in one survivor reached 104°F prior to death. One reported adult survivor had a recorded pulse rate of 150 beats/minute, and one child had a pulse rate recorded as 164 beats/minute.

As discussed in Section 2.2 (Death), an increased risk of death from cardiovascular disease was observed in a cohort of Icelandic fishermen accidently exposed to high levels of chloromethane from a refrigeration leak (Rafnsson and Gudmundsson 1997; Rafnsson and Kristbjornsdottir 2014). This risk was increased in both the 32- and 47-year follow-up rates of cardiovascular related mortality, including both acute coronary heart disease and cerebrovascular disease, compared with a reference group of seamen from the Icelandic registries. This excess was only significant for the deckhands who were estimated to have received the highest exposure to chloromethane due to the proximity of their sleeping quarters to the leaking refrigerator. In contrast, the risk of death due to circulatory diseases was not increased in an occupational cohort of synthetic rubber workers exposed to chloromethane (Holmes et al. 1986). While neither cohort study reported exposure levels, it is expected that the accidental exposure concentration in Icelandic fishermen was higher (potentially much higher) than occupational levels in the rubber plant. The risk of bias in these studies is increased given that they did not explicitly control for smoking or diet and there were relatively small numbers of individuals with significant exposure.

In a human controlled exposure experiment, volunteers were exposed for 1, 3 or 7.5 hours/day for 2–5 days per exposure group and no abnormalities of cardiac function or electrocardiograms were found for any of the exposure durations at concentrations up to 150 ppm (Stewart et al. 1980). However, a man exposed to an unknown acute dose of chloromethane presented for medical examination the day of

exposure with a pale, ashen face complaining of a headache. The patient died the following day, and the necropsy demonstrated capillary engorgement and chloromethane throughout the tissues examined (Baird 1954).

Only one study evaluating cardiovascular function in animals following inhalation exposure to chloromethane was identified. In dogs acutely exposed to lethal concentrations ≥14,661 ppm for 4–6 hours, an initial rise in blood pressure was followed by a precipitous drop in blood pressure after 2.5–3 hours (von Oettingen et al. 1949, 1950). Blood pressure continued to fall and was accompanied by a marked decrease in heart rate until death, which occurred within 4–6 hours. Changes observed in blood pressure and respiration were closely related for most of the observation period. Just prior to death, there was a transient increase in heart rate. The initial increase in blood pressure may have been due to residual anesthesia from the surgical procedure to cannulate the artery and vein for monitoring of cardiovascular function, while drastic reductions in blood pressure and heart rate were attributed to vasodilation in response to CNS depression.

Several additional studies evaluated heart weight and/or histology in animals. No exposure-related changes in heart histology were observed at acute-duration concentrations up to approximately 500 ppm in dogs and cats (McKenna et al. 1981a), intermediate-duration concentrations up to 399 pm in dogs or 1,473 ppm in rats or mice (CIIT 1981; McKenna et al. 1981b; Mitchell et al. 1979), or chronic-duration concentrations up to 998 ppm in rats and mice (CIIT 1981). However, some studies reported exposure-related increases in heart weight after intermediate- or chronic-duration exposure. In mice, increased relative heart weights were reported in males exposed to 399 ppm for 94 days in one study (McKenna et al. 1981b), while no exposure-related changes in heart weight were observed in mice in other studies at concentrations up to 1,473 ppm for 90 days (Mitchell et al. 1979) or 997 ppm for 6 months (CIIT 1981). In chronic-duration studies, exposure-related increases in absolute and/or relative heart weights were observed in male rats exposed to 997 ppm for 12 or 24 months, female mice exposed to 997 for 12 months, and female mice exposed to ≥224 ppm for 24 months (CIIT 1981). Increases in relative heart weight were also observed in female rats exposed to 997 ppm at 12 and 24 months; however, these effects were considered to be secondary to decreases in body weights due to a lack of concurrent increase in absolute heart weight. No exposure-related changes in heart weight were observed in male mice exposed to concentrations up to 997 ppm for up to 24 months (CIIT 1981).

2.6 GASTROINTESTINAL

Available human studies indicate that reported gastrointestinal effects following inhalation exposure may be secondary to neurological effects. Based on inhalation studies in animals, chloromethane does not appear to have direct adverse effects on the gastrointestinal system. No studies were located regarding gastrointestinal effects in humans or animals after oral or dermal exposure to chloromethane.

Numerous case reports of humans exposed to chloromethane have described symptoms of pain in the abdomen, hiccups, nausea, and vomiting (Baird 1954; Baker 1927; Battigelli and Perini 1955; Borovska et al. 1976; Hansen et al. 1953; Jones 1942; Kegel et al. 1929; Mackie 1961; Spevak et al. 1976; Verriere and Vachez 1949; von Raalte and van Velzen 1945; Weinstein 1937). In all cases, these symptoms were accompanied by CNS toxicity, which was usually severe. It is not clear, therefore, if the abdominal pain, nausea, and vomiting were secondary to the neurotoxic effects of chloromethane. Two of the reports (Battigelli and Perini 1955; Jones 1942) provided refrigerator chloromethane capacity and room size from which exposures of 75–1,282 ppm could be calculated.

In animals, no exposure-related histopathological changes in the gastrointestinal tract were observed at acute-duration exposures up to approximately 500 ppm in dogs and cats (McKenna et al. 1981a) or 5,000 ppm in rats (Morgan et al. 1982); intermediate-duration exposures up to 399 ppm in dogs (McKenna et al. 1981b), 1997 ppm in rats (CIIT 1981; McKenna et al. 1981b) or 1,473 ppm in mice (CIIT 1981; McKenna et al. 1981b; Mitchell et al. 1979); or chronic-duration exposures up to 997 ppm in rats and mice (CIIT 1981; McKenna et al. 1981b). One acute-duration study reported foul-smelling diarrhea in male and female rats within 2-days of exposure to 5,000 ppm (Morgan et al. 1982). In another study, decreased ingesta were observed in the gastrointestinal tract of male rats exposed to 1,000 ppm chloromethane for 72 hours (Burek et al. 1981). As observed in human case reports, gastrointestinal distress was observed at an exposure level associated with severe neurotoxicity.

2.7 HEMATOLOGICAL

Available human studies are too limited to determine if inhalation exposure to chloromethane affects hematological endpoints. Based on inhalation studies in animals, the hematological system does not appear to be a sensitive target of chloromethane toxicity. No studies were located regarding hematological effects in humans or animals after oral or dermal exposure to chloromethane.

No hematological effects were found in volunteers who participated in a controlled human exposure study of neurological and neurobehavioral effects of acute-duration inhalation exposure of up to 150 ppm chloromethane (Stewart et al. 1980). This study, however, had several limitations such as small sample size and subjects lost to attrition. Additionally, measured blood and breath concentrations in several participants were much higher than for other participants.

In a series of case reports, Kegel et al. (1929) reported decreases in reticulocyte count, hemoglobin, red blood cell count, and white blood cell count among several cases of poisonings in Chicago in 1928 and 1929 associated with chloromethane leaks in a refrigerator manufacturing plant and in kitchenette apartments. However, other case reports of human exposure to chloromethane have generally not found an association between chloromethane exposure and hematological effects (Gudmundsson 1977; Jones 1942). For example, in a group of Icelandic fishermen exposed accidentally to chloromethane due to a refrigeration leak, no evidence of long-term impacts on the hematological system was seen in the 10 patients the researchers evaluated 13 years post-exposure (Gudmundsson 1977).

Increased red blood cell (RBC) parameters (RBC count, hematocrit, hemoglobin) that were observed in rats continuously exposed to chloromethane at 1,968 ppm for 2 days or 972 ppm for 3 days were considered secondary to dehydration (and subsequent hemoconcentration) in lethargic or moribund animals, rather than a direct effect on the hematological system (Burek et al. 1981). In other inhalation studies, no exposure-related effects on hematological parameters were found in acute-duration studies in dogs or cats at concentrations up to 496 or 501 ppm, respectively (McKenna et al. 1981a); intermediate-duration studies in dogs at concentrations up to 299 ppm (McKenna et al. 1981b) or in rats or mice at concentrations up to 1,473 ppm (CIIT 1981; McKenna et al. 1981b; Mitchell et al. 1979); or chronic-duration studies in rats or mice at concentrations up to 997 ppm (CIIT 1981).

Spleen enlargement, suggestive of extramedullary hematopoiesis, and hemoglobinuria without hematuria, suggestive of intravascular hemolysis, were found in female 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 (200 ppm) (Landry et al. 1985). This study did not evaluate hematological parameters in blood or examine male mice.

2.8 MUSCULOSKELETAL

No studies were located regarding musculoskeletal effects in humans exposed to chloromethane. In inhalation studies in animals, no musculoskeletal effects were observed following intermediate-duration exposures to concentrations up to 399 ppm in dogs (McKenna et al. 1981b) or 1,473 ppm in rats or mice (CIIT 1981) McKenna et al. 1981b; Mitchell et al. 1979), or chronic-duration exposure to concentrations up to 997 ppm in rats or mice (CIIT 1981). No studies were located regarding musculoskeletal effects in animals after oral or dermal exposure to chloromethane.

2.9 HEPATIC

Available human studies are too limited to determine if inhalation exposure to chloromethane affects the liver; no oral or dermal studies in humans were identified. A systematic evaluation of the literature (Appendix C) determined that hepatic toxicity is a presumed health effect associated with inhalation exposure to chloromethane based on a high level of evidence from laboratory animals. Only one study evaluating hepatic effects in animals following oral exposure was identified; no studies were located regarding hepatic effects in animals after dermal exposure to chloromethane.

Evidence from human studies is limited to case reports of people exposed to chloromethane via inhalation (Jones 1942; Kegel et al. 1929; Mackie 1961; Spevak et al. 1976; Weinstein 1937; Wood 1951). Jones (1942) reported large amounts of coproporphyrin III in the urine (initially 6 times normal, increased to 30 times normal, and then slowly fell to normal) which was suggestive of liver damage. Spevak et al. (1976) reported jaundice in 3 women exposed to chloromethane from a commercial refrigerator leak. Other case reports found marked hyperemia, lipoid granules in Kupffer cells, thickened capsule, and Glisson septums with lymphocyte accumulations (Kegel et al. 1929), clinical jaundice (Weinstein 1937), and cirrhosis of the liver (Wood 1951). While these case reports lacked exact exposure data, it is likely that the liver effects were due to exposure to chloromethane rather than to alcohol, another chemical, a virus, or a parasite.

Hepatic effects have also been observed in animals exposed to chloromethane via inhalation, including liver weight effects, alterations in serum clinical chemistry parameters, and mild histopathological lesions. However, there is some inconsistency between studies and species. In general, mice appear to be the most susceptible species.

A transient decrease in absolute and/or relative liver weight was observed in male rats continuously exposed to ≥196 ppm for 48 or 72 hours; this effect was no longer observed after a 12-day recovery period (Burek et al. 1981). Liver weights were unaltered in female rats similarly exposed up to 1,968 ppm. In contrast to decreased liver weight observed in the rat study by Burek et al. (1981), an increase in relative liver weights was observed in male rats exposed intermittently to 399 ppm for 93 days (McKenna et al. 1981b). In other studies in rats, no exposure-related changes in liver weight were observed following intermittent exposure to chloromethane at acute-duration concentrations up to 5,004 ppm (Chellman et al. 1986a), intermediate-duration concentrations up to 1,473 ppm (CIIT 1981; Mitchell et al. 1979), or chronic-duration concentrations up to 997 ppm (CIIT 1981). In mice, inconsistent findings were observed following acute-duration exposure to chloromethane. In a study by Landry et al. (1985) that evaluated both intermittent (5.5 hours/day) and continuous (22 hours/day) exposures in four separate 11-day experiments, one intermittent study reported increased absolute and relative liver weight in female mice exposed to 1,600 ppm. However, the second intermittent study did not observe adverse changes in liver weight in female mice at concentrations up to 2,400 ppm. In continuous-exposure paradigms, no adverse changes in liver weight were observed in female mice at concentrations up to 150 ppm (Landry et al. 1985). In another acute-duration study, no changes in liver weight were observed in mice at concentrations up to 749 ppm for 2 days (Wolkowski-Tyl et al. 1981b, 1983b). In longer-duration mouse studies, intermittent exposure was associated with increased absolute and/or relative liver weights in several studies, including male and female mice exposed to ≥741 ppm for 90 days (Mitchell et al. 1979), female mice exposed to 399 ppm for 93 days (McKenna et al. 1981b), and female mice exposed to 997 ppm for 12 or 18 months (CIIT 1981). However, no exposure-related changes in liver weight were observed in mice following intermittent exposure to concentrations up to 997 ppm for up to 24 months (CIIT 1981). In other species, no exposure-related changes in liver weights were observed in dogs or cats exposed to concentrations of 496 or 501 ppm, respectively, for 3 days (McKenna et al. 1981a) or dogs exposed to concentrations up to 399 ppm for 93 days (McKenna et al. 1981b).

Evidence for altered hepatic clinical chemistry parameters following inhalation exposure to chloromethane are limited and inconsistent between studies and exposure durations. In an acute-duration study, rats exposed continuously to 1,950 ppm for up to 72 hours showed decreased alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin levels just prior to death (Burek et al. 1981). No changes in hepatic clinical chemistry were observed at nonlethal concentrations in this study. Increased serum ALT was also observed following nonlethal exposures in mice at 1,500 ppm for 6 hours (Chellman et al. 1986b) and in male rats and male and female mice at 997 ppm for

12 months (CIIT 1981). However, in chronic-duration studies, only male mice showed increased serum ALT after exposure to 997 ppm for 18 months; this effect was no longer observed at 24 months (CIIT 1981). In other animal inhalation studies, no exposure-related changes in hepatic clinical chemistry were noted at acute-duration exposures up to 1,968 ppm in rats (Burek et al. 1981) and approximately 500 ppm in dogs and cats (McKenna et al. 1981a), or intermediate-duration exposures up to 1,473 ppm in rats and mice (CIIT 1981; McKenna et al. 1981b; Mitchell et al. 1979).

In an acute lethality study, dark, congested, and mottled livers were observed in rats that died following continuous exposure to 1,950 ppm for up to 72 hours (Burek et al. 1981). At nonlethal doses, slight liver effects observed following exposure to ≥198 ppm for 48 or 72 hours included lipid accumulation, slight extramedullary hematopoiesis, and altered tinctorial appearance of hepatocytes. These effects resolved after 12 days of recovery (Burek et al. 1981). Hepatic effects (centrilobular necrosis and/or fatty accumulation) were also noted in several species following acute exposure to lethal concentrations, including ≥1,000 ppm in guinea pigs and ≥2,000 ppm in rats and mice (Dunn and Smith 1947).

In rats, nonlethal, acute-duration exposures to concentrations ≥2,000 ppm were generally associated with mild effects, such as minimal hepatocellular degeneration and cloudy swelling of hepatocytes (Chellman et al. 1986a; Morgan et al. 1982). In mice, a single 6-hour exposure to 1,500 ppm was associated with hepatocellular necrosis and cytoplasmic vacuolation (Chellman et al. 1986b). An 11-day study in mice reported decreased hepatocyte size and evidence of glycogen depletion following exposure to ≥400 ppm for 5.5 hours/day or ≥100 ppm for 22 hours/day; in mice exposed for 22 hours/day, “higher exposure levels” (unspecified) were associated with focal hepatic necrosis (Landry et al. 1985). While adverse effects were observed at a lower exposure concentration with continuous exposure, when exposures are adjusted for duration (5.5 hours or 22 hours/24 hours), the duration-adjusted concentrations are equivalent (92 ppm). Minimal hepatocellular degeneration progressed to severe hepatocellular degeneration and necrosis in mice exposed to concentrations ranging from 500 to 2,000 ppm for 12 days (Morgan et al. 1982).

No exposure-related hepatic lesions were observed in rats following intermediate-duration exposure to concentrations up to 1,473 ppm (CIIT 1981; Dunn and Smith 1947; McKenna et al. 1981a; Mitchell et al. 1979) or chronic-duration exposure concentrations up to 997 ppm (CIIT 1981). In mice, no exposure-related lesions were observed in mice exposed to concentrations up to 1,473 ppm for approximately 3 months (McKenna et al. 1981b; Mitchell et al. 1979). However, exposure to 997 ppm resulted in hepatocellular degeneration in mice after 6 months, which progressed to necrosis, cytomegaly,

karyomegaly, and polykaryocytes at ≥12 months (CIIT 1981). In dogs, exposure to 51, 149, or 399 ppm for 3 months resulted in swollen hepatocytes in 2/4, 1/4, and 2/4 dogs, respectively, compared to 0/4 controls (McKenna et al. 1981b). Since a clear dose-response was not observed, and no other liver effects were observed, the toxicological significance of these effects are unlikely to be treatment-related.

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 mg/kg and no centrilobular hepatic necrosis was found. Chloromethane neither suppressed glucose 6-phosphatase activity in the centrilobular portion of the liver lobule, nor increased cell sap ribonucleic acid content, indicating that oral exposure to chloromethane is unlikely to induce hepatic necrosis.

2.10 RENAL

Available human studies are too limited to determine if inhalation exposure to chloromethane affects the renal system; no oral or dermal studies in humans were identified. Animal data indicate that the renal system is a toxicity target following inhalation exposure to high concentrations. No studies were located regarding renal effects in animals after oral or dermal exposure to chloromethane.

Case reports of humans exposed to chloromethane have described indicators of renal toxicity such as albuminuria, red blood cells in the urine, increased serum creatinine and blood urea nitrogen (BUN), proteinuria, granular or hyaline casts, anuria, and the presence of acetone, diacetic acid, and occasionally formic acid in the urine (Jones 1942; Kegel et al. 1929; Mackie 1961; Spevak et al. 1976; Verriere and Vachez 1949). Exposure concentrations at which these effects occurred are not known. Microscopic examination of the kidney of an individual who died following chloromethane exposure revealed marked capillary hyperemia, dilated glomerular and interstitial capillaries packed with blood cells, swollen epithelial lining of the convoluted tubules, and narrowing of the lumen (Kegel et al. 1929). In individuals exposed to less chloromethane, symptoms of renal damage disappeared after 2 weeks after admission (Spevak et al. 1976).

Studies in rodents have consistently observed renal damage following acute-duration inhalation exposures to high concentrations of chloromethane. In acute-duration studies with continuous inhalation exposure, renal failure was cited as the cause of death in rats exposed to ≥972 ppm continuously for up to 78 hours

(Burek et al. 1981). Renal lesions observed in rats exposed to ≥972 ppm for ≥48 hours included renal tubule necrosis, increased renal tubular cytoplasmic homogeneity, and increased lipid accumulation in renal tubular cells; multifocal renal tubules were also observed in male rats exposed to 504 ppm for 72 hours (Burek et al. 1981). Clinical chemistry and urinalysis findings were also indicative of renal toxicity, including elevated serum BUN in females exposed to 972 ppm for 72 hours and in males and females exposed to 1,968 ppm for 48 hours and increased protein, ketones, glucose, and blood in the urine at ≥972 ppm for both durations (Burek et al. 1981). Dunn and Smith (1947) also report renal tubule necrosis and/or fatty metamorphosis in several species at acute concentrations associated with lethality, including guinea pigs at ≥1,000 ppm rats and mice at ≥2,000 ppm. Mice also displayed hemoglobinuria at ≥1,000 ppm (Dunn and Smith, 1947; Smith and von Oettingen 1947b).

Renal lesions have also been consistently observed in rats and mice following intermittent (6 hours/day), acute-duration exposures, with reports of necrosis and degeneration of the proximal convoluted tubules at concentrations ≥2,000 ppm (Chellman et al. 1986a; Landry et al. 1985; Morgan et al. 1982). A few acute-duration studies in mice reported effects at lower concentrations, including slight degeneration of the proximal tubules and increased renal cell regeneration at 1,500 ppm for 2 weeks (Chellman et al. 1986b; Jiang et al. 1985) and minimal-to-moderate basophilic renal tubules with hematuria at ≥1,000 ppm for 12 days (Morgan et al. 1982).

In longer-duration studies, no adverse renal effects were noted in rats or mice at concentrations up to 1,473 ppm for 3 months (McKenna et al. 1981b; Mitchell et al. 1979), in rats at concentrations up to 1,000 ppm for 175 days (Dunn and Smith 1947), or in rats at concentrations up to 997 ppm for 6–24 months (CIIT 1981). In mice, decreased absolute and relative kidney weights were seen in male mice (but not female mice) following exposure to 997 ppm for 6 months; these findings were not accompanied by any changes in clinical chemistry, urinalysis, or histology (CIIT 1981). However, renal tubule hyperplasia was observed in male mice (but not female mice) exposed to 997 ppm for ≥12 months (CIIT 1981).

In non-rodent species, no adverse renal effects were noted at acute-duration exposures up to approximately 500 pm in dogs or cats (McKenna et al. 1981a) or intermediate-duration exposures up to 399 ppm in dogs (McKenna et al. 1981b).

2.11 DERMAL

No studies were located regarding dermal effects in humans after exposure to chloromethane. Based on inhalation studies in laboratory animals, the skin is not a target of chloromethane toxicity. No studies were located regarding dermal effects in animals after oral or dermal exposure to chloromethane.

No dermal effects were observed from acute-duration inhalation exposure to chloromethane at concentrations up to approximately 500 ppm in dogs or cats (McKenna et al. 1981a), although one dog with approximately 200 ppm of exposure had multiple areas of alopecia. The study authors noted that this may have been "secondary to fighting with cage mates." In 3-month inhalation studies, no dermal effects were observed at concentrations up to 1,473 ppm in rats or mice (McKenna et al. 1981b; Mitchell et al. 1979) or 399 ppm in dogs (McKenna et al. 1981b). Similarly, no dermal effects were noted in rats or mice exposed to concentrations up to 997 ppm for 6–24 months (CIIT 1981).

2.12 OCULAR

Available human case reports indicate that reported ocular effects following inhalation exposure are likely secondary to neurological effects. Based on inhalation studies in animals, chloromethane does not appear to have adverse effects on the eyes. No studies were located regarding ocular effects in humans or animals after oral or dermal exposure to chloromethane.

Case reports of humans exposed to chloromethane via inhalation have described such symptoms as blurred and double vision and dilated and slowly reacting pupils (Baker 1927; Borovska et al. 1976; Kegel et al. 1929; Mackie 1961). These symptoms likely reflect effects on the nervous system rather than effects on the eye itself.

No exposure-related ocular effects were observed during ophthalmological or histopathological examinations of male cats and Beagle dogs exposed to concentrations up to approximately 500 ppm continuously for 3 days (McKenna et al. 1981a), dogs exposed to concentrations up to 399 ppm for 90 days (McKenna et al. 1981b), or rats or mice exposed to concentrations up to 1,473 ppm for 90 days (Mitchell et al. 1979) or 997 ppm for 6 months (CIIT 1981). In mice, mucopurulent conjunctivitis was observed at an increased incidence following exposure to 368 ppm for 90 days; however, incidences were not increased at 741 or 1,473 ppm, compared to control (Mitchell et al. 1979). Therefore, these findings are not considered toxicologically relevant.

In chronic-duration inhalation studies, no exposure-related ocular effects were noted in mice at concentrations up to 997 ppm (CIIT 1981). Some effects were noted in rats; however, findings lacked a clear duration-related response and may have been secondary to a sialodacryo-adenitis (SDA) infection in the colony. Therefore, the adversity of these findings is unclear. After 12 months of exposure to chloromethane vapor, a corneal lesion described as a haze elliptically patterned over a central portion of the eye was seen in the majority of exposed rats at ≥51 ppm (CIIT 1981). This corneal haze may have been the result of chemical effects upon the eyes in which the lacrimal function was compromised by the undercurrent SDA infection, which was histopathologically diagnosed at 12 months. The study authors hypothesized that this disease reduced lacrimal function, making the eye more vulnerable to irritation from chloromethane. After exposure for 18 months, this haze was no longer apparent; however, an increase in the incidence of corneal opacity was observed in female rats at ≥224 ppm (CIIT 1981). By 24 months, the incidence of corneal opacity was no longer different between rats in the control and exposure groups. Minimal vacuolar degeneration of the anterior lens fibers was also observed in 7/10 male and 6/10 female rats exposed to 997 ppm for 18 months; however, this lesion was not observed in excess at 24 months, so its relationship to chloromethane exposure is unclear.

2.13 ENDOCRINE

No studies were located regarding endocrine effects in humans after exposure to chloromethane. Exposure-related endocrine effects (outside the reproductive system) were only observed in rats following acute exposure to very high concentrations. Reproductive effects, including alterations in serum reproductive hormone levels, are discussed in Section 2.16. No studies were located regarding endocrine effects in animals after oral or dermal exposure to chloromethane.

Observed effects following inhalation exposure in rats included vacuolation of cell cytoplasm in the adrenal cortex in rats exposed to 5,004 ppm for 5 days (Chellman et al. 1986a) and clear droplets in endothelial cytoplasm indicative of fatty degeneration in rats exposed to ≥3,500 ppm for 9 days (Morgan et al. 1982). In other studies, no exposure-related changes in the endocrine system were observed at acute-duration exposures up to approximately 500 ppm in dogs and cats (McKenna et al. 1981a), intermediate-duration exposures up to 1,473 ppm in rats and mice (CIIT 1981; Mitchell et al. 1979), or chronic-duration exposures up to 997 ppm in rats and mice (CIIT 1981).

2.14 IMMUNOLOGICAL

No studies were located regarding immunological effects in humans after exposure to chloromethane. Evidence from animal inhalation studies is inconsistent but suggests that the thymus and spleen may be toxicity targets at high concentrations. No studies were located regarding immunological effects in animals after oral or dermal exposure to chloromethane.

No studies evaluating immunological function in animals following exposure to chloromethane were located; however, several studies evaluated immune organ weight and/or histology. In a series of 11-day inhalation studies in mice, thymus atrophy and decreased absolute and relative thymus weight were observed at ≥1,600 ppm when exposure was 5.5 hours/day (Landry et al. 1985). When exposure was nearly continuous (22 hours/day), one set of experiments reported decreased absolute and relative thymus weight at 15, 50, and 150 ppm, compared to control, while another did not observe exposure-related changes in thymus weight at concentrations up to 100 ppm (Landry et al. 1985). Observed changes in the spleen (enlarged spleen) in mice exposed to 2,400 ppm for 11 days were attributed to extramedullary hematopoiesis by the study authors (Landry et al. 1985).

In longer-duration studies, no exposure-related changes in immune organ weight or histology were observed in rats or mice exposed to concentrations up to 1,473 ppm for 3 months (McKenna et al. 1981b; Mitchell et al. 1979), dogs exposed to concentrations up to 399 ppm for 3 months (McKenna et al. 1981b), or rats exposed to 997 ppm for 6–24 months (CIIT 1981). Mice exposed to 997 ppm for 6 months showed lymphoid depletion of the spleen and thymic lymphoid necrosis (CIIT 1981). Splenic atrophy and splenic and thymic lymphoid depletion were also observed in mice after exposure to 997 ppm for 18–24 months; however, these effects were not observed at the 12-month interim sacrifice (CIIT 1981).

2.15 NEUROLOGICAL

A systematic evaluation of the literature (Appendix C) determined that neurological effects are a presumed outcome associated with inhalation exposure to chloromethane based on a low level of evidence from human studies and high level of evidence from inhalation studies in laboratory animals. No studies were located regarding neurological effects in humans or animals after oral or dermal exposure to chloromethane.

Numerous case reports of humans exposed to chloromethane vapors as a result of industrial, refrigeration leaks, or other household exposures have described neurological effects. In general, symptoms develop within a few hours after exposure and include fatigue, progressive drowsiness, staggering, headache, nausea and vomiting, abdominal pain, slurred speech, blurred and double vision, mental confusion, disorientation, combativeness, tremor, vertigo, muscular weakness, muscular cramping and rigidity, sleep disturbances, ataxia, convulsions, cyanosis alternating with coma, delirium, and restlessness (Baird 1954; Baker 1927; Battigelli and Perini 1955; Borovska et al. 1976; Hansen et al. 1953; Hartman et al. 1955; Jones 1942; Kegel et al. 1929; Lanham 1982; MacDonald 1964; McNally 1946; Minami 1998; Scharnweber et al. 1974; Spevak et al. 1976; von Raalte and van Velzen 1945; Wood 1951). In some cases, symptoms persisted for several hours after exposure ended but disappeared completely within a few days. In other cases, symptoms lasted for several months, and depression and personality changes developed. In cases of more severe poisoning, convulsion, coma, and death ensued; or neurological effects remained (Kegel et al. 1929; McNally 1946; MacDonald 1964). In one lethal case, a gradual onset of headache and nausea occurred the day of exposure and improved the following day, but the symptoms worsened to coma, convulsions, and death (Baird 1954). 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).

Additional evidence of the neurotoxic effects of chloromethane comes from the crew of an Icelandic fishing boat that were exposed for up to 4 days in 1963 to chloromethane that leaked from a refrigerator on board a fishing trawler (Gudmundsson 1977). Initial effects of exposure in the crew were signs of intoxication that continued after exposure ended (no estimates of exposure levels were reported). Four of the 15 crew members with symptoms of severe chloromethane poisoning died within 10 years of exposure; 1 died 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 post exposure at the age of 34 years. Autopsy revealed a recent coronary occlusion, which was not necessarily connected with the primary illness. In a 13-year follow-up of this cohort (Gudmundsson 1977), 5 out of the 10 patients that were alive 13 years post-exposure still exhibited abnormal neurological signs upon examination. Ten survivors stated they had a reduced tolerance to alcohol (compared with 5 at 20 months post-exposure), while 4 admitted excessive alcohol intake. Regarding the progress or reversibility of the symptoms, one patient who had considerable muscle atrophy and fasciculations 20 months after the accident, had improved by 13 years

post-exposure, 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 another occupational cohort, there were no associations between chloromethane exposure in fabricating plants (mean 33.57 ppm) and neurological function (Table 2-1) (NIOSH 1976). Endpoints evaluated included electroencephalogram (EEG) recordings and numerous measures of neurological function or behavior. While no exposure estimates are available for the Icelandic fisherman cohort, exposure to fabricating workers is likely lower than the acute-duration exposure experienced in the fishing boat accident.

Three human controlled trials evaluated exposure to chloromethane and potential neurotoxic effects and did not find any association (Putz-Anderson et al. 1981a, 1981b; Stewart et al. 1980). In Putz-Anderson et al. (1981a, 1981b), exposure to concentrations up to 200 ppm for up to 3.5 hours did not impact hand-eye coordination or alertness, with the only finding being a slight time delay in an auditory time-discrimination test, which could be due to solvent effects on ear hairs rather than a neurological effect. In Stewart et al. (1980), exposure to concentrations up to 150 ppm for 1, 3, and 7.5 hours/day on 2 or 5 consecutive days resulted in no exposure-related neurological abnormalities, abnormal EEG observations, effects on cognitive test, or significant subjective responses were observed, other than a slight time delay in a light-stimulus time-discrimination test, which was determined by the authors to not be related to chemical exposure. These studies, however, had several limitations such as small sample size, subjects lost to attrition, and multiple exposure schemes.

Chloromethane exposure at sufficiently high levels also results in neurological effects in animals. Consistent findings across numerous studies include clinical signs of neurotoxicity, some severe, and histopathological changes in the brain (cerebellum) and spinal cord. In general, mice and dogs appear to be the most sensitive species, with similar, but more severe, responses at lower exposure concentrations. There are no mechanistic data to explain the marked difference in neurotoxicity between species and strains, see Section 2.21, Mechanisms of Toxicity for general mechanisms of toxicity.

As discussed in Section 2.2, deaths associated with exposure to chloromethane for acute or intermediate durations were preceded by clinical signs of neurotoxicity in several species, predominantly neuromuscular and sensorimotor effects (Smith and von Oettingen 1947a, 1947b; von Oettingen et al. 1949, 1950). These results demonstrate a universal response of animals to the neurotoxic effects of chloromethane. Effects observed following acute-duration exposures included neuromuscular

abnormalities, impaired gait, and hindlimb drag in mice at ≥500 ppm; tremors, spasticity, and impaired gait in dogs at ≥500 ppm; backward arching of the head, neck, and spine, lost righting reflex, and convulsions in guinea pigs at ≥1,000 ppm; incoordination, motor impairments, seizures, and/or loss of consciousness in monkeys at 2,000 ppm; agitation and hunched posture in rats at ≥2,000 ppm; and neuromuscular dysfunction of hind legs and spastic adduction. While all cats survived an acute-duration exposure to 2,000 ppm, weakness, ataxia, and loss of righting reflex were observed. Effects became more severe (e.g., inability to walk) and/or were observed at lower concentrations following intermediate-duration exposure in monkeys, mice, and dogs at ≥500 ppm, guinea pigs at 1,000 ppm, and cats at 2,000 ppm (Smith and von Oettingen 1947a, 1947b). However, no clinical signs were noted in rabbits exposed to ≥500 ppm or rats exposed to 1,000 ppm for up to 266 days (despite ≥50% mortality). In dogs exposed to extremely high concentrations for 4–6 hours (14,661 or 40,560 ppm), decreased corneal and pupillary reflexes and complete muscle relaxation were observed prior to death, indicating CNS depression (von Oettingen et al. 1949, 1950).

Additionally, studies have reported clinical signs of neurotoxicity and motor deficits in rats and mice following inhalation exposure, with mice more susceptible than rats. Studies with continuous exposure (22 hours/day), showed lethargy in rats at ≥972 ppm after 48 or 72 hours (Burek et al. 1981), motor incoordination in mice at ≥150 ppm within 11 days (Landry et al. 1985), and ataxia in mice at ≥200 ppm within 5 days (Landry et al. 1985). With intermittent exposure (6 hours/day) for 5–9 days, severe signs of neurotoxicity were observed in rats at ≥5,000 ppm, including incoordination, ataxia, hindlimb paralysis, and sedation (Chellman et al. 1986a; Morgan et al. 1982). In longer-duration intermittent exposure studies, no clinical signs of neurotoxicity were reported in rats at concentrations up to 1,473 ppm for 3 months (McKenna et al. 1981b; Mitchell et al. 1979) or 997 ppm for 6–24 months (CIIT 1981). In mice, intermittent (5.5–6 hours/day) exposure for up to 2 weeks was associated with motor incoordination, altered activity levels (increased, then decreased), hypersensitivity to touch and sound, piloerection, tremors, convulsions, ataxia, front and hindlimb paralysis or rigidity, and/or sedation at concentrations ≥502 ppm (Chellman et al. 1986b; Jiang et al. 1985; Landry et al. 1985; Wolkowski-Tyl et al. 1981a, 1981b, 1983a, 1983b). In mice, severe neurological signs and motor impairment were observed following intermittent exposure to 997 ppm for ≥18 months, including tremor, hindlimb rigidity, paralysis, altered gait, and impaired reflexes (CIIT 1981). However, no clinical signs of neurotoxicity were observed at intermittent concentrations up to 1,473 ppm for 3 months (McKenna et al. 1981b; Mitchell et al. 1979) or 997 ppm for 6 or 12 months (CIIT 1981).

Consistent with the observed motor impairments described above, the granular layer of the cerebellum, which controls posture and coordination, appears to be a target of chloromethane toxicity in rodents. As observed with clinical signs, mice appear to be markedly more sensitive than rats, and mouse strains showed differences in susceptibility. Degeneration of the cerebellum was observed in rats following acute-duration inhalation exposure to ≥5,000 ppm; severity of lesions ranged from minimal to severe (Chellman et al. 1986a; Morgan et al. 1982). However, no histopathological changes in the brain or spinal cord were noted in rats following continuous exposure to concentrations up to 1,968 ppm for 48 hours or 1,950 ppm for 72 hours (Burek et al. 1981), up to 1,473 ppm for 3 months (McKenna et al. 1981b; Mitchell et al. 1979), or up to 997 ppm for 6–24 months (CIIT 1981).

In mice, the most sensitive mouse strain appears to be C57BL/6. In this strain, continuous exposure (22 hours/day) for 11 days resulted in slight cerebellar degeneration at ≥150 ppm (Landry et al. 1985). Intermittent exposure (5.5–6 hours/day) for 11–14 days showed slight cerebellar degeneration at 400 ppm, minimal-to-severe cerebellar degeneration at 1,000 ppm, focal necrosis at 1,492 ppm, and severe cerebellar degeneration at 1,500 ppm (Jiang et al. 1985; Landry et al. 1985; Morgan et al. 1982; Wolkowski-Tyl et al. 1981a, 1983a). Landry et al. (1985) addressed an apparent greater sensitivity to continuous exposure and hypothesized that it might be related to the conversion of chloromethane to an active metabolite, 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. However, when neurological effects are compared on the basis of total chloromethane inhaled per day, they appear to be similar for intermittent and continuous exposures. No longer-duration studies in C57BL/6 mice were identified. In B6C3F1 mice, minimal cerebellar degeneration was observed after intermittent exposure to concentrations ≥1,500 ppm for 12–14 days (Chellman et al. 1986b; Morgan et al. 1982). No exposure-related lesions were observed in B6C3F1 mice after intermittent exposure to concentrations up to 1,473 ppm for 3 months (Mitchell et al. 1979) or 997 ppm for 6 or 12 months (CIIT 1981). However, after 18–24 months, axonal swelling and degeneration of axons in spinal cord were observed in B6C3F1 mice exposed to ≥51 ppm; minimal-to-mild cerebellar degeneration was observed at 997 ppm (CIIT 1981). In other mouse strains, no histopathological changes in the brain or spinal cord were observed in C3H mice intermittently exposed to concentrations up to 2,000 ppm for 12 days (Morgan et al. 1982) or CD-1 mice intermittently exposed to concentrations up to 399 ppm for 94 days (McKenna et al. 1981b).

While mice appear more susceptible than rats with respect to overt clinical signs of neurotoxicity and histopathological findings, sensorimotor response testing during a 93-day inhalation study revealed

sensorimotor impairments in female rats, including impairments in the wire maneuver task at ≥149 ppm (inability of the animals to raise their hindquarters to the top of the wire while grasping with forelimbs) and decreased hindlimb clasp at 399 ppm (McKenna et al. 1981b). These effects were not observed in mice at concentrations up to 399 ppm (McKenna et al. 1981b). No other studies evaluating sensorimotor responses were identified.

In dogs, clinical signs of neurotoxicity and various CNS lesions were observed following continuous (23.5 hours/day) exposure to 496 ppm for 3 days (McKenna et al. 1981a). Clinical signs included severe limb stiffness, tremors, salivation, and incoordination. Unlike rodents, the cerebellum was not a target of chloromethane toxicity in dogs. However, observed brain and spinal cord lesions included vacuolization, swollen eosinophilic axons, loss of axons, demyelination, 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. In a 93-day study, intermittent (6 hours/day) exposure to concentrations up to 399 ppm did not result in neurological effects in dogs.

In cats, no signs of neurotoxicity were observed after continuous exposure (23.5 hours/day) to concentrations up to 501 ppm for 3 days (McKenna et al. 1981a).

2.16 REPRODUCTIVE

Available human studies are too limited to determine if inhalation exposure to chloromethane affects the reproductive system; no oral or dermal studies in humans were identified. A systematic evaluation of the literature (Appendix C) determined that toxicity to the male reproductive system is a presumed health effect associated with inhalation exposure to chloromethane based on a high level of evidence from laboratory animals. No studies were located regarding reproductive effects in animals after oral or dermal exposure to chloromethane.

One case report of a human with a history of exposure to chloromethane described sexual impotence as a possible indicator of reproductive toxicity. The individual owned a refrigeration plant and reported high exposures to chloromethane along with signs and symptoms typically associated with acute overexposure. In addition, during a 1-year period, he began experiencing morning urethral discharge and sexual impotence that gradually increased to completeness in a 3–4-month period (Mackie 1961). No additional studies were located regarding reproductive effects in humans after exposure to chloromethane.

Impaired male fertility has been observed in rats following inhalation exposure. In acute-duration studies, exposure to concentrations ≥3,000 ppm for 5 days prior to breeding with unexposed female rats resulted in decreased fertilization rate, decreased number of live and total implants, and increased pre- and post-implantation loss (Chellman et al. 1986c; Working and Bus 1986; Working et al. 1985a, 1985b). Exposed males showed a reversible disruption of spermatogenesis, transient reduction in testes weights, and increased infiltration of neutrophils and macrophages into the interstitium of the cauda epididymis (Chellman et al. 1986c; Working et al. 1985b). In a 2-generation study in which males were exposed for 10 weeks prior to mating, the number of fertile males and the number of litters per copulation plug were reduced in F0 males at 472 ppm following mating with similarly exposed or unexposed females (Hamm et al. 1985). Complete male sterility, atrophy of the seminiferous tubules, and epididymal granulomas were observed in F0 rats at 1,502 ppm (Hamm et al. 1985). A nonsignificant decrease in F1 male fertility was observed at 472 ppm.

Several additional studies evaluated male reproductive organ weight and/or histology in rodents following inhalation exposure to chloromethane. Continuous exposure to >500 ppm for 48 hours was associated with numerous histopathological changes in the testes, including sperm granulomas, decreased sperm in the tubule lumen, interstitial edema, and coagulated proteinaceous obstruction of lumen in rats; testicular atrophy and inflammation was also observed after exposure for 72 hours (Burek et al. 1981). In acute-duration inhalation studies with intermittent exposure for 2–12 days, testicular changes were consistently observed at ≥3,500 ppm. Observed changes include reduced testes weight, sperm effects (delayed spermiation, reduction in spermatids and sperm, reduced sperm motility, altered sperm maturation), and/or various testicular lesions (seminiferous epithelium vacuolation, bilateral epididymal granulomas, multinucleated giant cells) (Chapin et al. 1984; Chellman et al. 1986a; 1987; Morgan et al. 1982). One acute study in rats also reported decreased serum testosterone after exposure to 3,500 ppm for 12 days (Chapin et al. 1984). In longer-duration studies in rats and mice, no adverse testicular effects were noted at concentrations exposed up to 1,473 ppm for 3 months (McKenna et al. 1981b; Mitchell et al. 1979). However, degeneration and atrophy of the seminiferous tubules and sperm granulomas were observed following exposure to 997 ppm for 6, 12, 18, or 24 months in rats and 18 or 24 months in mice (CIIT 1981).

No histopathological changes in male reproductive organs were noted in dogs or cats exposed continuously (23.5 hours/day) to concentrations up to approximately 500 ppm for 3 days (McKenna et al. 1981a) or dogs exposed intermittently (6 hours/day) for 3 months (McKenna et al. 1981b).

The female reproductive system does not appear to be a target of chloromethane toxicity. No exposure-related changes in female reproductive organ weight or histology were observed in F0 or F1 rats exposed to concentrations up to 1,502 ppm during a 2-generation study (Hamm et al. 1985). However, due to clear deficits in male fertility in this study (and lack of a mating study between unexposed males and exposed females), female reproductive function could not be adequately assessed. In gestational exposure studies, no adverse reproductive effects were observed in rats exposed to concentrations up to 1,492 ppm on GDs 7–19 (Wolkowski-Tyl et al. 1981a, 1983a), mice exposed to concentrations up to 502 ppm on GDs 6–17 (Wolkowski-Tyl et al. 1981a, 1981b, 1983b, 1983b), or rabbits exposed to concentrations up to 1,012 ppm on GDs 6–28 (Theuns-van Vliet 2016). In other studies, no changes in female reproductive organ weight or histology were observed in rats or mice exposed to concentrations up to 1,473 ppm for 3 months (McKenna et al. 1981b; Mitchell et al. 1979) or 997 ppm for 6–24 months (CIIT 1981).

2.17 DEVELOPMENTAL

No studies were located regarding developmental effects in humans after exposure to chloromethane. A systematic review of the literature (Appendix C) determined that chloromethane is not classifiable as it relates to developmental toxicity following inhalation exposure, based on a low level of evidence in laboratory studies. Potential developmental effects following gestational exposure have been examined in rats, mice, and rabbits; findings suggest differences in species susceptibility and developmental targets. No studies were located regarding developmental effects in animals after oral or dermal exposure to chloromethane.

In rats, decreased fetal body weight, decreased crown-rump length (females only), and delayed skeletal development were observed after maternal exposure to 1,492 ppm on GDs 7–19 (Wolkowski-Tyl et al. 1981a, 1983a). These findings may have been secondary to maternal toxicity (marked reduction in body weight gain), which was observed at ≥479 ppm. In a 2-generation study in rats, no adverse effects on survival, growth, or development were observed in F1 or F2 offspring at parental exposure concentrations up to 472 ppm (Hamm et al. 1985).

In mice, an exposure-related increase in heart defects was observed following maternal exposure to concentrations ≥479 ppm on GDs 6–17, characterized by small right ventricle, globular heart, white spots (assumed to be calcium deposits, in the left ventricular wall), and/or absent or abnormal atrioventricular valves, chordae tendinea, and papillary muscles (Wolkowski-Tyl et al. 1981a, 1981b, 1983a, 1983b). In the mouse studies, maternal toxicity (reduced survival and body weight) was not observed until

≥749 ppm. However, in a letter to the journal from the same research organization, John-Greene et al. (1985) suggested that the heart anomalies reported by Wolkowski-Tyl et al. (1983a) may have been an artifact of the sectioning technique, due to the examination of the fixed as opposed to unfixed fetal tissue, or a misdiagnosis. They also suggested that, though Wolkowski-Tyl et al. (1983b) used a more appropriate sectioning technique, the papillary muscle effects reported were rare and should not have occurred without other expected cardiovascular malformations. In pilot exposures of 250–300 ppm on GDs 11.5–12.5, John-Greene et al. (1985) observed inter-animal variability in the appearance of the papillary muscles in control mice and could not reproduce the results of Wolkowski-Tyl et al. (1983a, 1983b). However, in a response to the John-Greene et al. (1985) letter, Wolkowski-Tyl (1985) countered that the inability of John-Greene et al. (1985) to detect the abnormality was due to the lower exposure concentrations, shorter exposure durations, and difference in timing of exposure during gestation, arguing that the most critical day is GD 14.

No exposure-related changes in litter outcomes, fetal body weight, or malformations or anomalies were observed in rabbits exposed to concentrations up to 1,012 ppm on GDs 6–28 (Theuns-van Vliet 2016).

2.18 OTHER NONCANCER

No studies were located regarding other systemic effects in humans after exposure to chloromethane. The only other systemic effect reported in inhalation studies in animals was inanition (exhaustion caused by lack of nourishment) associated with a decrease in food consumption in mice exposed to ≥200 ppm for up to 11 days (22 hours/day) or 2,400 ppm for up to 9 days (5.5 hours/day) (Landry et al. 1985). No studies were located regarding other noncancer effects in animals after oral or dermal exposure to chloromethane.

2.19 CANCER

Available human data regarding carcinogenicity of chloromethane following inhalation exposure are limited and findings are mixed. Cancer bioassays in animals are available for rats and mice via inhalation exposure. Increased renal tumors were reported in male mice; no neoplastic changes were noted in female mice or male or female rats. No studies were located regarding carcinogenicity in animals after oral or dermal exposure to chloromethane.

Several epidemiological studies have evaluated the potential association between occupational exposure to chloromethane and risk of cancer (Table 2-1). Most available studies did not have direct measures of

chloromethane exposure and used job exposure matrices instead to estimate the probability and intensity of chloromethane exposure. Of these, the occupational cohort study with the highest probability and intensity of exposure is most likely the Icelandic fisherman cohort (discussed in Section 2.2) accidentally exposed to high levels of chloromethane due to a refrigerant leak. At the 32-year follow-up, the risk of death from cancer was not increased in this cohort, compared to a referent group of Icelandic fishermen (Rafnsson and Gudmundsson 1997). However, at the 47-year follow-up, the risk of death specifically from kidney cancer was increased in the exposed cohort, compared to the referent group (Rafnsson and Kristbjornsdottir 2014). It is noted that the Icelandic fisherman cohort is small (<30 men), had high but unmeasured exposure levels, and was not adjusted for other lifestyle factors such as smoking and diet. Therefore, the generalizability of these results is unclear. In another occupational cohort study, the risk of death due to cancer was not increased in synthetic rubber workers exposed to chloromethane (Holmes et al. 1986). While no exposure estimates are available, exposure to rubber workers is likely lower than the acute-duration exposure experienced in the Icelandic fisherman cohort.

In general, case-control studies did not observe associations between estimated occupational exposure to chloromethane and overall risk of cancer. Barry et al. (2011) did not observe an increased risk of NHL in women with occupational exposure to chloromethane, compared to unexposed women (Barry et al. 2011). However, the risk was increased specifically for follicular lymphoma in exposed women, and exposed women with a specific CYP2E1 rs2070673 polymorphism (TT but not TA genotype) had an increased risk of both total NHL and follicular lymphoma. No associations were observed for diffuse large B-cell lymphoma. Kernan et al. (1999) did not observe an increased risk of death from pancreatic cancer in patients with occupational exposure to chloromethane, compared to unexposed patients, when sexes and races were combined. Based on a small sample of black men, there was an increased risk of death from pancreatic cancer when there was a high probability of exposure to chloromethane. This association was not observed in white men, white women, or black women. Dosemeci et al. (1999) did not observe any associations between renal cell carcinoma and occupational exposure to chloromethane.

A high incidence of renal tumors was found in male mice that were exposed primarily to approximately 997 ppm chloromethane and died or were killed at 12 months or later (primarily between 18 and 24 months) in a 2-year oncogenicity study (CIIT 1981). Tumors consisted of renal cortex adenomas, adenocarcinomas, papillary cystadenomas, tubular cystadenomas, and a papillary cystadenocarcinoma. Renal cortex adenomas were also observed in two male mice at 225 ppm after 24 months; while this incidence did not differ significantly compared to controls, they were considered treatment-related by the study authors due to similarity with findings at 1,000 ppm. No evidence of carcinogenicity was found in

similarly exposed female mice or male or female rats exposed to concentrations up to 997 ppm (CIIT 1981).

IARC and EPA have both determined that chloromethane is not classifiable as to its carcinogenicity in humans (EPA 2001; IARC 2019). HHS (NTP 2016) has not evaluated the potential for chloromethane to cause carcinogenicity in humans.

2.20 GENOTOXICITY

Available evidence indicates that chloromethane is mutagenic and clastogenic and has the potential to directly interact with deoxyribonucleic acid (DNA). Results of *in vitro* and *in vivo* genetic testing are presented in Tables 2-4 and 2-5, respectively.

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

Species (test system)	Endpoint	Results Activation With	Results Activation Without	Reference
Prokaryotic organisms				
Salmonella typhimurium	Gene mutation	+	+	Simmon et al. 1977
S. typhimurium strain TA1535	Gene mutation	+	+	Andrews et al. 1976
S. typhimurium strains TA18, TA100, TA1535, TA1537	Gene mutation	+	+	DuPont 1977
S. typhimurium strain TA677	Gene mutation	NT	+	Fostel et al. 1985
S. typhimurium strains TA98, TA100	Gene mutation	+	+	NTP 2019
Mammalian cells				
Human lymphoblasts	Gene mutation	NT	+	Fostel et al. 1985
Human lymphoblasts	Sister-chromatid exchange	NT	+	Fostel et al. 1985
Chinese hamster lung cells	Chromosomal aberrations	+	+	Asakura et al. 2008
Human lymphoblasts	DNA strand breaks	NT	–	Fostel et al. 1985
Rat hepatocytes	Unscheduled DNA synthesis	NT	+	Working et al. 1986
Rat spermatocytes	Unscheduled DNA synthesis	NT	+	Working et al. 1986
Rat tracheal epithelial cells	Unscheduled DNA synthesis	NT	–	Working et al. 1986
Primary hamster embryo cells	Unscheduled DNA synthesis	NT	+	Hatch et al. 1982, 1983

+ = positive result; — = negative result; DNA = deoxyribonucleic acid; NT = not tested

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

Species (test system)	Endpoint	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)	Unscheduled DNA synthesis in hepatocytes	(+)	Working et al. 1986
Rat (inhalation)	Unscheduled DNA synthesis in spermatocytes	–	Working et al. 1986
Rat (inhalation)	Unscheduled DNA synthesis in tracheal epithelial cells	–	Working et al. 1986
Mouse (inhalation)	DNA damage in kidney cells (single strand breaks)	+	Jager et al. 1988
Mouse (inhalation)	DNA damage in kidney cells (single strand breaks)	+	Ristau et al. 1990
Drosophila (inhalation)	Recessive lethal	+	University of Wisconsin 1986

– = negative results; + = positive results; (+) = marginally positive result; DNA = deoxyribonucleic acid

Chloromethane is mutagenic in *Salmonella typhimurium* both with and without metabolic activation (Andrews et al. 1976; DuPont 1977; Fostel et al. 1985; NTP 2019; Simmon et al. 1977) and human lymphoblasts without metabolic activation (not tested with metabolic activation) (Fostel et al. 1985). In *in vivo* studies, chloromethane induced recessive lethal mutations in *Drosophila melanogaster* (University of Wisconsin 1986) and dominant lethal mutations in rats (Chellman et al. 1986c; Rushbrook 1984) following inhalation exposure.

Chloromethane is also clastogenic, inducing sister chromatid exchanges and in human lymphoblast cells without metabolic activation (not tested with metabolic activation) (Fostel et al. 1985) and chromosomal aberrations in Chinese hamster lung cells both with and without metabolic activation (Asakura et al. 2008).

Findings regarding direct interactions with DNA are mixed. Chloromethane did not induce DNA strand breaks in human lymphoblasts following *in vitro* exposure without metabolic activation (Fostel et al. 1985); however, DNA strand breaks were observed in kidney cells of mice follow *in vivo* inhalation exposure (Jager et al. 1988; Ristau et al. 1990). Chloromethane also induced unscheduled DNA synthesis (UDS) in cultured primary hamster embryo cells without metabolic activation (Hatch et al. 1982, 1983). Working et al. (1986) evaluated the potential for chloromethane to induce UDS in rat hepatocytes, spermatocytes, and tracheal epithelial cells both *in vitro* and *in vivo*. UDS was induced in cultured rat

hepatocytes and spermatocytes without metabolic activation at near-cytotoxic concentrations; however, *in vivo* exposure only marginally induced UDS in hepatocytes (Working et al. 1986).

2.21 MECHANISMS OF TOXICITY

Lethal and toxic effects associated with chloromethane have been attributed to common mechanisms across organ systems. Chellman et al. (1986b) proposed that glutathione (GSH) conjugation of chloromethane into toxic metabolites underlies its effects, as evidenced by reduced lethality, hepatotoxicity, renal toxicity, and neurotoxicity in mice following pre-treatment with the GSH deplete, L-buthionine-S,R-sulfoximine (BSO). For example, the LC_{50} in the non-pretreated mice was 2,200 ppm, while the LC_{50} for the pretreated rats was 3,200 ppm. Additionally, a single, 8-hour exposure to 1,000 ppm chloromethane significantly reduced glutathione-S-transferase (GST) activity in the liver and kidney in female and male mice and in male rats, but when exposure was repeated 6 hours/day for 4 days, GST activity was only significantly reduced in the liver of male mice (Jager et al. 1988). Additionally, several studies have provided evidence of dose-related depletion of nonprotein sulfhydryls (NPSH) in the rat liver and/or kidney following exposure to chloromethane, which is likely the result of GSH conjugation of chloromethane (Chapin et al. 1984; Dodd et al. 1982; Landry et al. 1983a).

Pro-inflammatory changes may also contribute to systemic toxicity of chloromethane. Chellman et al. (1986a) showed that pre- and post-treatment with an anti-inflammatory agent (3-amino-l-[m-(trifluoromethyl)phenyl]-2-pyrazoline [BW775C]) reduced lethality, hepatotoxicity, neurotoxicity, and testicular toxicity. Both incidence and severity of chloromethane-induced lesions were reduced following treatment with BW775C. The study authors concluded that protection from toxic effects 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 GSH content. The protection afforded by BW755C may have been related to an inhibition of leukotriene and prostaglandin synthesis.

However, decreased fertility in male rats exposed to chloromethane does not appear to be related to inflammatory changes. Multiple studies showed that BW755C did not protect against sperm damage or pre-implantation loss in females mated with exposed males (Chellman et al. 1986c, 1987). Instead, the study authors proposed that these outcomes were related to the cytotoxicity of chloromethane, not chloromethane-induced inflammation. Other studies speculated that while inflammation-derived reactive metabolites (e.g., superoxide anion) could damage DNA or sperm in epididymides, chloromethane may

not reach the testes in sufficient concentrations to produce detectable DNA damage (Working et al. 1985a). The study authors concluded that preimplantation losses observed in acute-duration inhalation studies in rats could be explained by a cytotoxic effect resulting in failure of fertilization, rather than a genotoxic effect resulting in early embryonic death (Working and Bus 1986).