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 chloroform. 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 chloroform, 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 chloroform was also conducted; the results of this review are presented in Appendix C.

Animal and human inhalation studies are presented in Table 2-1 and Figure 2-2, animal oral studies are presented in Table 2-2 and Figure 2-3; and animal dermal studies are presented in Table 2-3.

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. Effects have been classified into “less serious LOAELs” or “serious LOAELs (SLOAELs).” “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause

significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an 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 chloroform are indicated in Tables 2-1 and 2-2 and Figures 2-2 and 2-3.

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

The health effects of chloroform have been evaluated in 86 human and 146 animal studies. As illustrated in Figure 2-1, most of the health effects data come from inhalation and oral exposure studies in animals. For the purposes of Figure 2-1, all human studies with exposure to chloroform as a tap water disinfection byproduct were classified as oral, despite potential for multi-route exposure (e.g., inhalation and dermal via showering and bathing activities). Similarly, human studies evaluating exposure to chloroform when swimming in chlorinated pools are classified as inhalation exposure, despite concurrent dermal exposure, because exposure via inhalation is expected to contribute more to body burden. Lastly, human studies that evaluated blood levels of chloroform as a biomarker of exposure but did not have any information pertaining to possible exposure sources are not included in Figure 2-1 due to unknown route(s) of exposure.

For animal data, inhalation and oral studies are available for all health effect and exposure duration categories. The dermal animal database is limited to two acute-duration studies. The most examined endpoints were body weight, hepatic, and renal effects. The available human studies include some epidemiological data (including occupational and evaluations of chlorinated by products in water), but available data are predominantly from case studies and case-series reports. Human studies were predominantly focused on hepatic, cancer, developmental, and neurological effects.

As outlined in Chapter 1, the respiratory, hepatic, renal, and neurological systems as well as the developing organism appear to be sensitive targets of toxicity following inhalation or oral exposure to chloroform. 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 chloroform toxicity:

- **Respiratory Endpoints.** Respiratory effects are a presumed health effect associated with chloroform exposure via inhalation based on inadequate evidence in human epidemiology studies and a high level of evidence in animal studies. In humans, epidemiological data with exposure-route information are limited to one study reporting a lack of alterations in respiratory function in adults after a 40-minute swim in a chlorinated pool. In case reports, depression of respiratory rates and/or respiratory arrest has been reported at high exposure levels; these effects are likely secondary to CNS depression. Lung damage has been reported in fatal cases of inhalation or oral exposure. In animals, the nasal epithelium and underlying nasal bones are consistent targets of toxicity in rodents following acute-, intermediate-, and chronic-duration inhalation exposure and acute- and intermediate-duration gavage exposure. Damage to the lower respiratory tract in animals was generally only observed at lethal exposure levels.

- **Hepatic Endpoints.** Hepatic effects are a known health effect for humans exposed to chloroform based on a low level of evidence from human epidemiology studies, high level of evidence from animal studies, and other relevant data consisting of the extensive database of case reports and case series documenting hepatic effects of chloroform in exposed humans. Evidence from occupational studies in humans is inconsistent. However, numerous case series and case reports indicate that the liver is a clear target of toxicity in humans following oral and inhalation exposure to high levels of chloroform. In animal studies, hepatic lesions have been observed following acute-, intermediate-, and chronic-duration inhalation and oral studies in rodents; intermediate- and chronic-duration oral studies in dogs; and an acute-duration oral study in rabbits. Hepatic enzyme changes have also been observed in some studies. In acute- and intermediate-duration oral studies, rodents exposed via gavage are more susceptible to hepatotoxicity than those exposed via drinking water.

- **Renal Endpoints.** Renal effects are a presumed health effect associated with chloroform exposure via inhalation based on inadequate evidence in human epidemiology studies and a high level of evidence in animal studies. Limited epidemiological data did not report adverse renal effects in one occupational cohort or a group of competitive swimmers. However, several case studies reported renal effects in humans associated with exposure to high levels of chloroform via inhalation or oral routes. In animal studies, renal lesions have been observed following acute-, intermediate-, and chronic-duration inhalation and oral studies in rodents; intermediate- and chronic-duration oral studies in dogs; and acute-duration oral and dermal studies in rabbits. In acute- and intermediate-duration oral studies, rodents exposed via gavage are more susceptible to renal toxicity than those exposed via drinking water.

- **Neurological Endpoints.** Neurological effects are a known health effect associated with chloroform exposure based on a low level of evidence in human epidemiology studies, high level of evidence in animal studies, and other relevant data including chloroform's historical use as a general anesthetic, case reports and case series documenting marked neurological effects of chloroform in exposed humans, and a plausible mechanism of action. Chloroform was previously a common general anesthetic, so it is a known CNS depressant at high exposure levels in both

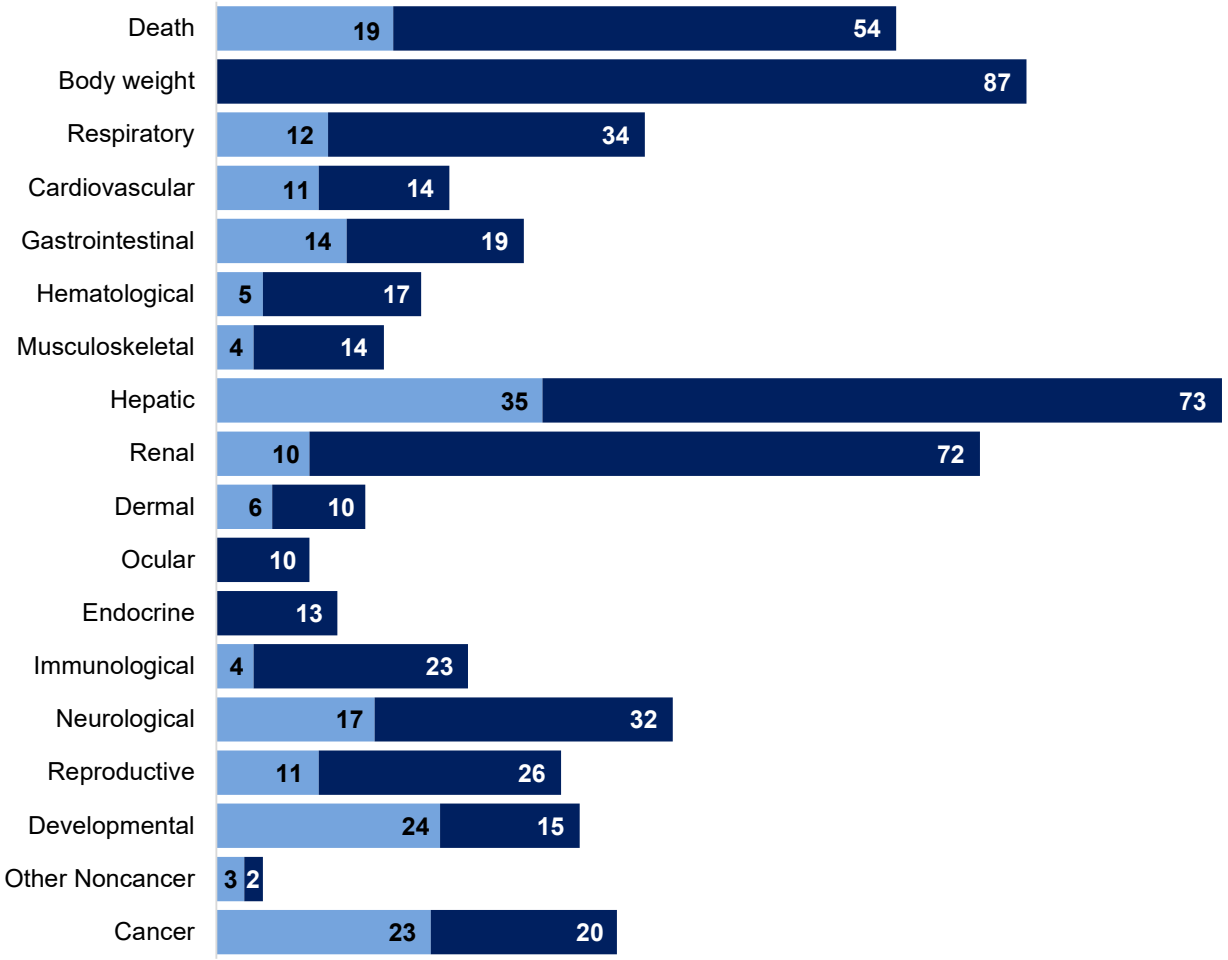
humans and animals. There is limited evidence for neurological effects at exposure levels below those associated with frank CNS depression. One epidemiological study in humans reported neurobehavioral impairments at low occupational exposure levels, and a limited number of animal studies reported alterations in neurobehavioral testing following acute-duration oral exposure. The only histopathological change reported in the neurological system is olfactory nerve loss in rats following acute-duration inhalation exposure; this finding is likely in response to degeneration of the nasal olfactory epithelial tissue observed at the same exposure levels.

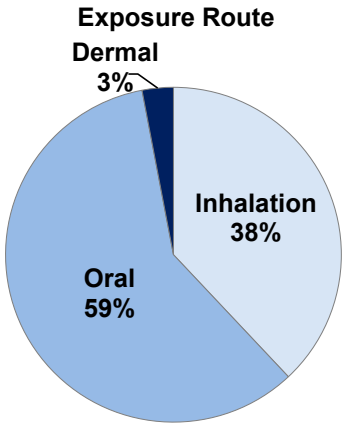
- **Developmental Endpoints.** Developmental effects are a suspected health effect for humans based on inadequate evidence in human epidemiology studies and a moderate level of evidence in animal studies. Epidemiological studies evaluating developmental effects associated with exposure to disinfection byproducts in chlorinated water, including chloroform, provide inconsistent evidence of adverse pregnancy outcomes (low birth weight, intrauterine growth restriction, small for gestational age). There is also inconsistent evidence for fetal malformations or variations in animals following inhalation or oral exposure. Decreased fetal growth was reported in many developmental studies at inhalation or oral exposure levels associated with maternal toxicity (e.g., decreased maternal body weight gain).

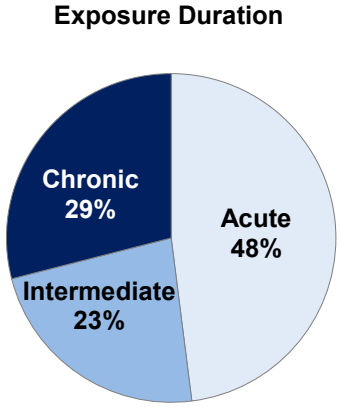
Figure 2-1. Overview of the Number of Studies Examining Chloroform Health Effects*

Most studies examined the potential hepatic, body weight, and renal effects of chloroform

Fewer studies evaluated health effects in humans than animals (counts represent studies examining endpoint)







*Includes studies discussed in Chapter 2. A total of 258 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints. Human studies with multi-route exposure were included only once in the figure; the studies were classified based on the most predominant route of exposure (e.g., tap water exposure classified as oral, despite potential for inhalation or dermal exposure via showering/bathing). Human studies with unknown route(s) of exposure (i.e., exposure assessed via biomarker) are not included in this figure or the study count reported above.

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Baeder and Hofmann 1988									
1	Rat (Wistar) 20 F	10 days GDs 7–16 7 hours/day (WB)	0, 32, 119, 311	LE, CS, BW, FI, OW, NX, RX, DX	Bd wt		32	119	LOAEL: 18% decrease in maternal body weight gain SLOAEL: 24% decrease in maternal body weight gain
					Hepatic	311			
					Renal	311			
					Immuno	311			
					Repro	119		311	Increased incidence of full litter resorption
					Develop	119	311		6% decrease in live fetus weight; 4% decrease in live fetus crown-rump length
DHA 2022									
2	Rat (Sprague-Dawley) 24 M	30 minutes (WB)	0, 401, 3,206, 6,411	BI, NX	Neuro	401	3,206		Increased overall distance travelled in an open field, decreased rearing, impaired motor coordination
EPA 1978									
3	Rat (Sprague-Dawley) 9–10 F	8 days GDs 7–14 1 hour/day (WB)	0, 942, 2,233, 4,117	LE, BW, FI, GN, RX, DX	Bd wt	2,233		4,117	25% decrease in maternal body weight
					Neuro	942		2,233	Narcosis
					Repro	2,233		4,117	Increased resorptions
					Develop	2,233	4,117		8% decrease in fetal body weight

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kasai et al. 2002									
4	Rat (Fischer-344) 10 M, 10 F	2 weeks 5 days/week 6 hours/day (WB)	0, 500, 1,000, 2,000, 4,000, 8,000	LE, HP	Death			2,000	100% mortality
					Resp		500		Desquamation and atrophy of olfactory epithelium; edema of the lamina propria
					Hepatic		500		Hepatic vacuolation (central area)
					Renal		500		Vacuolation in the proximal tubules
Larson et al. 1994c; Mery et al. 1994									
5	Rat (Fischer-344) 5 M	7 days 6 hours/day (WB)	0, 1.5, 3.1, 10.4, 29.3, 100, 271	CS, BW, GN, OW, HP	Bd wt	100		271	20% decrease in body weight gain
					Resp	3.1	10.4		Goblet cell hyperplasia in nasal respiratory epithelium, olfactory gland degeneration in lamina propria; nasal periosteal cell proliferation and new bone formation
					Hepatic	29.3	100		Hepatocellular proliferation
					Renal	10.4	29.3		Focal epithelial proliferation in the renal cortex
					Neuro	3.1		10.4	Olfactory neuron loss
Lundberg et al. 1986									
6	Rat (Sprague-Dawley) 10 F	4 hours (WB)	Not reported	LE	Death			9,770.6	LC_{50}
Schwetz et al. 1974									
7	Rat (Sprague-Dawley) 3–68 F	10 days GDs 6–15 7 hours/day (WB)	0, 30, 95, 291	CS, BW, FI, BC, OW, RX, DX	Bd wt		30	291	LOAEL: 10% decrease in maternal body weight on GD 13 SLOAEL: 38% decrease in maternal body weight on GDs 13 and 21
					Repro	95		291	Increased resorptions, decreased number of live fetuses/litter

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Develop		30	95	LOAEL: Delayed ossification and wavy ribs SLOAEL: Missing ribs; acaudate fetuses with imperforate anus
Smyth et al. 1962									
8	Rat (Albino) 6 B	4 hours (NS)	8,000	LE	Death			8,000	86% mortality
Templin et al. 1996b									
9	Rat (Fischer-344) 5 M	4 days 6 hours/day (WB)	0, 2, 10, 30, 90, 300	CS, BW, GN, HP	Bd wt	90	300		17% decrease in body weight gain
					Resp	2[b]	10		Loss of olfactory glands, periosteal hypercellularity and proliferation, mineralization of the basal lamina, new nasal bone growth
					Hepatic	90	300		Hepatocellular proliferation
					Renal	90	300		Minimal vacuolation of proximal convoluted tubule
Aranyi et al. 1986									
10	Mouse (CD-1) 140 F	3 hours (WB)	0, 10.6	LE, IX	Immuno	10.6			
Aranyi et al. 1986									
11	Mouse (CD-1) 112 F	5 days 3 hours/day (WB)	0, 10.6	LE, IX	Immuno		10.6		Increased susceptibility to succumb to infection
Ban et al. 2006									
12	Mouse (BALB/c) 12 F	4 days 6 hours/day (WB)	0, 20	IX	Immuno	20			

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Constan et al. 1999									
13	Mouse (B6C3F1) 5 M	4 days 6 hours/day (WB)	0, 92	LE, CS, BW, OW, GN, HP	Bd wt	92			
					Resp		92		Submucosal edema and periosteal cell proliferation in the ethmoid turbinates and nasal wall
					Hepatic		92		Moderate vacuolar degeneration, increased cell proliferation, increased relative liver weight
					Renal			92	Severe necrosis in proximal convoluted tubules, increased cell proliferation, increased relative kidney weight
					Neuro		92		Lethargy
Constan et al. 1999									
14	Mouse (Sv/129) 4–5 M	4 days 6 hours/day (WB)	0, 92	LE, CS, BW, OW, GN, HP	Death			92	25% sacrificed moribund
					Bd wt	92			
					Resp		92		Submucosal edema and periosteal cell proliferation in the ethmoid turbinates and nasal wall
					Hepatic			92	Marked centrilobular degeneration and necrosis, increased cell proliferation, increased relative liver weight
					Renal			92	Severe necrosis in proximal convoluted tubules, increased cell proliferation, increased relative kidney weight
					Neuro		92		Lethargy

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
de Oliveira et al. 2015									
15	Mouse (C57BL/6) 10 M, 10 F	5 days 20 minutes 3 times/day, totaling 1 hour/day (WB)	0, 7	BW, OW, HP	Bd wt	7			
					Resp		7		Increased white blood cells in BALF, increased alveolar area, and decreased density of alveolar septa; decreased relative lung weight in females
Deringer et al. 1953									
16	Mouse (C3H) 3–22 M, 3–20 F	1 hour (WB)	0, 942, 983	LE	Death			983 M	100% mortality of adult males within 5–8 days
Deringer et al. 1953									
17	Mouse (C3H) 3–22 M, 3–20 F	2 hours (WB)	0, 942, 1,004	LE	Death			942 M	100% mortality of adult males within 2–11 days
Deringer et al. 1953									
18	Mouse (C3H) 3–22 M, 3–20 F	3 hours (WB)	0, 692, 1,106	LE	Death			692 M	100% mortality of adult males within 7–8 days
Deringer et al. 1953									
19	Mouse (C3H) 3–22 M, 3–20 F	2 hours (WB)	0, 942, 963	LE	Death			963 M	100% mortality of young mice within 2–7 days
Deringer et al. 1953									
20	Mouse (C3H) 3–22 M, 3–20 F	3 hours (WB)	0, 786, 901	LE	Death			786 M	100% mortality of young mice within 8–11 days

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Gehring 1968									
21	Mouse (Swiss-Webster) 20 F	12 hours (WB)	0, 4,500	LE, CS	Death			4,500	LT_{50} of 560 minutes
					Neuro			4,500	ET_{50} of 35 minutes for anesthesia
Kasai et al. 2002									
22	Mouse (Crj:BDF1) 10 M, 10 F	2 weeks 5 days/week 6 hours/day (WB)	0, 500, 1,000, 2,000, 4,000, 8,000	LE	Death			1,000 F 500 M	90% mortality 90% mortality
Larson et al. 1994c; Mery et al. 1994									
23	Mouse (B6C3F1) 5 F	7 days 6 hours/day (WB)	0, 1.2, 3, 10, 29.5, 101, 288	CS, BW, GN, OW, HP	Bd wt	288			
					Resp	3	10		Nasal periosteal cell proliferation
					Hepatic	1.2	3	101	LOAEL: Increased relative liver weight SLOAEL: Extensive necrosis; severe vacuolar degeneration
					Renal	101	288		Proximal tubule epithelial regeneration, cellular proliferation in renal cortex and medulla outer stripe
Larson et al. 1996									
24	Mouse (B6C3F1) 5 F	4 days 6 hours/day (WB)	0, 0.3, 2, 10, 30, 88	CS, BW, GN, HP	Resp	2[b]	10		Connective tissue proliferation in the nasal lamina propria, periosteal cell proliferation in the nasal cavity
					Hepatic	2	10		Mild-to-moderate diffuse lipid hepatocytic vacuolation, scattered hepatocyte necrosis
					Renal	88			
Lehmann and Flury 1943									
25	Mouse (NS) NS	0.5–2 hours (NS)	2,500, 3,100, 4,100	CS	Neuro	2,500		3,100	Slight narcosis after 1 hour

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Murray et al. 1979									
26	Mouse (CF1) 34–35 F	8 days GDs 1–7, 7 hours/day (WB)	0, 97	LE, CS, BW, FI, WI, GN, RX, DX	Repro			97	Decreased number of dams with implantation sites; increased resorptions/litter
					Develop			97	10% decrease in fetal body weight, decreased crown-rump length, delayed skull and sternebrae ossification
Murray et al. 1979									
27	Mouse (CF1) 34–35 F	8 days GDs 6–15 7 hours/day (WB)	0, 99	LE, CS, BW, FI, WI, GN, RX, DX	Bd wt	99			
					Repro			99	Decreased number of dams with implantation sites
					Develop		99		Delayed skull ossification
Murray et al. 1979									
28	Mouse (CF1) 40 F	8 days GDs 8–15 7 hours/day (WB)	0, 97	LE, CS, BW, FI, WI, GN, RX, DX	Repro	97			
					Develop			97	Cleft palate, 15% decrease in fetal body weight, decreased crown-rump length, delayed skull and sternebrae ossification
Selgrade and Gilmour 2010									
29	Mouse (CD-1) 10 F	3 hours (WB)	0, 100, 500, 1,000, 2,000	LE, IX	Immuno	100	500		Decreased bacterial clearance in lung following infection
Selgrade and Gilmour 2010									
30	Mouse (CD-1) 6 F	3 hours (WB)	0, 100, 500, 1,000, 2,000	IX	Immuno		100		Decreased phagocytic activity of alveolar macrophages following infection
Templin et al. 1996c									
31	Mouse (BDF1) 5 M	2 weeks 4–5 days/week 6 hours/day (WB)	0, 30, 90	LE, CS, BW, OW, GN, HP	Death			30	40% mortality
					Bd wt		30		13% decrease in body weight gain
					Hepatic	30	90		Minimal swelling in midzonal hepatocytes

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Renal			30	Severe tubular necrosis and tubular degeneration
Templin et al. 1996c									
32	Mouse (BDF1) 4–5 M, 5 F	4 days 6 hours/day (WB)	0, 0.3, 5, 30, 90	LE, CS, BW, OW, GN, HP	Bd wt	5 M 90 F	30 M		14% decrease in body weight gain
					Hepatic	5 M 30 F	30 M 90 F		Hepatocellular proliferation in males at ≥30 ppm and females at 90 ppm; focal necrosis in both sexes at 90 ppm
					Renal	5 M 90 F	30 M	90 M	LOAEL: Mild-to-moderate proximal tubular necrosis and dilation; hyaline casts and tubular degeneration; cell proliferation SLOAEL: Moderate-to-severe necrosis
Lehmann and Flury 1943									
33	Cat (NS) NS	5–93 minutes (NS)	7,200, 10,800, 14,300, 21,500	CS	Neuro			7,200	Disturbed equilibrium after 5 minutes, light narcosis after 78 minutes, and deep narcosis after 93 minutes
INTERMEDIATE EXPOSURE									
Kasai et al. 2002									
34	Rat (Fischer-344) 10 M, 10 F	13 weeks 5 days/week 6 hours/day (WB)	0, 25, 50, 100, 200, 400	LE, CS, BW, BC, UR, OW, GN, HP	Bd wt	25	50		Unspecified decrease in body weight gain
					Resp		25		Mineralization and atrophy of olfactory epithelium
					Hepatic	100 M 50 F	200 M 100 F		Localized hepatocyte loss
					Renal	50 F	50 M 100 F		Occult blood in urine (males) and increased absolute and relative kidney weight (females)

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Templin et al. 1996b									
35	Rat (Fischer-344) 10–13 M, 5–8 F	3 weeks 7 days/week 6 hours/day (WB)	0, 2, 10, 30, 90, 300	LE, CS, BW, OW, GN, HP	Bd wt	30 M 10 F	90 M 30 F	300	LOAEL: Decreased body weight gain in males (11%) and females (12%) SLOAEL: Decreased body weight gain in males (31%) and females (28%)
					Resp	2	10		Loss of olfactory glands, edema, and cellular proliferation in the nasal lamina propria
					Cardio	300			
					Gastro	300			
					Musc/skel	300			
					Hepatic	90 M 30 F	300 M 90 F		Hepatocellular vacuolation, cell necrosis in females at ≥90 ppm and males at 300 ppm; hepatocellular proliferation in both sexes at 300 ppm
					Renal	10	30		Renal cell proliferation in both sexes; vacuolation in the proximal convoluted tubule in males
					Dermal	300			
					Ocular	300			
					Endocr	300			
					Immuno	300			
					Neuro	300			
					Repro	300			
Templin et al. 1996b									
36	Rat (Fischer-344) 10–13 M	6 weeks 7 days/week 6 hours/day (WB)	0, 2, 10, 30, 90, 300	LE, CS, BW, OW, GN, HP	Bd wt	30	90	300	LOAEL: 19% decrease in body weight gain SLOAEL: 42% decrease in body weight gain

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Resp		2		Loss of olfactory glands and edema in the nasal lamina propria; atrophy of ethmoid turbinates
					Musc/skel	300			
					Hepatic	90	300		Hepatocellular vacuolation and proliferation, cell necrosis
					Renal	10	30		Renal cell proliferation; vacuolation in the proximal convoluted tubule
Templin et al. 1996b									
37	Rat (Fischer-344) 14–15 M, 14–15 F	13 weeks 7 days/week 6 hours/day (WB)	0, 2, 10, 30, 90, 300	LE, CS, BW, OW, GN, HP	Bd wt	30	90	300	LOAEL: Decreased body weight gain in males (13%) and females (16%) SLOAEL: Decreased body weight gain in males (45%) and females (31%)
					Resp		2[c]		Loss of olfactory glands and edema in the nasal lamina propria; atrophy of ethmoid turbinates
					Cardio	300			
					Gastro	300			
					Musc/skel	300			
					Hepatic	30	90		Hepatocellular vacuolation and hepatocyte degeneration and/or necrosis
					Renal	10	30		Renal cell proliferation
					Dermal	300			
					Ocular	300			
					Endocr	300			
					Immuno	300			
					Neuro	300			
					Repro	300			

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Templin et al. 1996b									
38	Rat (Fischer-344) 13–15 M, 13–14 F	13 weeks 5 days/week 6 hours/day (WB)	0, 30, 90, 300	LE, CS, BW, GN, HP	Bd wt		30	300	LOAEL: Decreased body weight gain in males (18%) and females (12%) SLOAEL: Decreased body weight gain in males (48%) and females (20%)
					Resp		30		Loss of olfactory glands, edema, and cellular proliferation in the nasal lamina propria; atrophy of ethmoid turbinates
					Musc/skel	300			
					Hepatic	90	300		Hepatocellular vacuolation and proliferation; hepatocyte degeneration and cell necrosis
					Renal	30	90		Renal cell proliferation
Torkelson et al. 1976									
39	Rat (NS) 10–12 M, 10–12 F	6 months 5 days/week 7 hours/day (WB)	0, 25, 50, 85	LE, BW, HE, BC, UR, GN, OW, HP	Bd wt	25 M 85 F	50 M		14% decrease in body weight
					Hemato	85			
					Hepatic	25 F	25 M 50 F		Lobular degeneration, focal necrosis
					Renal		25		Increased relative kidney weight in both sexes; cloudy swelling of the renal tubular epithelium in males
					Immuno	85			
					Repro	85 M			

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Torkelson et al. 1976									
40	Rat (NS) 10–12 M	6 months 5 days/week 1, 2, or 4 hours/day (WB)	0, 25	LE, BW, HE, BC, UR, GN, OW, HP	Bd wt	25			
					Hemato	25			
					Hepatic	25			
					Renal	25			
					Immuno	25			
					Repro	25			
Kasai et al. 2002									
41	Mouse (Crj:BDF1) 10 M, 10 F	13 weeks 5 days/week 6 hours/day (WB)	0, 12, 25, 50, 100, 200	LE, CS, BW, BC, UR, OW, GN, HP	Death			12 M	20% mortality
					Bd wt	200			
					Resp		12		Thickening of nasal bones in both sexes; eosinophilic changes in olfactory and respiratory epithelia of females
					Hepatic	50 F 100 M	100 F 200 M	200 F	LOAEL: Hepatocellular swelling in males; hepatic cell atypia in females SLOAEL: Liver necrosis; increased absolute and relative liver weights; increased serum AST and ALT
					Renal	100 F	12 M 200 F	25 M	LOAEL: Necrosis and cytoplasmic basophilia in the proximal tubules and proteinuria in males; increased absolute and relative kidney weights in females SLOAEL: Severe proximal tubular necrosis and degeneration
Larson et al. 1996									
42	Mouse (B6C3F1) 5–8 M, 10–13 F	3 weeks 7 days/week 6 hours/day (WB)	0, 0.3, 2, 10, 30, 88	CS, BW, GN, OW, HP	Bd wt	88			
					Resp	88			
					Cardio	88			
					Gastro	88			

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Musc/skel	88			
					Hepatic	10	30		Hepatocyte vacuolation and swelling in both sexes, hepatocellular proliferation in females
					Renal	10 M	30 M		Enlarged nuclei and renal cell proliferation in the proximal convoluted tubules
						88 F			
					Ocular	88			
					Endocr	88			
					Immuno	88			
					Neuro	88			
					Repro	88			
Larson et al. 1996									
43	Mouse (B6C3F1) 10–13 F	6 weeks 7 days/week 6 hours/day (WB)	0, 0.3, 2, 10, 30, 88	CS, BW, GN, OW, HP	Bd wt	88			
					Resp	88			
					Musc/skel	88			
					Hepatic	10	30		Mild degenerative changes, hepatocellular proliferation
					Renal	88			
Larson et al. 1996									
44	Mouse (B6C3F1) 12–15 M, 14–15 F	13 weeks 7 days/week 6 hours/day (WB)	0, 0.3, 2, 10, 30, 88	CS, BW, OW, GN, HP	Bd wt	88			
					Resp	88			
					Cardio	88			
					Gastro	88			
					Musc/skel	88			
					Hepatic	10	30		Centrilobular hepatocyte swelling and vacuolation

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Renal	10 M	30 M		Focal regeneration, enlarged nuclei, and renal cell proliferation in the proximal convoluted tubules
						88F			
					Ocular	88			
					Endocr	88			
					Immuno	88			
					Neuro	88			
					Repro	88			
Larson et al. 1996									
45	Mouse (B6C3F1) 8–15 M, 8–15 F	13 weeks 5 days/week 6 hours/day (WB)	0, 10, 88	CS, BW, GN, HP	Bd wt	88			
					Resp	88			
					Musc/skel	88			
					Hepatic	10	88		Mild hepatocyte vacuolation; hepatocellular proliferation
					Renal		10 M		Renal cell proliferation in the proximal convoluted tubules
						88 F			
Templin et al. 1998									
46	Mouse (BDF1) 5 M, 5 F	3 weeks 5 days/week 6 hours/day (WB)	M: 0, 1, 5 F: 0, 5, 30, 90	LE, CS, BW, OW, HP	Bd wt	5 M 90 F			
					Hepatic	5 M	90 F		Increased relative liver weight; hepatocellular proliferation
						30 F			
					Renal	5 M 90 F			

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Templin et al. 1998									
47	Mouse (BDF1) 5 M	7 weeks 5 days/week 6 hours/day (WB)	0, 1, 5, 17, 26	LE, CS, BW, OW, HP	Bd wt	26			
					Hepatic	5	17		Increased relative liver weight, centrilobular swelling
					Renal	5	17		Cellular proliferation and regenerative lesions in proximal convoluted tubule
Templin et al. 1998									
48	Mouse (BDF1) 8 M, 8 F	13 weeks 5 days/week 6 hours/day (WB)	M: 0, 1, 5, 23, 55 F: 0, 5, 30, 90	LE, CS, BW, OW, HP	Bd wt	1 M 90 F	5 M	23 M	LOAEL: 17% decrease in percent body weight gain SLOAEL: 23% decrease in percent body weight gain
					Hepatic	5	23 M 30 F		Centrilobular swelling
					Renal	5 M	23 M		Cellular proliferation and regenerative lesions in proximal convoluted tubule
						90 F			
CHRONIC EXPOSURE									
Li et al. 1993									
49	Human 9–26 M, 14–35 F	1–15 years (occupational)	0, 2.76, 6.04	CS, BC, OF, NX	Hepatic	6.04			
					Renal	6.04			
					Neuro		2.76		Impaired hand-eye coordination in pursuit aiming task
Nagano et al. 2006									
50	Rat (Fischer-344) 50 M	104 weeks 5 days/week 6 hours/day (WB)	0, 25, 50, 100	LE, CS, BW, BC, UR, GN, HP	Bd wt	100			
					Renal	25	50		Cytoplasmic basophilia, tubular lumen dilation, and nuclear enlargement in the proximal tubule; glycosuria

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Yamamoto et al. 2002									
51	Rat (Fischer-344) 50 M, 50 F	104 weeks 5 days/week 6 hours/day (WB)	0, 10.1, 30.0, 90.1	LE, CS, BW, FI, HE, BC, UR, OW, GN, HP	Bd wt	30.0	90.1		Unspecified suppression of body weight gain
					Resp		10.1		Atrophy and respiratory metaplasia of the olfactory epithelium; thickening of nasal bones
					Cardio	90.1			
					Gastro	90.1			
					Hemato	90.1			
					Musc/skel	90.1			
					Hepatic	30.0	90.1		Decreased serum total cholesterol, triglycerides, and phospholipids in males; decreased serum triglycerides and vacuolated cell foci in females
					Renal	10.1	30.0		Nuclear enlargement of the proximal tubules and dilation of the tubular lumen; glycosuria
					Dermal	90.1			
					Ocular	90.1			
					Endocr	90.1			
					Immuno	90.1			
					Neuro	90.1			
					Repro	90.1			

Additional information obtained from unpublished study (MHLW 1994a, 1994b)

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Yamamoto et al. 2002									
52	Mouse (Crj:BDF1) 50 M; 50 F	104 weeks 5 days/week 6 hours/day (WB)	0, 5.0, 29.1, 85.8	LE, CS, BW, FI, HE, BC, UR, OW, GN, HP	Bd wt	29.1	85.8		Unspecified decrease in body weight
					Resp		5.0[d]		Atrophy and respiratory metaplasia of the olfactory epithelium in females; thickening of nasal bone in both sexes
					Cardio	85.8			
					Gastro	85.8			
					Hemato	85.8			
					Musc/skel	85.8			
					Hepatic	29.1	85.8		Fatty change in the liver
					Renal	5 M 29.1 F	29.1 M 85.8 F		Renal tubular lesions in males at ≥29.1 ppm; increased cytoplasmic basophilia in females at 85.8 ppm; increased BUN in both sexes at 85.8 ppm
					Dermal	85.8			
					Ocular	85.8			
					Endocr	85.8			
					Immuno	85.8			
					Neuro	85.8			

Table 2-1. Levels of Significant Exposure to Chloroform – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	85.8			
					Cancer			29.1 M	CEL: Renal adenoma or carcinoma (combined)
Additional information obtained from unpublished study (MHLW 1994a, 1994b)									

Studies selected for derivation of inhalation MRLs.

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an acute-duration inhalation MRL of 0.001 ppm. The NOAEL of 2 ppm was adjusted for continuous exposure and converted into a $NOAEL_{HEC}$ of 0.04 ppm and then divided by a total uncertainty factor of 30 (3 for extrapolation of animal to humans with dosimetric adjustment, 10 for human variability); see Appendix A for more detailed information regarding the MRL.
[c]Used to derive an intermediate-duration inhalation MRL of 0.0008 ppm. The LOAEL of 2 ppm was adjusted for continuous exposure and converted into a $LOAEL_{HEC}$ of 0.07 ppm and then divided by a total uncertainty factor of 90 (3 for use of a minimal LOAEL, 3 for extrapolation of animal to humans with dosimetric adjustment, 10 for human variability); see Appendix A for more detailed information regarding the MRL.
[d]Used to derive a chronic-duration inhalation MRL of 0.0004 ppm. The LOAEL of 5 ppm was adjusted for continuous exposure and converted into a $LOAEL_{HEC}$ of 0.11 ppm and then divided by a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation of animal to humans with dosimetric adjustment, 10 for human variability); see Appendix A for more detailed information regarding the MRL.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BALF = bronchioalveolar lavage fluid; BC = serum (blood) chemistry; Bd wt or BW = body weight; BI = biochemical changes; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; ET_{50} = median time to observed effect; F = female(s); FI = food intake; Gastro = gastrointestinal; GD = gestational day; GN = gross necropsy; HE = hematology; HEC = human equivalent concentration; Hemato = hematological; HP = histopathology; Immuno = immunological; LC_{50} = median lethal concentration; LE = lethality; LOAEL = lowest-observed-adverse-effect level; LT_{50} = median lethal time; 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; OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive function; SLOAEL = serious lowest-observed-adverse-effect level; UR = urinalysis; (WB) = whole-body; WI = water intake

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

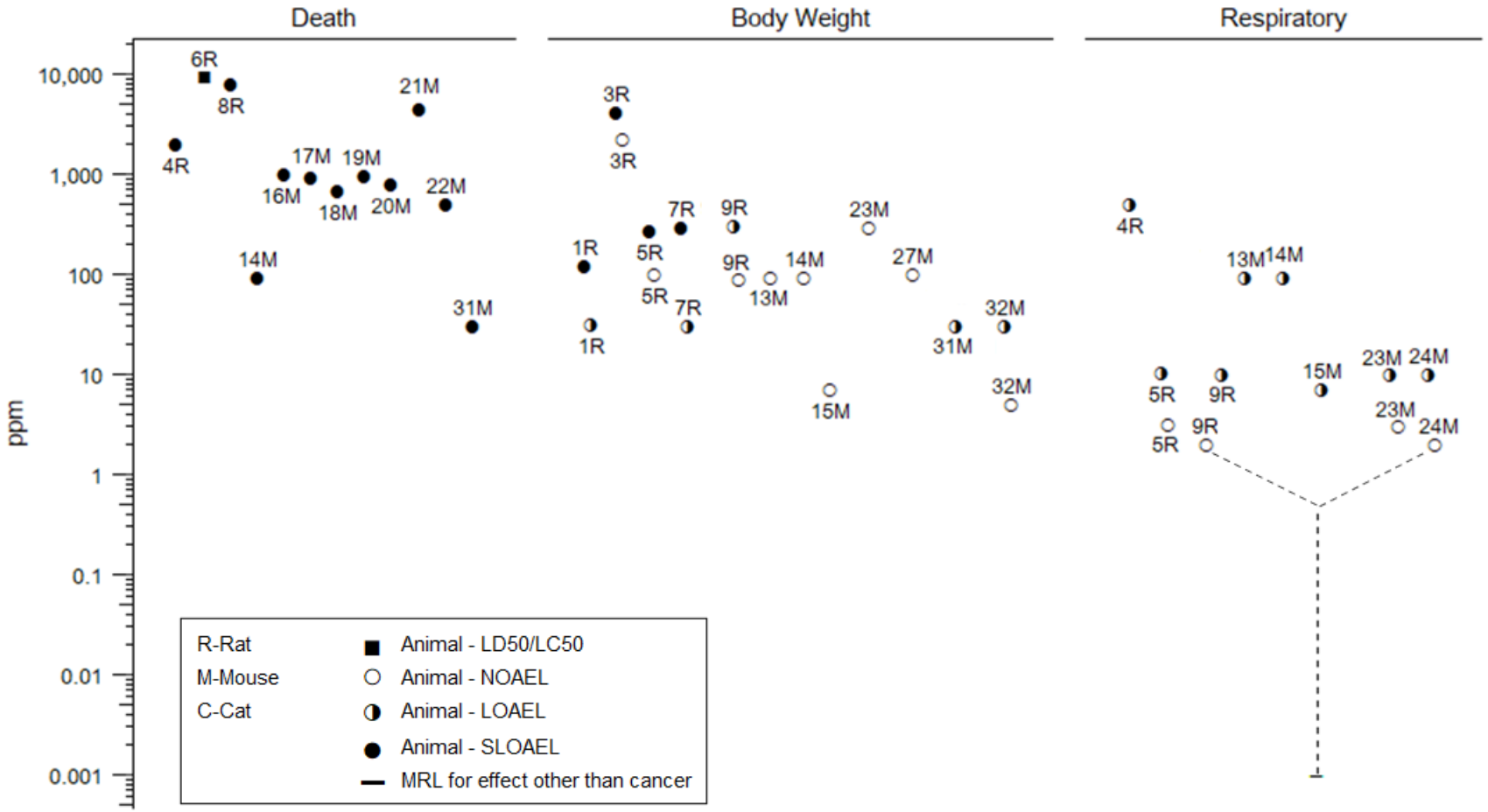

Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Acute (≤14 days)

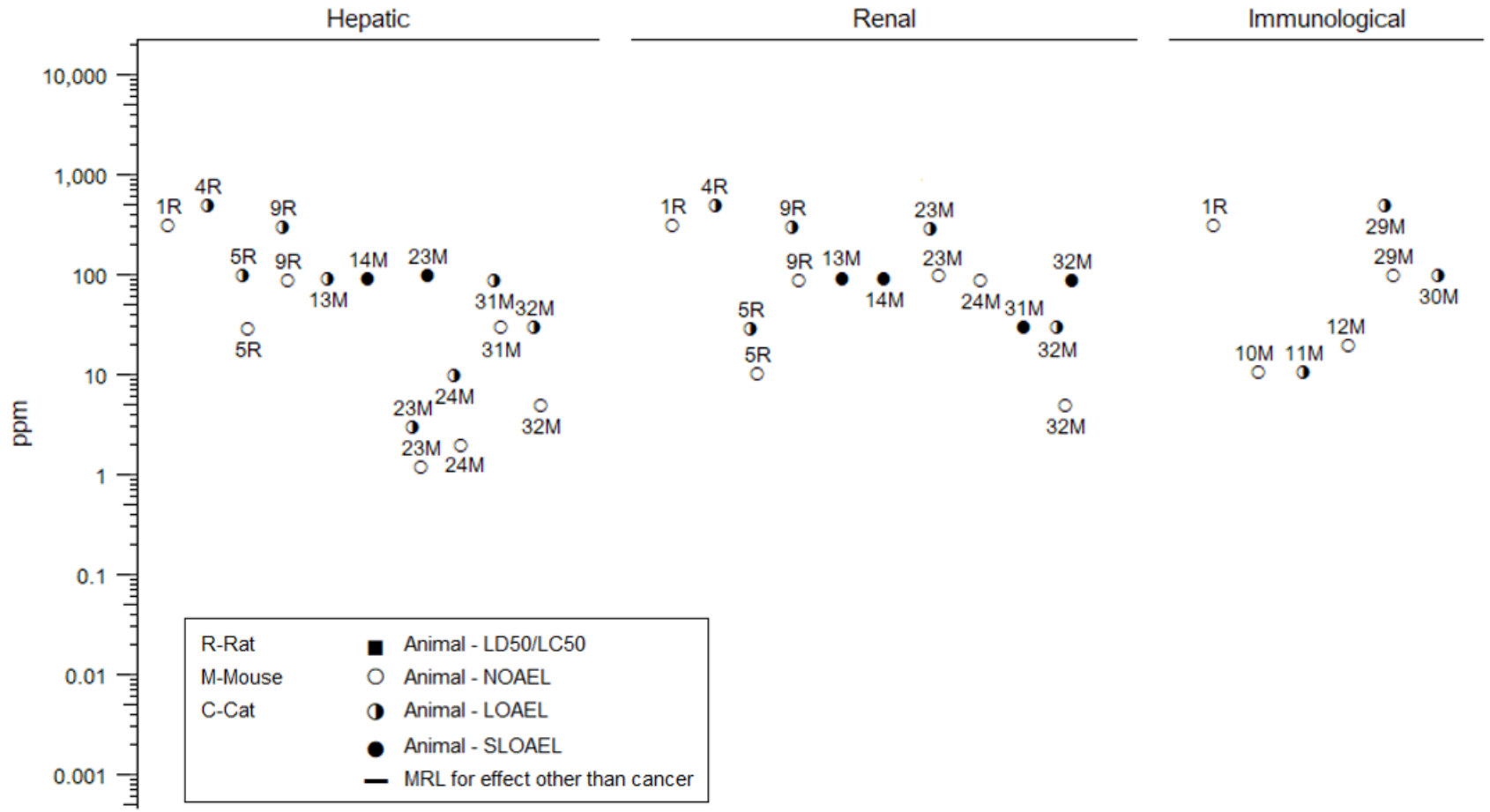

Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Acute (≤14 days)

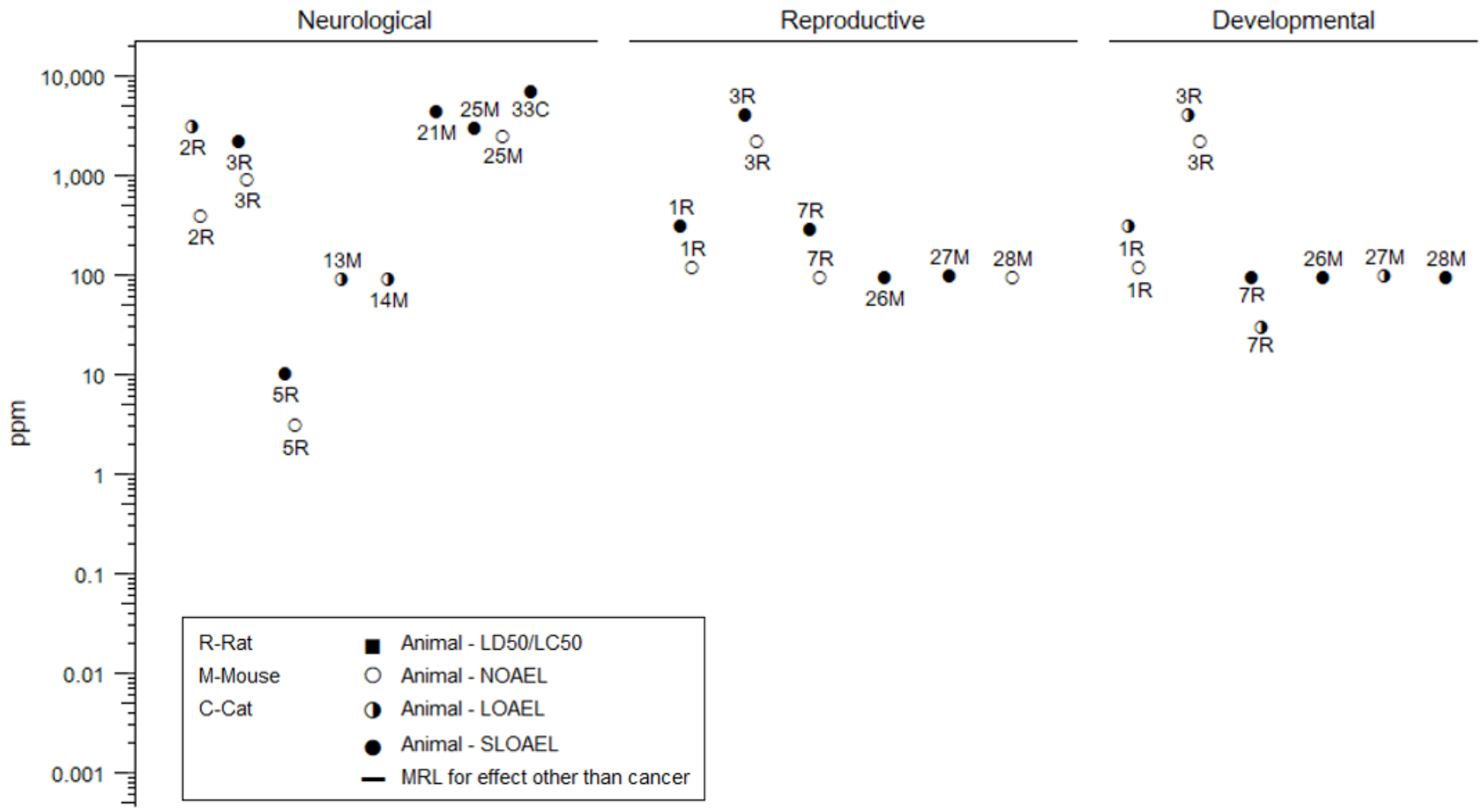

Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Intermediate (15–364 days)

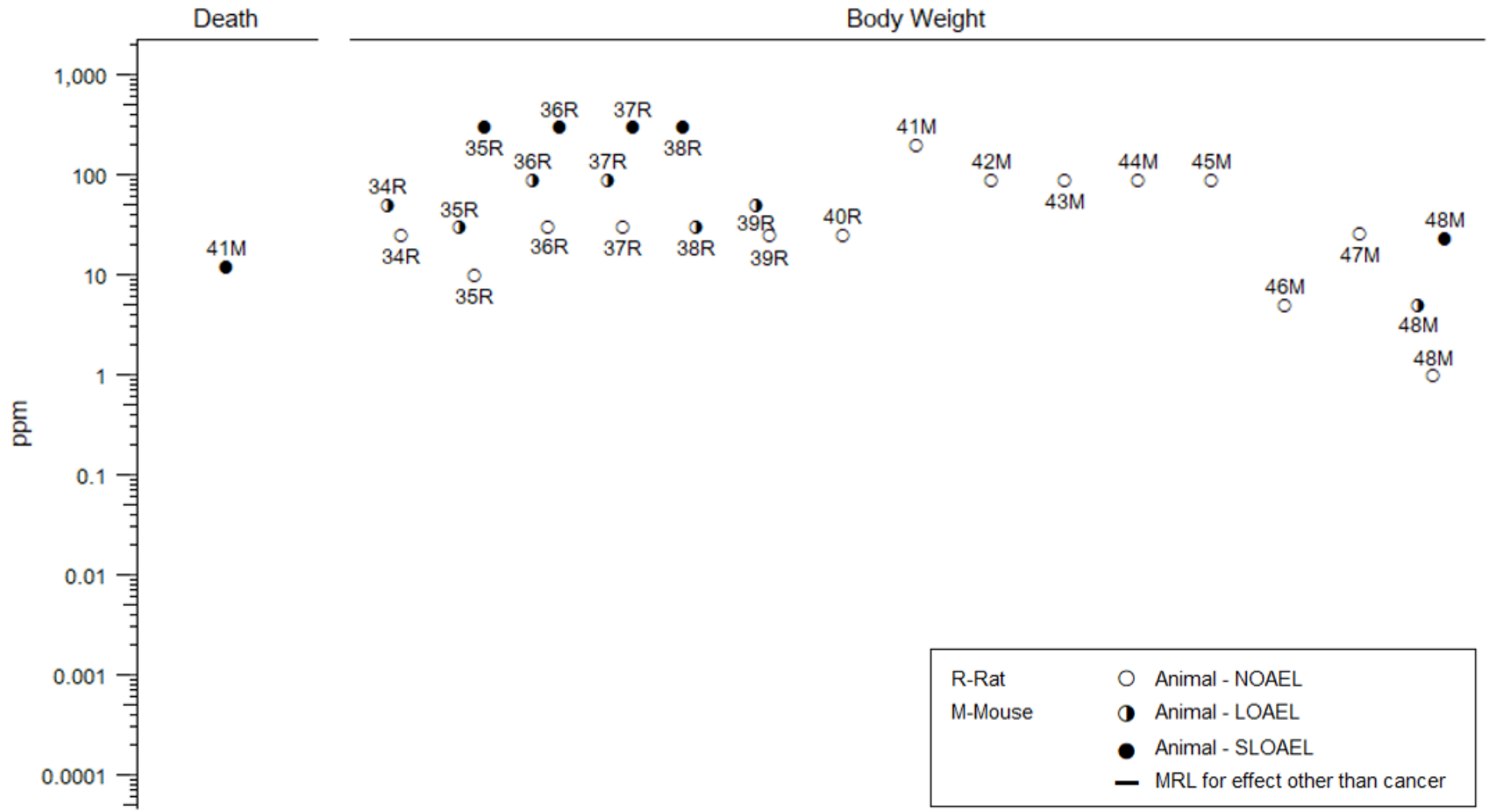

Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Intermediate (15–364 days)

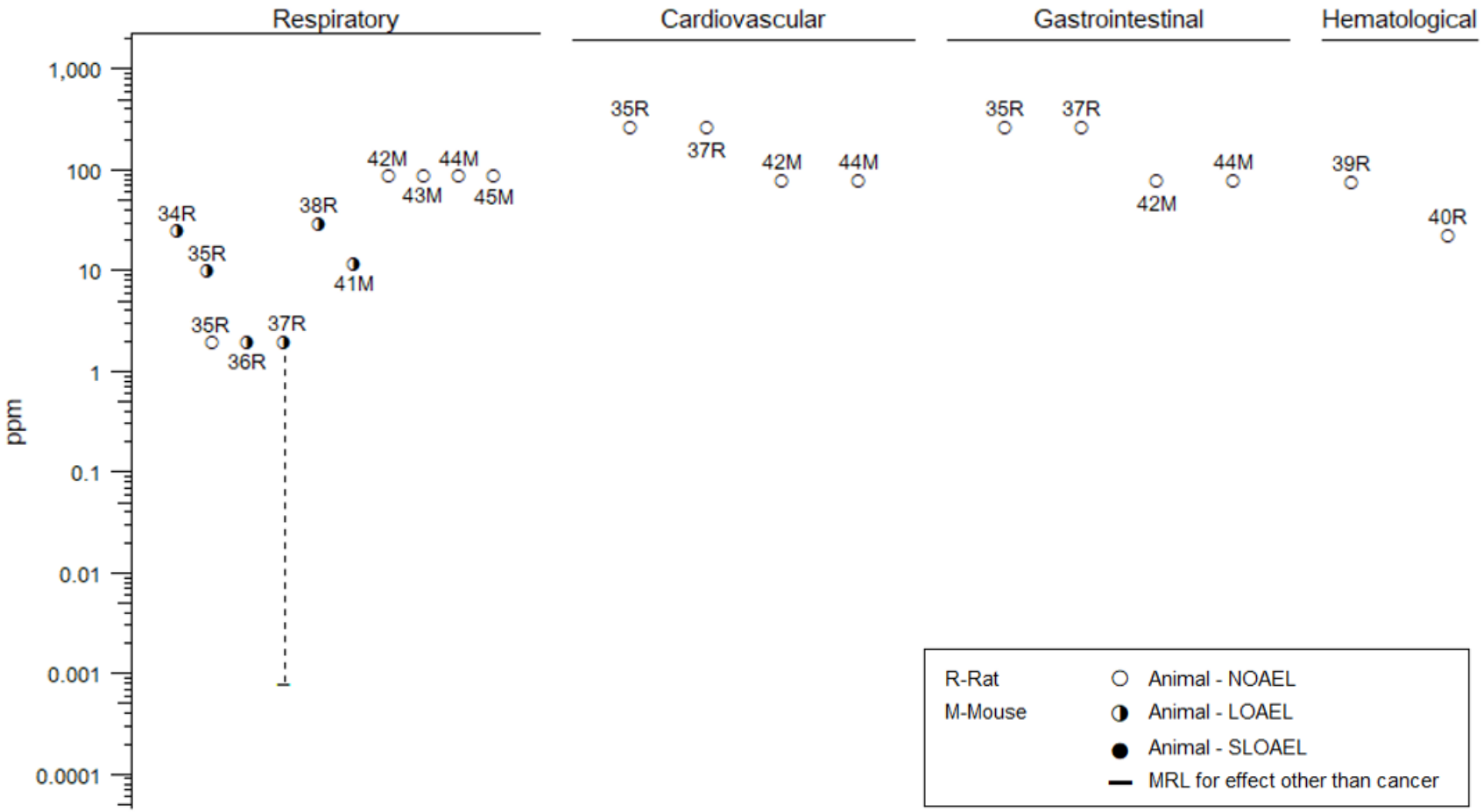

Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Intermediate (15–364 days)

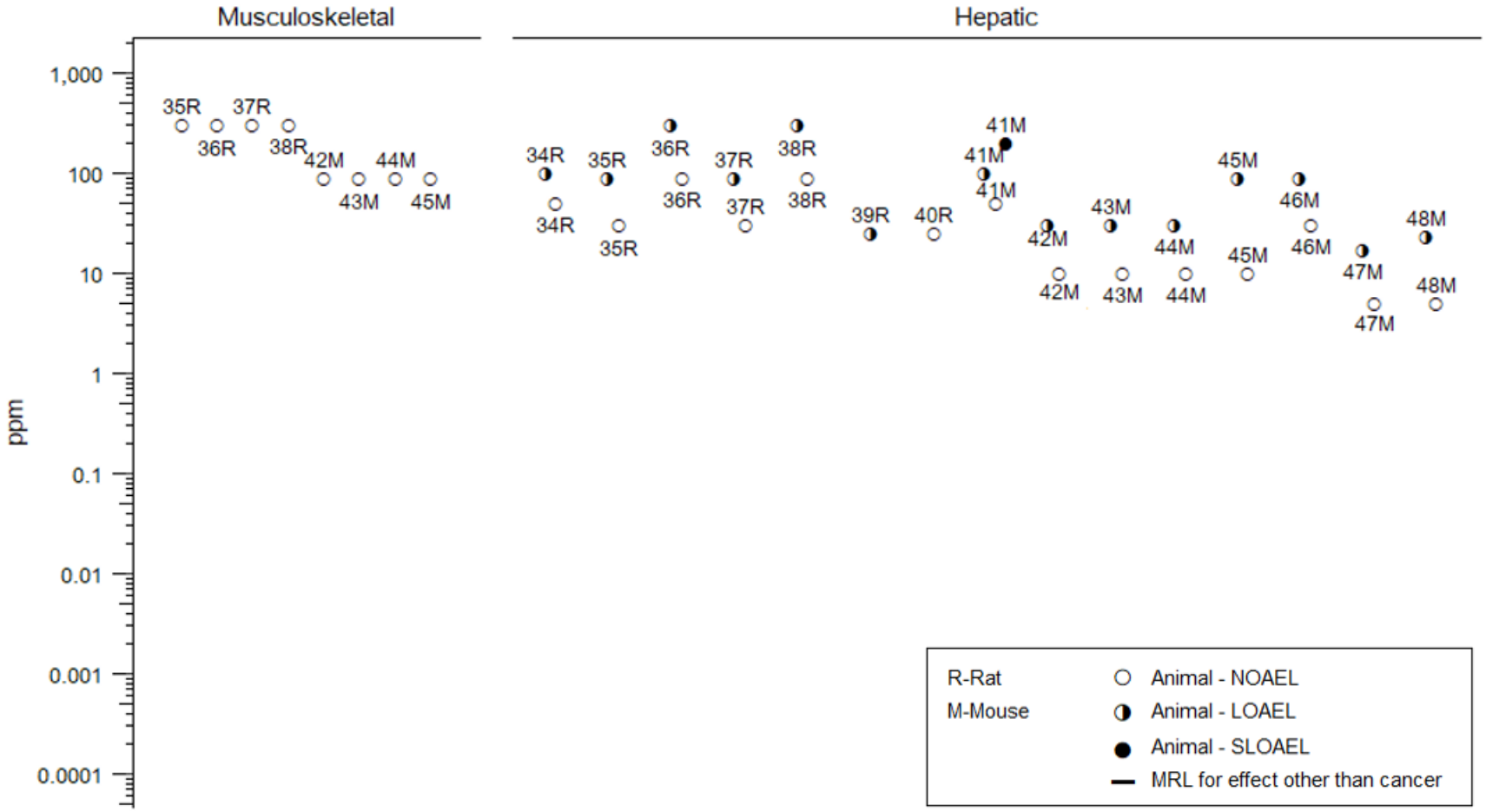

Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Intermediate (15–364 days)

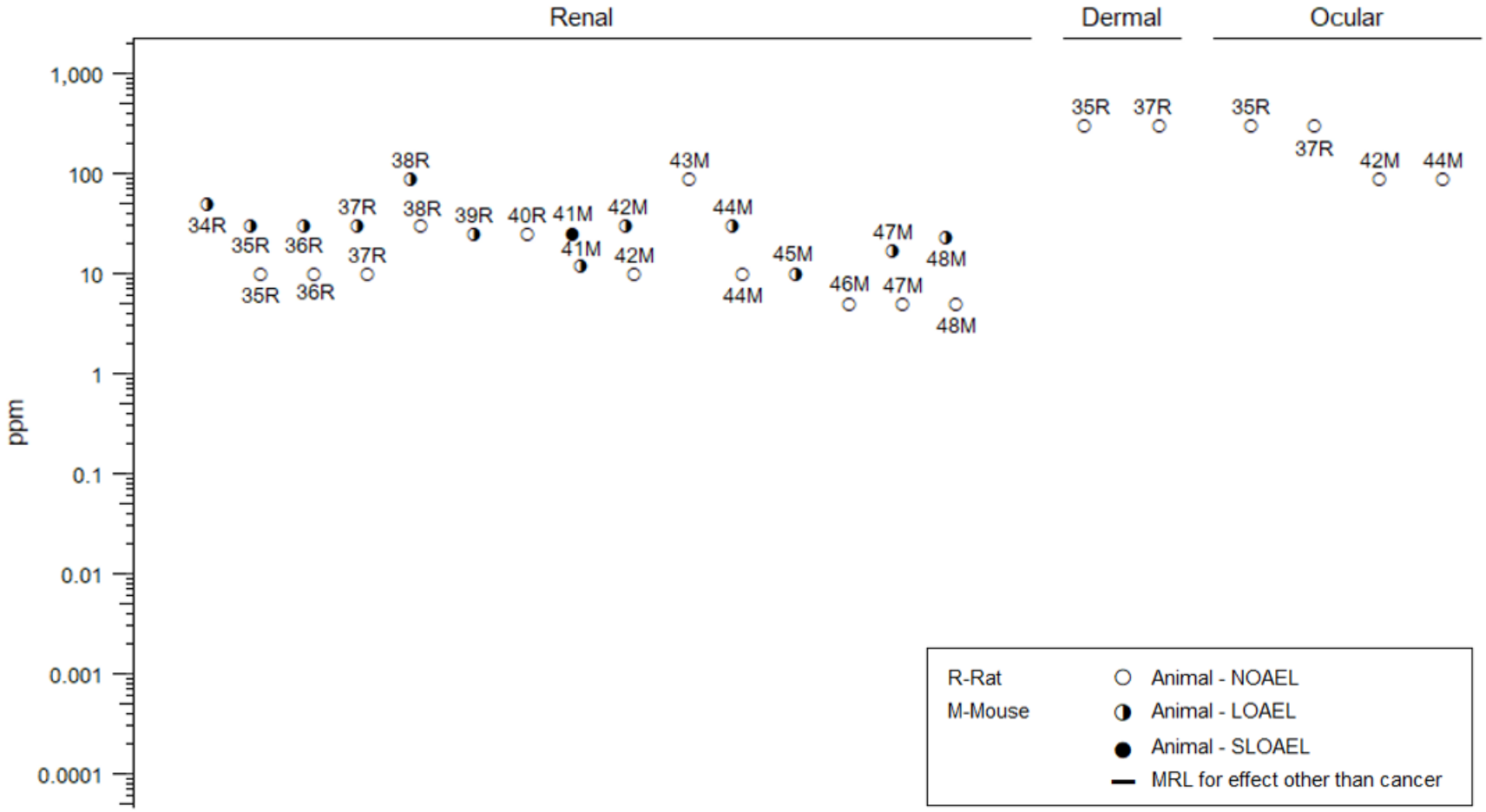

Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Intermediate (15–364 days)

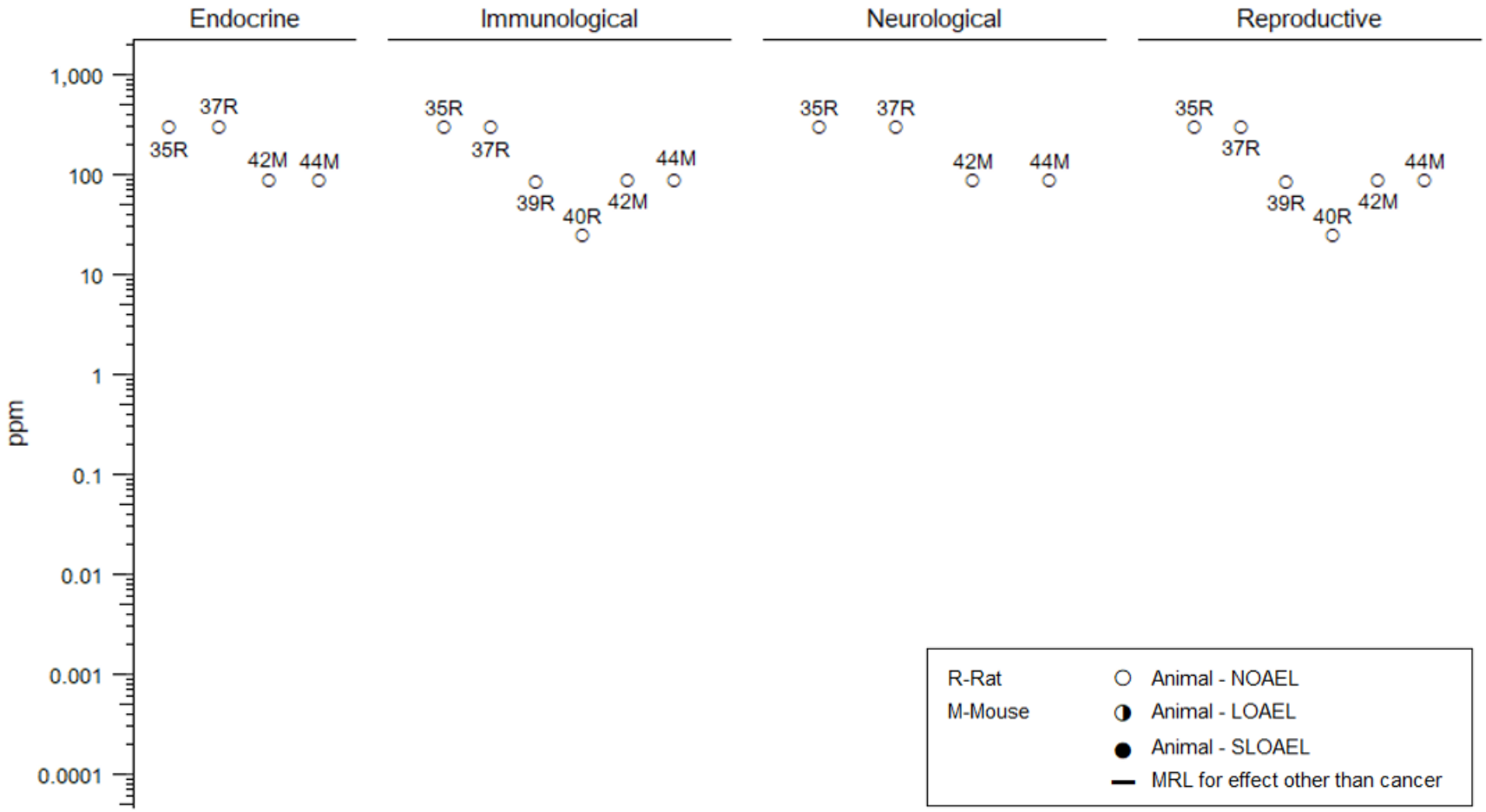

Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Chronic (≥365 days)

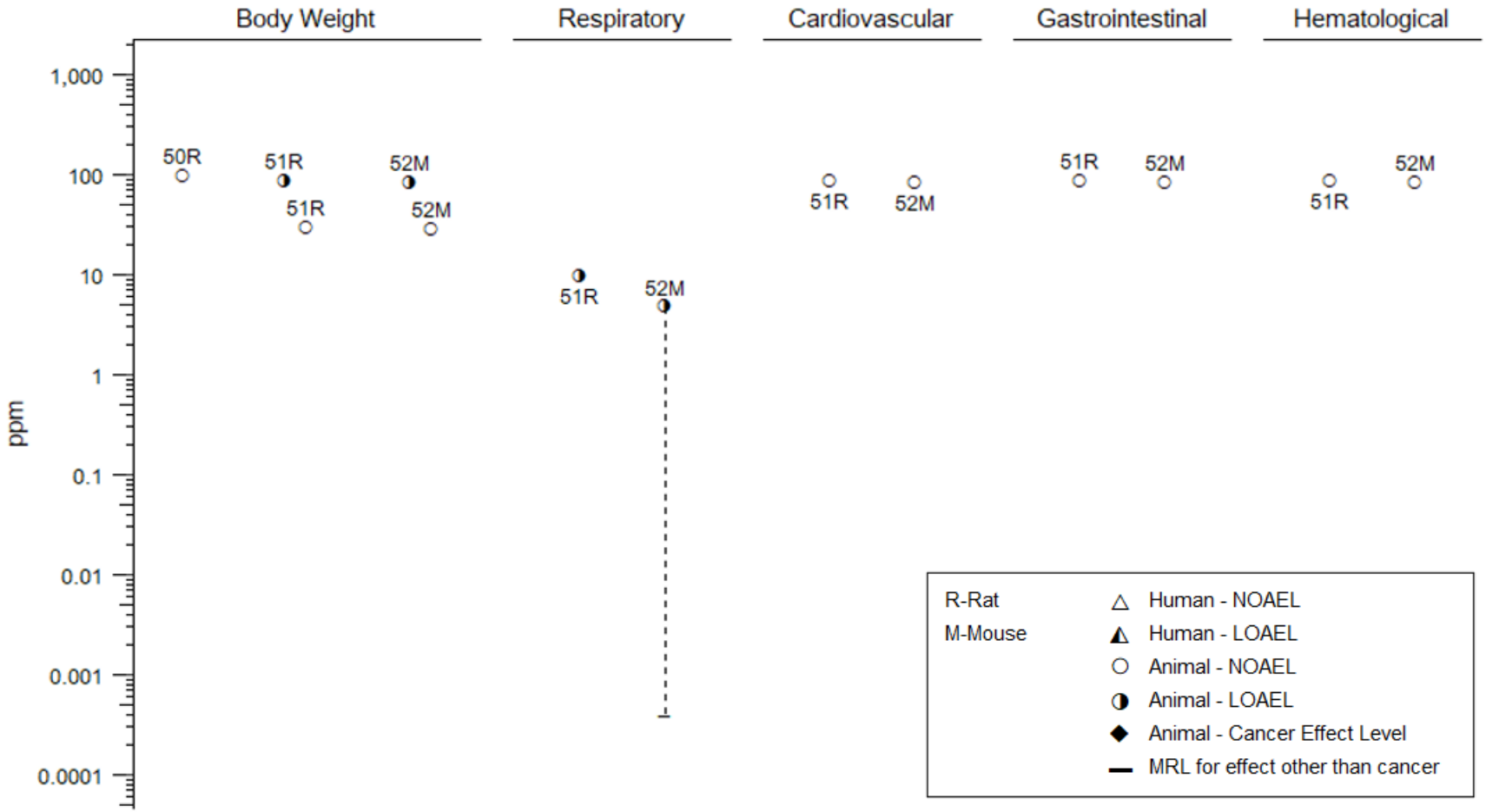

Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Chronic (≥365 days)

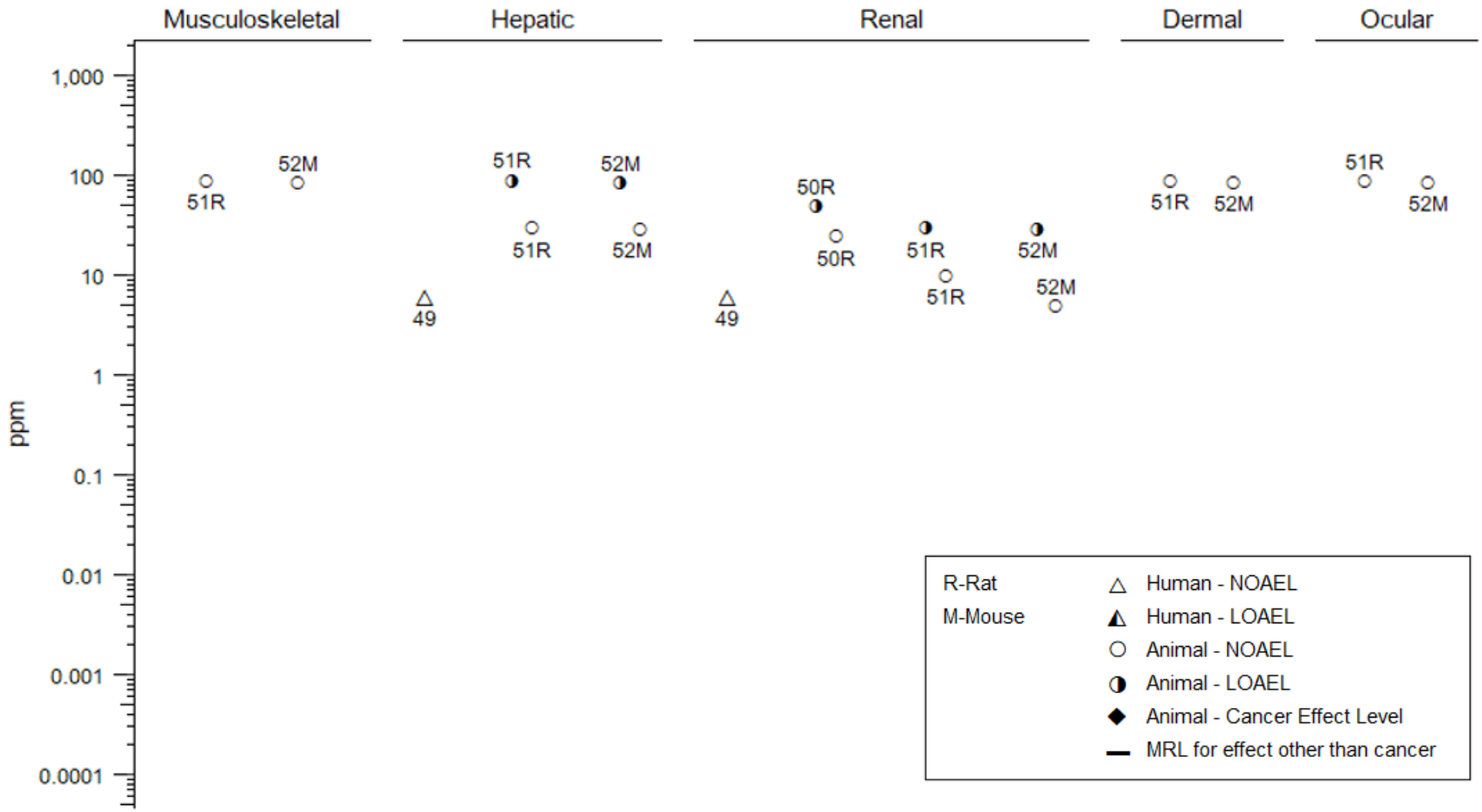

Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Chronic (≥365 days)

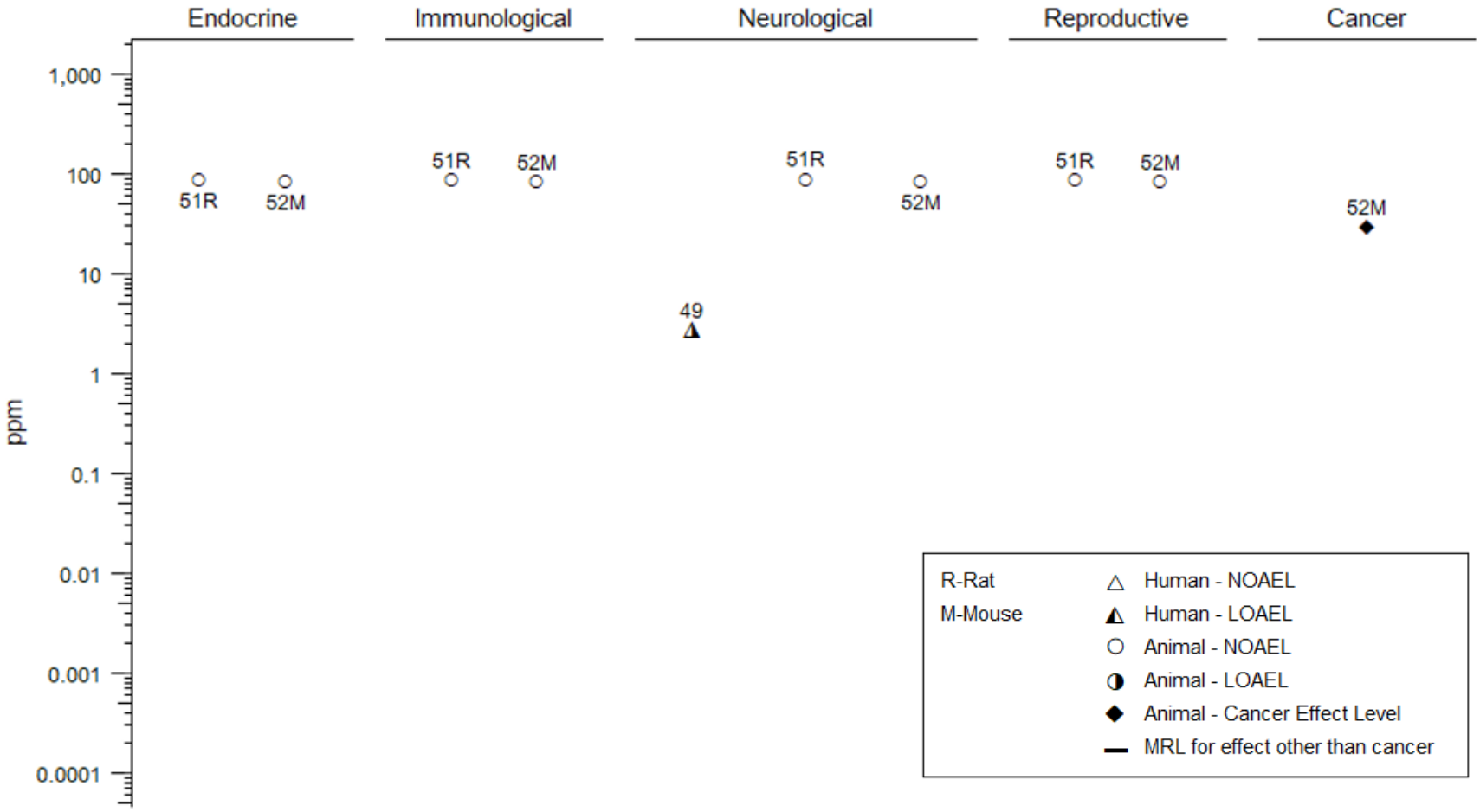

Table 2-2. Levels of Significant Exposure to Chloroform – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Chu et al. 1982b									
1	Rat (Sprague-Dawley) 10 M, 10 F	Once (GO)	0, 546, 765, 1,071, 1,500, 2,100	LE, CS, BW, FI, WI, HE, BC, BI, GN, OW, HP	Death			1,117 F 908 M	LD_{50}
					Bd wt	1,071 M 1,500 F			
					Hemato		546		Mild reduction in hematocrit and RBC count in males and hemoglobin in both sexes
					Hepatic	765 M 1,071 F	1,071 M 1,500 F		Increased serum cholesterol
					Renal	1,071 M			
							546 F		Increased relative kidney weight
Ito et al. 2000									
2	Rat (Sprague-Dawley) 12–18 M	Once (GO)	0, 220	HP	Hepatic		220		Increased leukocyte adherence to sinusoidal wall, hepatocyte swelling, reduced perfusion of sinusoids and increased phagocytosis activity of Kupffer cells
Keegan et al. 1998									
3	Rat (Fischer-344) 6–18 M	Once (GW)	0, 15, 22, 30, 60, 90, 119, 179	BW, BC, OW	Bd wt	179			
					Hepatic	30	60		Increased serum ALT and SDH
					Renal	179			
Kimura et al. 1971									
4	Rat (Sprague-Dawley) 6–12 M	Once (G)	Not reported	LE, CS	Death			445	LD_{50} in 14-day-old rats (5–8 g)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kimura et al. 1971									
5	Rat (Sprague-Dawley) 6 M	Once (G)	Not reported	LE, CS	Death			1,336	LD_{50} in young adult rats (80–160 g)
Kimura et al. 1971									
6	Rat (Sprague-Dawley) 6 M	Once (G)	Not reported	LE, CS	Death			1,187	LD_{50} in adult rats (300–470 g)
Larson et al. 1993									
7	Rat (Fischer-344) 2–5 M	Once (GO)	0, 34, 180, 477	CS, BW, BC, UR, GN, OW, HP	Bd wt	477			
					Hepatic	180	477		Mild hepatocyte necrosis, hepatocellular proliferation, elevated serum ALT, AST, and SDH
					Renal		34	180	LOAEL: Scattered necrosis of the renal proximal tubule SLOAEL: Severe renal proximal tubule necrosis; renal cell proliferation
Larson et al. 1995a									
8	Rat (Fischer-344) 12 M	4 days (GO)	0, 3, 10, 34, 90, 180	BW, WI, HE, BC, GN, OW, HP	Bd wt	180			
					Hepatic	10	34		Increased relative liver weight
					Renal	10	34	180	LOAEL: Mild-to-moderate degeneration of renal proximal tubules and tubule epithelial cell proliferation SLOAEL: Progressive degeneration of renal proximal tubules

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Larson et al. 1995a									
9	Rat (Fischer-344) 12 M	4 days (W)	0, 6.6, 19.3, 33.2, 57.5, 68.1	BW, WI, HE, BC, GN, OW, HP	Bd wt	33.2	57.5		17% decrease in body weight gain
					Hepatic	68.1			
					Renal	68.1			
Larson et al. 1995b									
10	Rat (Fischer-344) 5 F	4 days (GO)	0, 34, 100, 200, 400	CS, BW, OW, HP	Bd wt	200	400		18% decrease in body weight gain
					Resp		34		Degeneration of the olfactory epithelium and olfactory glands of lamina propria; periosteal hypercellularity; new nasal bone formation
					Hepatic	34	100		Slight hepatocyte vacuolation and hepatocellular proliferation
					Renal	100		200	Necrosis, degeneration, and regeneration of proximal tubule epithelium; proliferation of proximal tubule epithelial cells in renal cortex
Lilly et al. 1997									
11	Rat (Fischer-344) 10 M	Once (G)	0, 89.5, 119.4, 179.1, 238.8, 358.2	BW, BC, UR, OW	Bd wt	238.8	358.2		11% decrease in terminal body weight
					Hepatic		89.5		Increased serum SDH
					Renal	119.4	179.1		Increased urinary LDH and AST
Miyagawa et al. 1998									
12	Rat (Fischer-344) 9 M	Once (GO)	0, 50, 150, 500	BC, UR, HP	Hepatic	150	500		Centrilobular vacuolation, hepatocellular hypertrophy and proliferation; increased plasma AST

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Renal		50	500	LOAEL: Increased urinary NAG and LDH SLOAEL: Vacuolation and necrosis of tubular epithelial cells; cell proliferation in inner renal cortex; increased BUN
Müller et al. 1997									
13	Rat (Wistar) 16 M	Once (GO)	0, 149	CS, OF	Cardio		149		Decreased heart rate, increased blood pressure, and altered cardiac functional parameters
Potter et al. 1996									
14	Rat (Fischer-344) 12 M	7 days (G)	0, 90, 179	BW, BC, OW, GN, HP	Bd wt	179			
					Renal	179			
					Repro	90	179		Decreased serum testosterone
Ruddick et al. 1983									
15	Rat (Sprague-Dawley) 15 F	10 days GD 6–15 (GO)	0, 100, 200, 400	LE, BW, HE, BC, BI, GN, HP, DX	Bd wt			100	30% decrease in maternal body weight gain
					Hemato		100		Decreased hemoglobin and hematocrit
					Develop	200		400	19% decrease in fetal body weight; delayed ossification
Smyth et al. 1962									
16	Rat (Wistar) 5 F	Once (G)	Not reported	LE	Death			2,180	LD_{50}
Templin et al. 1996a									
17	Rat (Fischer-344) 5 M	Once (GO)	0, 10, 34, 90, 180, 477	CS, BW, OW, GN, HP	Bd wt	477			
					Resp	34	90		Vacuolation, edema, and loss of olfactory glands in the lamina propria; periosteal cell proliferation of nasal bones
					Hepatic	180	477		Mild hepatocellular vacuolation, hepatocellular proliferation

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Renal	34	90		Regenerative cell proliferation in the epithelial cells of the proximal tubules of the renal cortex
Templin et al. 1996a									
18	Rat (Osborne-Mendel) 6 M	Once (GO)	0, 10, 34, 90, 180, 477	CS, BW, OW, GN, HP	Bd wt	477			
					Resp	34	90		Vacuolation, edema, and loss of olfactory glands in the lamina propria; periosteal cell proliferation of nasal bones
					Hepatic	477			
					Renal		10		Regenerative cell proliferation in the epithelial cells of the proximal tubules of the renal cortex
Thompson et al. 1974									
19	Rat (Sprague-Dawley) 25 F	10 days GDs 6–15 2 divided doses/day (GO)	0, 20, 50, 126	LE, CS, BW, GN, HP, DX	Bd wt	20	50		Unspecified decrease in maternal body weight gain
					Dermal	50	126		Maternal alopecia
					Develop	50	126		8% decrease in fetal body weight
Thompson et al. 1974									
20	Rat (Sprague-Dawley) 6 F	10 days GDs 6–15 1 time or two divided doses/day (GO)	0, 79, 126, 300, 316, 516	LE, BW, GN, HP, RX, DX	Death			516	67% mortality
					Bd wt	79	126		Unspecified decrease in maternal body weight gain
					Gastro		516		Gastric erosions
					Hepatic			516	Acute toxic hepatitis
					Renal			516	Acute toxic nephrosis
					Repro	300		316	Increased resorptions
					Develop	300	316		Unspecified decrease in fetal weight

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Torkelson et al. 1976									
21	Rat (NS) 4 M	Once (G)	Not reported	LE	Death			2,000	LD_{50}
Wada et al. 2015									
22	Rat (Sprague-Dawley) 3 M	3 days (GO)	0, 125, 250, 500, 1,000, 2,000	LE, BW	Death			2,000	67% mortality
					Bd wt			1,000	Severe emaciation
Wada et al. 2015									
23	Rat (Sprague-Dawley) 5 M	3 days (GO)	0, 125, 250, 500	CS, BW, HP	Bd wt	250		500	10% body weight loss, compared to 3% gain in control
					Gastro	500			
					Hepatic		250		Hepatocellular enlargement and necrosis; centrilobular inflammatory cell infiltration and vacuolation (histology not assessed at 125 mg/kg/day)
					Neuro	250	500		Decreased spontaneous motor activity
Wang et al. 1997									
24	Rat (Wistar) 5 M	Once (GO)	0, 12.5, 200	BC, BI	Hepatic	12.5		200	Substantial increase in plasma AST and ALT
Balster and Borzelleca 1982									
25	Mouse (ICR) 6 M	Once (GW)	Not reported	CS, NX	Neuro		484		ED_{50} for impaired motor performance
Balster and Borzelleca 1982									
26	Mouse (ICR) 8 M	14 days (GW)	0, 3.1, 31.1	NX	Neuro	31.1			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Bowman et al. 1978									
27	Mouse (ICR Swiss) 10 M, 10 F	Once (GW)	500–4,000 (≥7 doses)	LE, CS, GN, HP	Death			1,120 M 1,400 F	LD_{50}
					Neuro			500	Ataxia, incoordination, and anesthesia; brain hemorrhage
Ewaid et al. 2020									
28	Mouse (BALB/c) 8 M	Once (G)	0, 50, 300, 700, 1,000, 1,500	LE, CS, BW, OW, HP	Death			550	LD_{50}
					Bd wt	300		700	20% decrease in body weight
					Hepatic	300	700		Elevated liver weight, centrilobular necrosis
					Renal	300	700		Hydropic degeneration
Jones et al. 1958									
29	Mouse (Swiss-Webster) 350 B	Once (GO)	7–1,100	LE, CS, HP	Death			1,100	Minimum lethal dose
					Hepatic		35	350	LOAEL: Minimal hepatotoxic dose (midzonal fatty changes) SLOAEL: Severe centrilobular necrosis
					Neuro			350	Minimal narcotic dose
Landauer et al. 1982									
30	Mouse (CD-1) 10 M	10 days (GW)	0, 3, 10, 30	CS, WI	Neuro	10	30		Conditioned taste aversion
Larson et al. 1993									
31	Mouse (B6C3F1) 9 F	Once (GO)	0, 34, 238, 350, 477	CS, BW, BC, GN, OW, HP, OF	Hepatic	34	238	350	LOAEL: Small, randomly scattered foci of hepatocyte necrosis; increased serum ALT and SDH SLOAEL: Marked hepatocellular swelling, vacuolation, degeneration and necrosis, hepatocellular proliferation
					Renal	477			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Larson et al. 1994b									
32	Mouse (B6C3F1) 14 F	4 days (GO)	0, 3, 10, 34, 90, 238, 477	BW, WI, BC, BI, GN, OW, HP	Bd wt	477			
					Hepatic	34	90	477	LOAEL: Mild vacuolation of hepatocytes, increased serum ALT SLOAEL: Severe coagulative necrosis and vacuolar degeneration
					Renal	238	477		Renal regenerative cell proliferation
Larson et al. 1994b									
33	Mouse (B6C3F1) 14 F	4 days (W)	0, 16, 26, 53, 81, 105	BW, WI, BC, BI, GN, OW, HP	Bd wt	53		81	23% decrease in body weight gain
					Hepatic	26[b]	53		Centrilobular hepatocyte eosinophilic cytoplasm
					Renal	105			
Larson et al. 1994d									
34	Mouse (B6C3F1) 5–12 M	4 days (GO)	0, 34, 90, 138, 277	LE, BW, WI, GN, OW, HP	Death			138	10% mortality
					Bd wt	277			
					Hepatic		34		Hepatocellular proliferation and mild hepatocellular swelling and vacuolation
					Renal			34	Extensive acute necrosis of the proximal convoluted tubule, regenerative cell proliferation in the renal cortex and medulla
Moore et al. 1982									
35	Mouse (CFLP Swiss) 3–5 M	Once (GO)	0, 17.3, 65.6, 273	BC, OW, HP	Hepatic	65.6	273		Hepatocellular proliferation, increased ALT
					Renal	17.3	65.6	273	LOAEL: Occasional tubular necrosis and renal regenerative cell proliferation SLOAEL: Widespread tubular necrosis, increased plasma urea, increased absolute kidney weight

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Moore et al. 1982									
36	Mouse (CFLP-Swiss) 3–5 M	Once (G)	0, 18.2, 59.2, 199	BC, OW, HP	Hepatic	199			
					Renal	59.2		199	Widespread tubular necrosis, renal regenerative cell proliferation, and increased absolute kidney weight
Chloroform was administered in a toothpaste vehicle.									
Munson et al. 1982									
37	Mouse (CD-1) 7–12 M, 8–12 F	14 days (GW)	0, 50, 125, 250	LE, BW, HE, BC, BI, GN, OW, IX	Bd wt	125 M 250 F	250 M		16% decrease in terminal body weight
					Hemato	250			
					Hepatic	50 M	125 M 50 F		Males: Increased absolute and relative liver weights Females: Increased relative liver weights
					Immuno		50		Suppressed humoral immunity
					Other noncancer	20 F 250 M	125 F		Decreased serum glucose
NTP 1988a									
38	Mouse (CD-1) 8 M, 8 F	14 days (GO)	0, 25, 50, 100, 250, 500	LE, CS, BW, GN, HP	Death			250 M	63% mortality
					Bd wt	100 M 500 F	250 M	500 M	LOAEL: >10% decrease in terminal body weight SLOAEL: >30% decrease in terminal body weight
					Dermal	50	100		Rough hair coat
					Ocular	100 M 500 F	250 M		Excessive tearing
					Neuro	100 M 500 F	250 M		Hunched posture, inactivity

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Philip et al. 2006									
39	Mouse (Swiss-Webster) 9–48 M	Once (G)	0, 750	LE	Death			750	90% mortality
Thompson et al. 1974									
40	Rabbit (Dutch Belted) 15 F	13 days GDs 6–18 (GO)	0, 20, 35, 50	LE, CS, BW, GN, HP, DX	Bd wt	35	50		Unspecified decrease in maternal body weight gain
					Develop		20		8% decrease in fetal body weight, delayed ossification
Thompson et al. 1974									
41	Rabbit (Dutch Belted) 5 F	13 days GDs 6–18 2 divided doses/day (GO)	0, 25, 63, 100, 159, 251, 398	LE, CS, BW, GN, HP, RX, DX	Death			63	20% mortality
					Bd wt	25	63		Unspecified maternal weight loss
					Gastro	25	63		Diarrhea
					Hepatic	63		100	Acute toxic hepatitis in does that died; mild fatty changes in 1/2 survivors
					Renal	63		100	Acute toxic nephrosis in does that died; mild fatty changes in 1/2 survivors
					Repro	25		63	2/4 surviving does aborted
					Develop	25			
INTERMEDIATE EXPOSURE									
Chu et al. 1982a									
42	Rat (Sprague-Dawley) 20 M, 20 F	90 days (W)	Males: 0, 0.65, 5.0, 46, 175 Females: 0, 0.71, 7.7, 53, 200	LE, BW, FI, WI, HE, BC, BI, OW, HP	Death			175 M 200 F	28% mortality during exposure and 90-day recovery period
					Bd wt	46 M 53 F		175 M 200 F	Decreased body weight gain in males (23%) and females (34%)
					Resp	175 M 200 F			
					Cardio	175 M 200 F			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Gastro	175 M 200 F			
					Hemato	175 M 200 F			
					Musc/skel	175 M 200 F			
					Hepatic	175 M 200 F			
					Renal	175 M 200 F			
					Endocr	46 M	175 M		Increased incidence and severity of thyroid lesions (reduced follicular size, colloid density, increased epithelial height)
						200 F			
					Immuno	175 M 200 F			
					Neuro	175 M 200 F			
					Repro	175 M			
Chu et al. 1982b									
43	Rat (Sprague-Dawley) 10 M	28 days (W)	0, 2.3, 23, 193	LE, CS, BW, FI, HE, BC, BI, OW, HP	Bd wt	193			
					Resp	193			
					Cardio	193			
					Gastro	193			
					Hemato	23	193		Decreased neutrophils
					Musc/skel	193			
					Hepatic	193			
					Renal	193			
					Endocr	193			
					Immuno	193			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro	193			
					Repro	193			
DeAngelo et al. 2002									
44	Rat (Fischer-344) 6 M	13 weeks (W)	0, 34	BW, WI, HP	Bd wt	34			
					Gastro	34			
Dorman et al. 1997									
45	Rat (Fischer-344) 6–10 F	3 weeks 5 days/week (GO)	Odor-cued: 0, 34, 100, 400 Tone-cued: 0, 400	CS, BW, NX, HP	Resp	34	100		Loss of olfactory glands in lamina propria; ethmoid periosteal proliferation
					Neuro	400			
EPA 1980									
46	Rat (Osborne-Mendel) 30–40 M	90 days (W)	0, 20, 38, 57, 81, 160	LE, CS, BW, FI, WI, BC, UR, GN, OW, HP	Bd wt	81	160		16% decrease in terminal body weight
					Resp	160			
					Gastro	160			
					Hemato	160			
					Hepatic	160			
					Renal	160			
					Endocr	160			
					Immuno	160			
					Repro	160			
Geter et al. 2004b									
47	Rat (Fischer-344)	26 weeks (W)	0, 35	BW, WI, HP	Bd wt	35			
					Gastro	35			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Hooth et al. 2002; McDorman et al. 2003a, 2003b									
48	Rat (Eker) 16–20 M, 8–10 F	10 months (W)	M: 0, 27, 102 F: 0, 158	LE, CS, BW, WI, OW, GN, HP	Gastro	158 F	27 M		Increased incidence of aberrant crypt foci in the colon
					Renal		27 M 158 F		Increased incidence of atypical tubules and hyperplasia
Larson et al. 1995a									
49	Rat (Fischer-344) 12 M	3 weeks 5 days/week (GO)	0, 3, 10, 34, 90, 180	BW, WI, HE, BC, GN, OW, HP	Bd wt	90	180		10% decrease in body weight gain
					Hepatic	34	90		Increased relative liver weight
					Renal	90	180		Progressive degeneration of the proximal tubules
Larson et al. 1995a									
50	Rat (Fischer-344) 12 M	3 weeks (W)	0, 6.0, 17.4, 32.0, 62.3, 106	BW, WI, HE, BC, GN, OW, HP	Bd wt	62.3		106	25% decrease in body weight gain
					Hepatic	106			
					Renal	106			
Larson et al. 1995b									
51	Rat (Fischer-344) 5 F	3 weeks 5 days/week (GO)	0, 34, 100, 200, 400	CS, BW, OW, HP, OF	Bd wt	400			
					Resp		34		New nasal bone formation and periosteal hypercellularity
					Hepatic	34	100		Increased hepatocellular proliferation
					Renal	34	100		Increased proliferation of proximal tubule epithelial cells in renal cortex
Lipsky et al. 1993									
52	Rat (Fischer-344) 6 M	4 weeks 5 days/week (GO)	0, 90, 180	BI, HP	Renal	90		180	Acute renal cell injury and necrosis; renal cell proliferation

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Lipsky et al. 1993									
53	Rat (Fischer-344) 6 M	4 weeks 5 days/week (GW)	0, 90, 180	BI, HP	Renal	180			
Müller et al. 1997									
54	Rat (Wistar) 16 M	4 weeks (GO)	0, 37	CS, BW, OF	Bd wt	37			
					Cardio		37		Decreased heart rate, increased blood pressure, and altered cardiac parameters
Auttachoat et al. 2009									
55	Mouse (B6C3F1) 48 F	28 days (W)	0, 0.35, 1.4, 3.5, 14, 35	BW, WI, HE, OW, IX	Bd wt	35			
					Hemato	35			
					Immuno	35			
Balster and Borzelleca 1982									
56	Mouse (ICR) 16 M	30 days (GW)	0, 100	NX	Neuro	100			
Balster and Borzelleca 1982									
57	Mouse (ICR) 6–13 M	60 days (GW)	0, 100, 400	LE, NX	Death			400	46% mortality
					Neuro		100		Impaired operant conditioning
Balster and Borzelleca 1982									
58	Mouse (ICR) 6–11 M	90 days (GW)	0, 3.1, 31.1	NX	Neuro	31.1			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Bull et al. 1986									
59	Mouse (B6C3F1) 10 M, 10 F	90 days (GO)	0, 60, 130, 270	BW, BC, GN, OW, HP	Bd wt	130 M 270 F		270 M	25% decrease in terminal body weight
					Hepatic		60	270	LOAEL: Fatty changes and increased absolute and relative liver weights SLOAEL: Extensive disruption of hepatic architecture, including mild to moderate early cirrhosis
Bull et al. 1986									
60	Mouse (B6C3F1) 10 M, 10 F	90 days (G)	0, 60, 130, 270	BW, BC, GN, OW, HP	Bd wt	130 M 270 F	270 M		13% decrease in terminal body weight
					Hepatic	60 M	130 M 60 F		Increased absolute and relative liver weight in females at ≥60 mg/kg/day and relative liver weight in males at ≥130 mg/kg/day; minimal-to-mild focal necrosis in both sexes at ≥130 mg/kg/day
Burkhalter and Balster 1979									
61	Mouse (ICR) 5 M, 5 F	10 weeks (premating – lactation) (GW)	0, 31.1	DX	Develop	31.1			
DeAngelo et al. 2002									
62	Mouse (B6C3F1) 6 M	13 weeks (W)	0, 89	BW, WI, HP	Bd wt	89			
					Gastro	89			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
EPA 1980									
63	Mouse (B6C3F1) 30–40 F	90 days (W)	0, 32, 64, 97, 145, 290, 435	LE, CS, BW, FI, WI, GN, OW, HP	Bd wt	435			
					Resp	435			
					Gastro	435			
					Hemato	435			
					Hepatic	145	290		Increased fat content of the liver; centrilobular fatty changes
					Renal	435			
					Endocr	435			
					Immuno	435			
Eschenbrenner and Miller 1945									
64	Mouse (Strain A) 5 M, 5 F	30 days (GO)	0, 149, 297, 594, 1188, 2376	LE, GN, HP	Hepatic	297		594	Cirrhosis
					Cancer			594	CEL: Hepatomas
Larson et al. 1994b									
65	Mouse (B6C3F1) 14 F	3 weeks 5 days/week (GO)	0, 3, 10, 34, 90, 238, 477	BW, WI, BC, BI, GN, OW, HP	Bd wt	477			
					Hepatic	10	34	238	LOAEL: Mild vacuolation of hepatocytes SLOAEL: Severe hepatocellular necrosis and vacuolar degeneration, increased serum ALT and SDH
					Renal	477			
Larson et al. 1994b									
66	Mouse (B6C3F1) 14 F	3 weeks (W)	0, 16, 43, 82, 184, 329	BW, WI, BC, BI, GN, OW, HP	Bd wt	329			
					Hepatic	43	82		Increased relative liver weight
					Renal	329			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Larson et al. 1994d									
67	Mouse (B6C3F1) >5 M	3 weeks 5 days/week (GO)	0, 34, 90, 138, 277	LE, BW, WI, GN, OW, HP	Bd wt	138	277		14% decrease in body weight gain
					Hepatic	34	90	277	LOAEL: Hepatocellular swelling SLOAEL: Degeneration and necrosis
					Renal		34	277	LOAEL: Regenerating proximal convoluted tubules SLOAEL: Severe degeneration and necrosis of the proximal tubules
Melnick et al. 1998									
68	Mouse (B6C3F1) 10 F	3 weeks 5 days/week (GO)	0, 55, 110, 238, 477	CS, BW, WI, BC, OW, HP	Bd wt	477			
					Hepatic		55		Increased incidence and severity of hepatocyte hydropic degeneration
Mostafa et al. 2009									
69	Mouse (Swiss) 18 B	54 days 5 days/week (GO)	0, 130, 238, 277, 477	BC, HP	Hepatic	130	238		Males: Marked cellular inflammatory infiltration Females: Focal necrosis
Munson et al. 1982									
70	Mouse (CD-1) 7–12 M, 7–12 F	90 days (GW)	0, 50, 125, 250	LE, BW, HE, BC, BI, OW, IX	Bd wt	250			
					Hemato	250			
					Hepatic	125 M	250 M 50 F		Increased relative liver weights
					Immuno	125	250		Males: Suppressed humoral immunity Females: Suppressed cell-mediated immunity
					Other noncancer	125	250		Decreased serum glucose

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NTP 1988a									
71	Mouse (CD-1) 20 M, 20 F	2-generation (continuous breeding); ~105 days (GO)	F0: 0, 6.6, 16, 41 F1: 0, 41	LE, BW, WI, GN, OW, HP, RX, DX	Bd wt	41			
					Resp	41			
					Hepatic	41 M	41 F		Increased relative liver weight and hepatocellular degeneration in F1 adult females
					Renal	41			
					Repro	41 F	41 M		Increased absolute and relative epididymal weight, degeneration of epididymal epithelium in F1 adult males
					Develop	41			
Roe et al. 1979									
72	Mouse (Swiss) NS B	6 weeks 6 days/week (G)	0, 60, 150, 425	LE, CS, BW	Death			150 M 425 F	80% mortality in males; 100% mortality in females
					Bd wt		60		Unspecified decrease in body weight gain
Chloroform was administered in a toothpaste vehicle.									
Sehata et al. 2002									
73	Mouse (CB6F1) 15 M, 15 F	26 weeks 5 days/week (GO)	M: 0, 140 F: 0, 240	LE, CS, BW, FI, HE, BC, OW, GN, HP	Bd wt	140 M 240 F			
					Resp		140 M 240 F		Increased incidence of bronchial epithelium degeneration
					Cardio	140 M 240 F			
					Hemato	140 M 240 F			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic		140 M 240 F		Increased incidence of hepatocyte vacuolation and swelling and hepatocellular foci, hepatocellular proliferation, increased absolute and relative liver weight, increased serum AST and ALT
					Renal	140 M	240 F		Increased renal cell proliferation
					Ocular	140 M 240 F			
					Endocr	140 M 240 F			
					Immuno	140 M 240 F			
					Neuro	140 M 240 F			
					Repro	140 M 240 F			
Heywood et al. 1979									
74	Dog (Beagle) 8–16 M, 8–16 F	up to 52 weeks 6 days/week (C)	0, 15, 30	CS, BW, WI, FI, BC, OP	Bd wt	30			
					Hemato	30			
					Hepatic	15[c]	30		Increased serum ALT from 26 to 52 weeks
					Renal	30			
					Ocular	30			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
CHRONIC EXPOSURE									
Dunnick and Melnick 1993; NCI 1976									
75	Rat (Osborne-Mendel) 20–50 M, 20–50 F	78 weeks 5 days/week (GO)	M: 0, 90, 180 F: 0, 100, 200	LE, BW, GN, HP	Death			90 M 100 F	17% decrease in male survival and 24% decrease in female survival at 78 weeks
					Bd wt		90 M 100 F	200 F	LOAEL: ≥10% decrease in body weight starting at 50 weeks in males and 18 weeks in females SLOAEL: ≥20% decrease in body weight starting at 8 weeks in females
					Resp			90 M 100 F	Wheezing; increased incidence and severity of inflammatory pulmonary lesions
					Cardio	180 M 200 F			
					Gastro	180 M 200 F			
					Hemato	180 M 200 F			
					Musc/skel	180 M 200 F			
					Hepatic	180 M			
						100 F	200 F		Necrosis of hepatic parenchyma
					Renal	180 M 200 F			
					Dermal	180 M 200 F			
					Endocr	180 M 200 F			
					Immuno	180 M 200 F			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro	180 M 200 F			
					Repro	180 M 200 F			
					Cancer			180 M	CEL: Kidney tubular cell adenomas and carcinomas
Hard et al. 2000; Jorgenson et al. 1985									
76	Rat (Osborne-Mendel) 50–330 M	104 weeks (W)	0, 19, 38, 81, 160	LE, CS, BW, WI, GN, HP	Bd wt	160			
					Renal	38	81		Renal tubule cell alterations (nuclear crowding, cytoplasmic vacuolation, faint basophilia; consistent with hyperplasia)
					Cancer			160	CEL: kidney tubular cell adenomas and adenocarcinomas
Non-neoplastic renal histology was evaluated in 18–49 males/group (except 19 mg/kg/day) and reported by Hard et al. (2000).									
Nagano et al. 2006									
77	Rat F344/DuCrj 50 M	104 weeks (W)	0, 45	LE, CS, BW, WI, BC, UR, GN, HP	Bd wt		45		11% decrease in terminal body weight
					Renal		45		Increased incidences of cytoplasmic basophilia and tubular lumen dilation in the proximal tubule
Tumasonis et al. 1985, 1987									
78	Rat (Wistar) 26–32 M, 22–45 F	180 weeks (W)	0, 200	BW, WI, GN, HP	Bd wt			200	50% decrease in body weight
					Cancer			200	CEL: hepatic neoplastic nodules and adenofibrosis
Dunnick and Melnick 1993; NCI 1976									
79	Mouse (B6C3F1) 50 M, 50 F	78 weeks 5 days/week (GO)	M: 0, 138, 277 F: 0, 238, 477	LE, BW, GN, HP	Death			477 F	17% decrease in survival
					Bd wt	277 M 477 F			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Resp	277 M			
						477 F			
					Cardio	277 M			
						238 F		477 F	Cardiac atrial thrombosis in nine mice that died
					Gastro	277 M			
						477 F			
					Hemato	277 M			
						477 F			
					Musc/skel	277 M			
						477 F			
					Hepatic		138 M 238 F		Nodular hyperplasia
					Renal	277 M			
						477 F			
					Dermal	277 M			
						477 F			
					Endocr	277 M			
						477 F			
					Immuno	277 M			
						477 F			
					Neuro	277 M			
						477 F			
					Repro	277 M			
						477 F			
					Cancer			238 F 138 M	CEL: Hepatocellular adenomas and carcinomas

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Jorgenson et al. 1985									
80	Mouse (B6C3F1) 50–430 F	104 weeks (W)	0, 34, 65, 130, 263	LE, BW, WI, GN, HP	Bd wt	263			
Roe et al. 1979									
81	Mouse (ICI) 52–104 M, 52–104 F	80 weeks 6 day/week (G)	0, 17, 60	LE, CS, BW, HE, GN, OW, HP	Bd wt	60			
					Resp	60			
					Hemato	60			
					Hepatic	60			
					Renal	60			
					Neuro	60			
					Cancer			60 M	CEL: Kidney tumors (malignant hypernephromas, benign adenomas)
Chloroform was administered in a toothpaste vehicle.									
Roe et al. 1979									
82	Mouse (ICI) 52–260 M	80 weeks 6 day/week (G)	0, 60	LE, CS, BW, FI, GN, OW, HP	Bd wt	60			
					Cancer			60	CEL: Kidney tumors (malignant hypernephromas, benign adenomas)
Chloroform was administered in a toothpaste vehicle.									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Roe et al. 1979									
83	Mouse (ICI) 52 M	80 weeks 6 day/week (GO)	0, 60	LE, CS, BW, FI, GN, OW, HP	Bd wt	60			
					Renal			60	Moderate-to-severe kidney disease
					Cancer			60	CEL: Kidney tumors (malignant hypernephromas, benign adenomas)
Heywood et al. 1979									
84	Dog (Beagle) 8–16 M, 8–16 F	7.5 years 6 days/week (C)	0, 15, 30	LE, CS, BW, FI, WI, HE, BC, OP, GN, OW, HP	Bd wt	30			
					Cardio	30			
					Hemato	30			
					Hepatic		15[d]		Moderate-to-marked fatty cysts; Increased serum ALT ($BMDL_{10}$ for moderate-to-marked fatty cysts in male dogs=2.15 mg/kg/day)
					Renal	15	30		Fat deposition in glomeruli
					Ocular	30			
					Endocr	30			
					Immuno	30			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro	30			
					Repro	30			

Studies selected for derivation of oral MRLs

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive the acute-duration oral MRL of 0.3 mg/kg/day. The NOAEL of 26 mg/kg/day was divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability); see Appendix A for more detailed information regarding the MRL.
[c]Used to derive the intermediate-duration oral MRL of 0.1 mg/kg/day. The NOAEL of 15 mg/kg/day was adjusted for continuous exposure (6 days/7 days) to a $NOAEL_{ADJ}$ of 13 mg/kg/day and then divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability); see Appendix A for more detailed information regarding the MRL.
[d]Used to derive a chronic-duration oral MRL of 0.02 mg/kg/day. The $BMDL_{10}$ of 2.15 mg/kg/day was adjusted for continuous exposure (6 days/7 days) to a $BMDL_{ADJ}$ of 1.84 mg/kg/day and was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability); see Appendix A for more detailed information regarding the MRL.

ADJ = adjusted for daily exposure; ALT = alanine aminotransferase; AST = aspartate aminotransferase; B = both males and females; BC = serum (blood) chemistry; Bd wt or BW = body weight; BI = biochemical indices; $BMDL_{10}$ = benchmark dose lower confidence limit 10%; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; ED_{50} = median dose to observed effect; F = female(s); FI = food intake; (G) = gavage; Gastro = gastrointestinal; GD = gestational day; GN = gross necropsy; (GO) = gavage in oil; (GW) = gavage in water; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; IX = immune function; LD_{50} = median lethal dose; LDH = lactate dehydrogenase; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Musc/skel = musculoskeletal; NAG = N-acetylglucosaminidase; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OF = organ function; OP = ophthalmology; OW = organ weight; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive function; SDH = sorbitol dehydrogenase; SLOAEL = serious lowest-observed-adverse-effect level; UR = urinalysis; (W) = water; WI = water intake

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

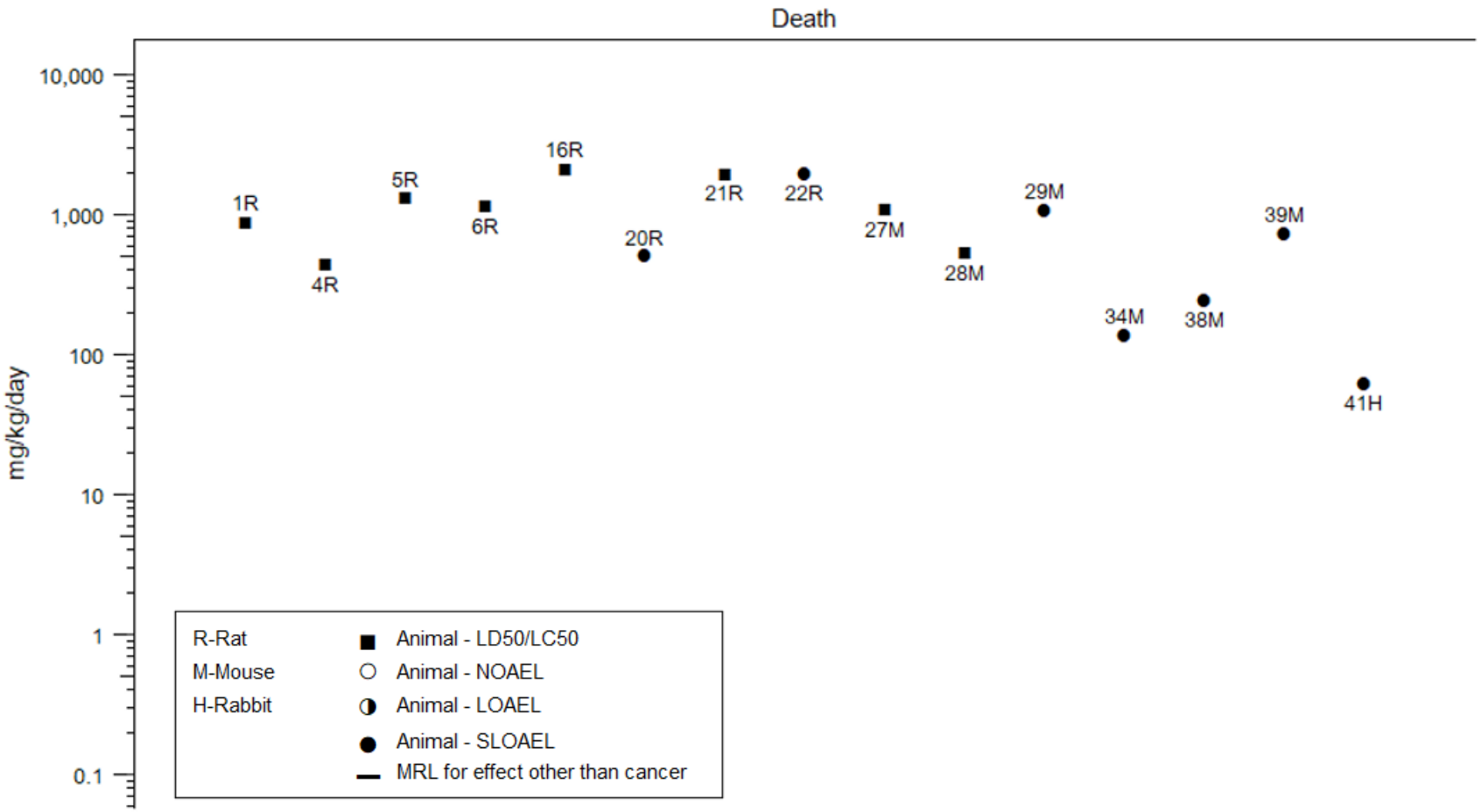

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

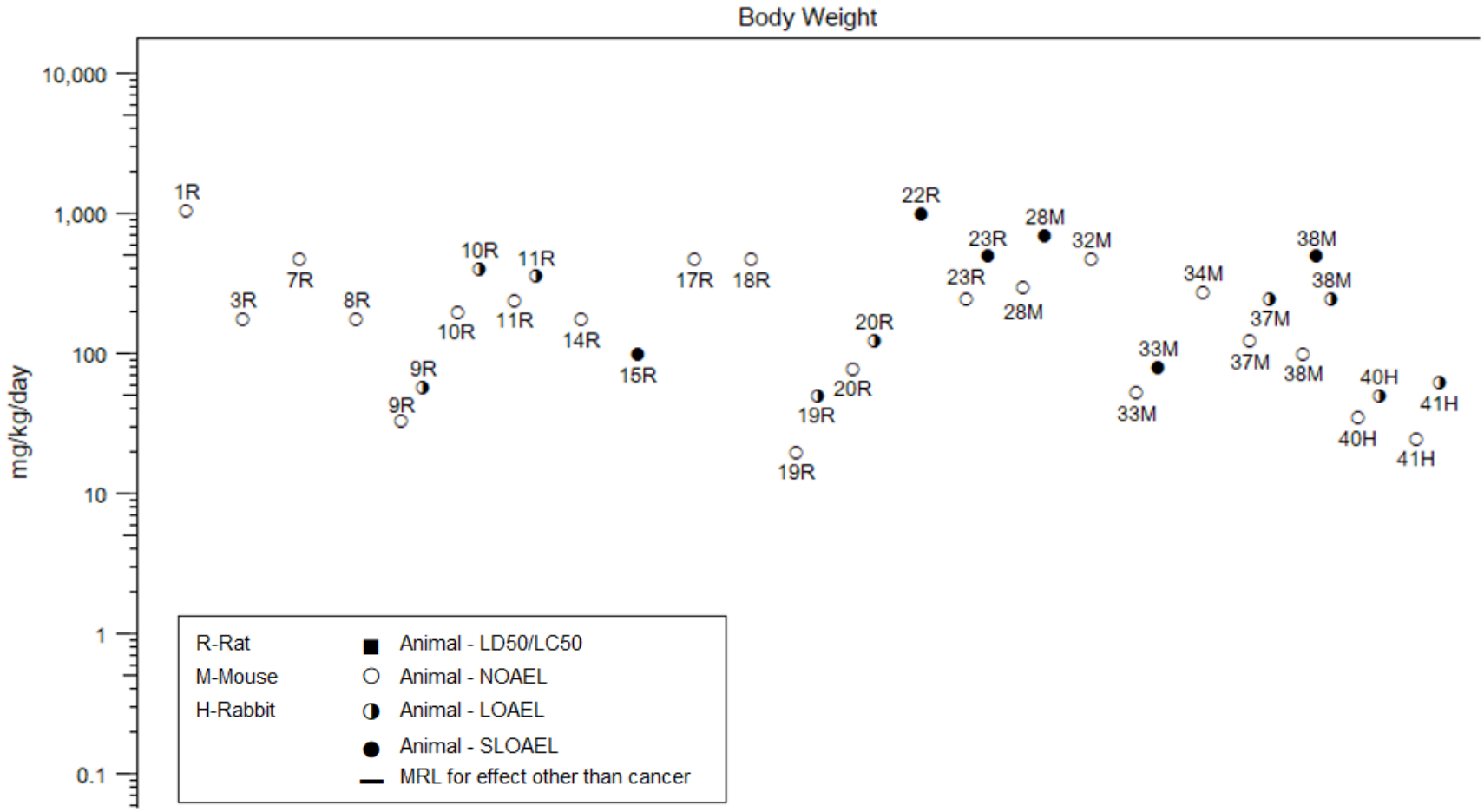

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

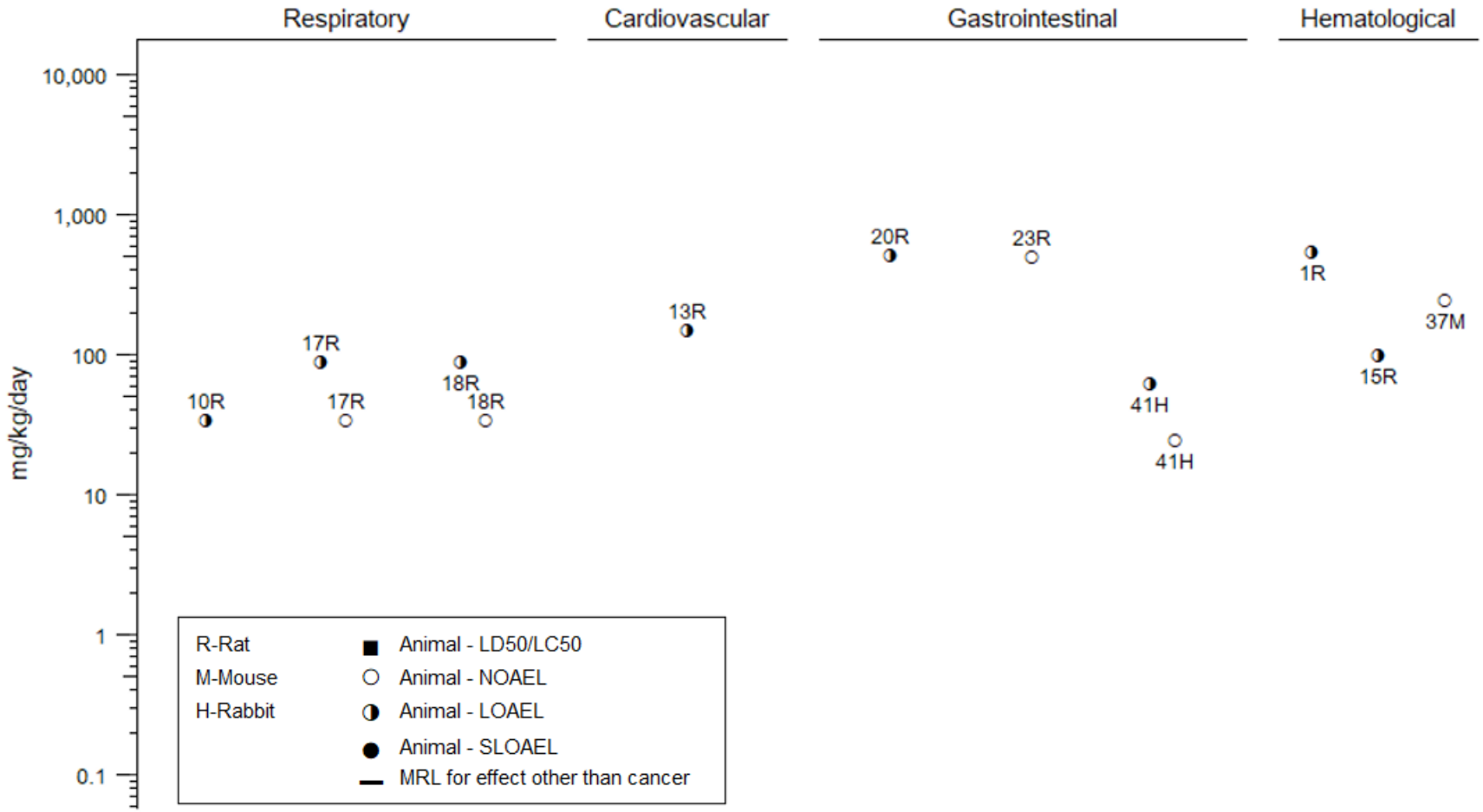

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

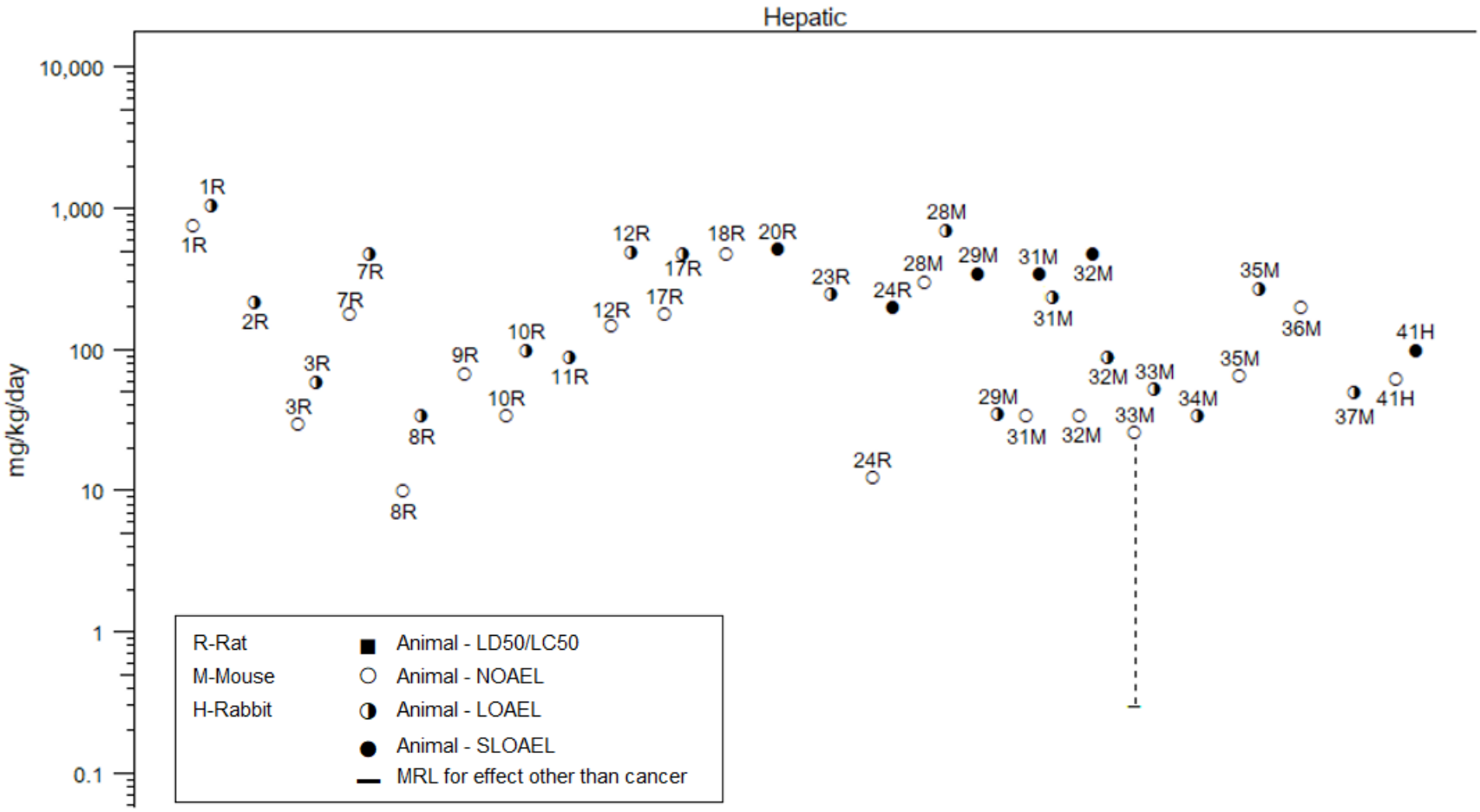

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

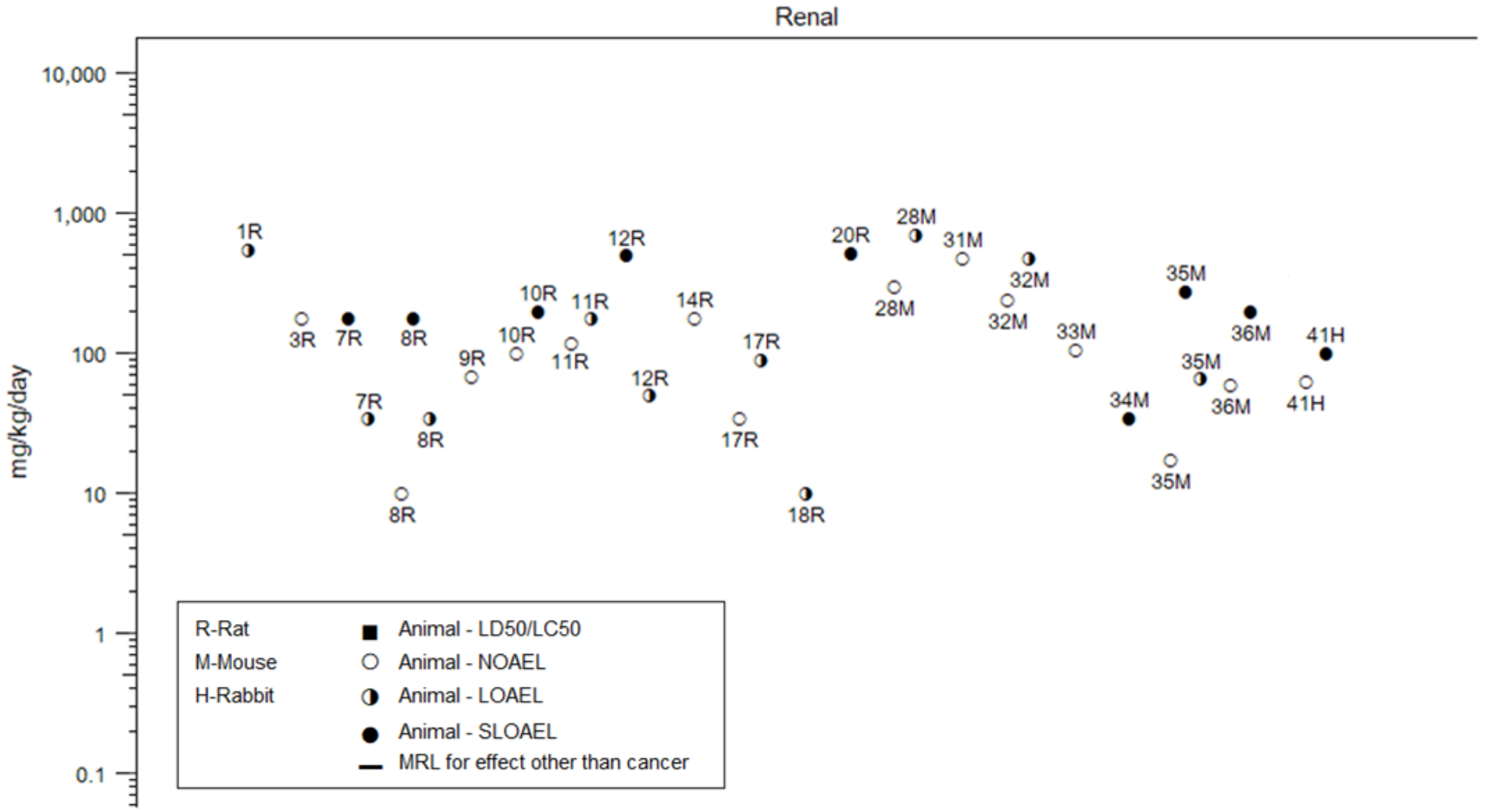

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

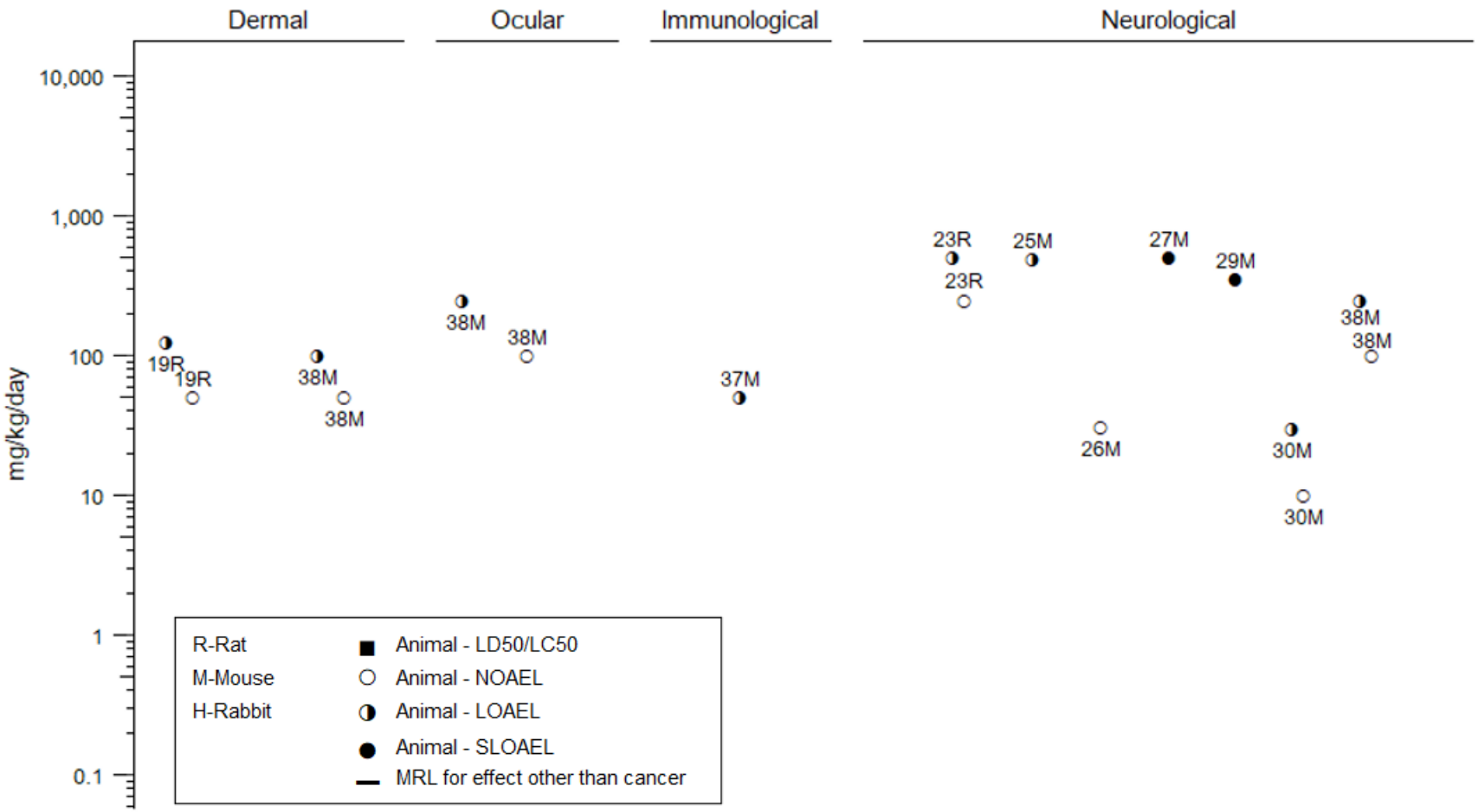

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

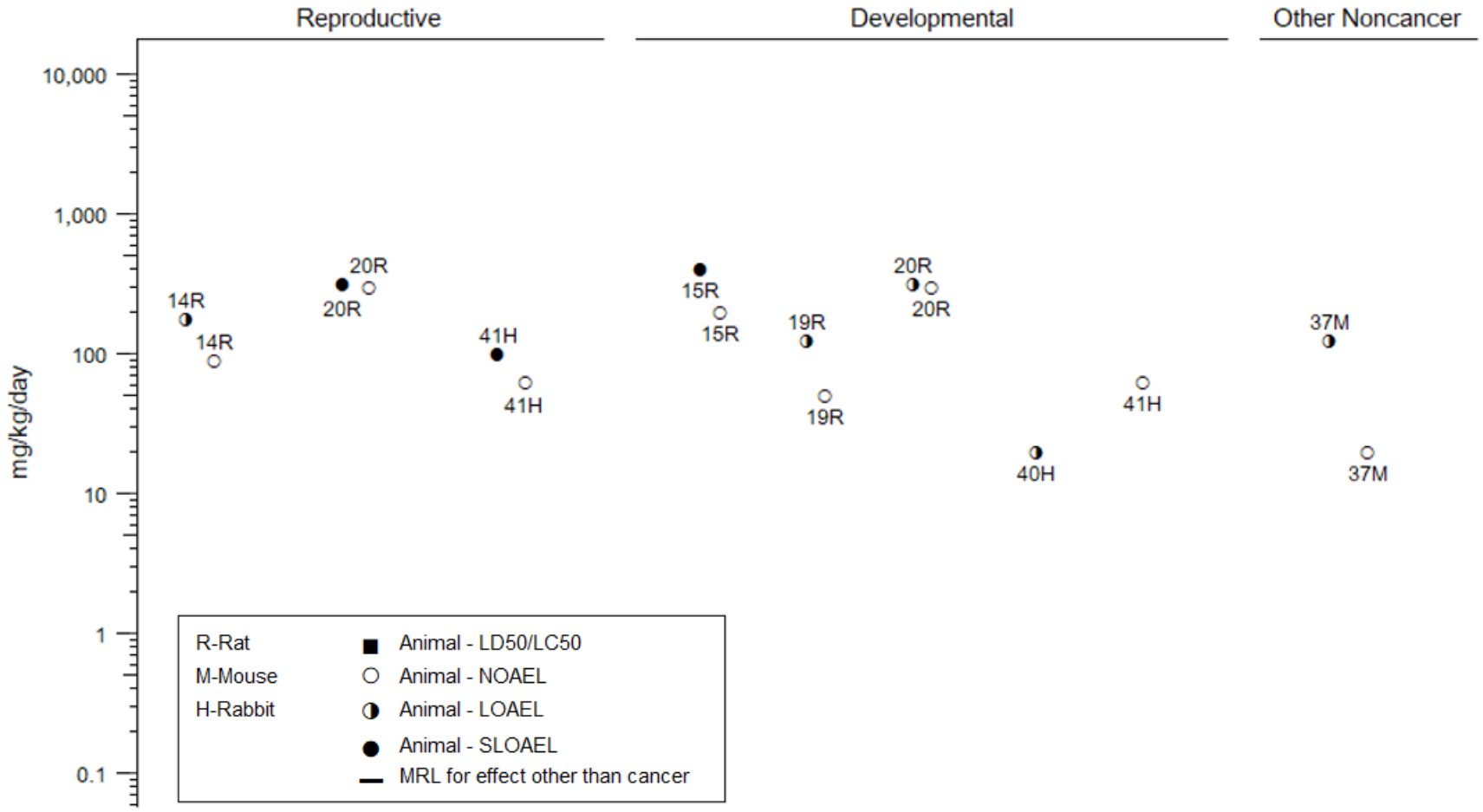

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

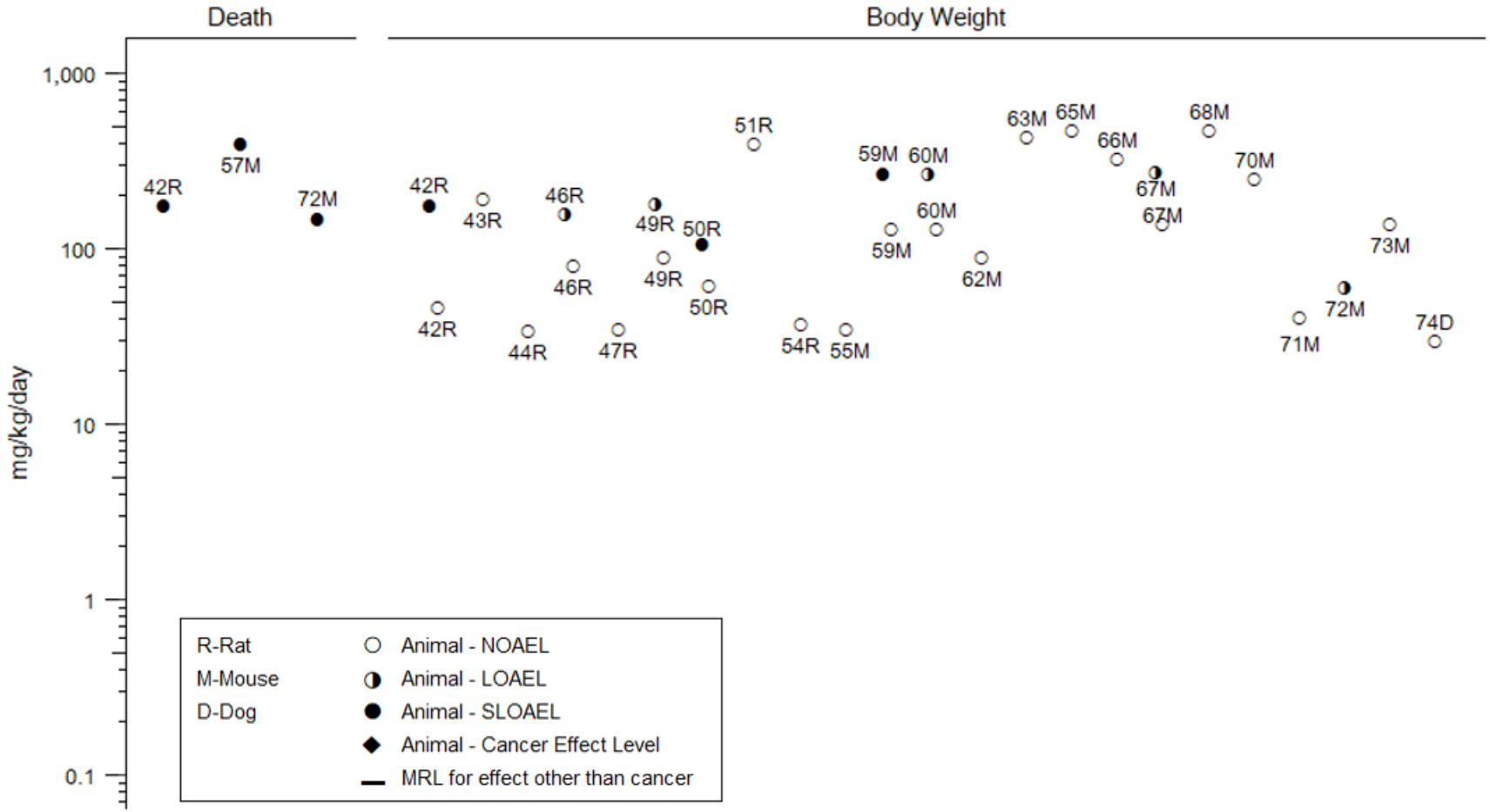

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

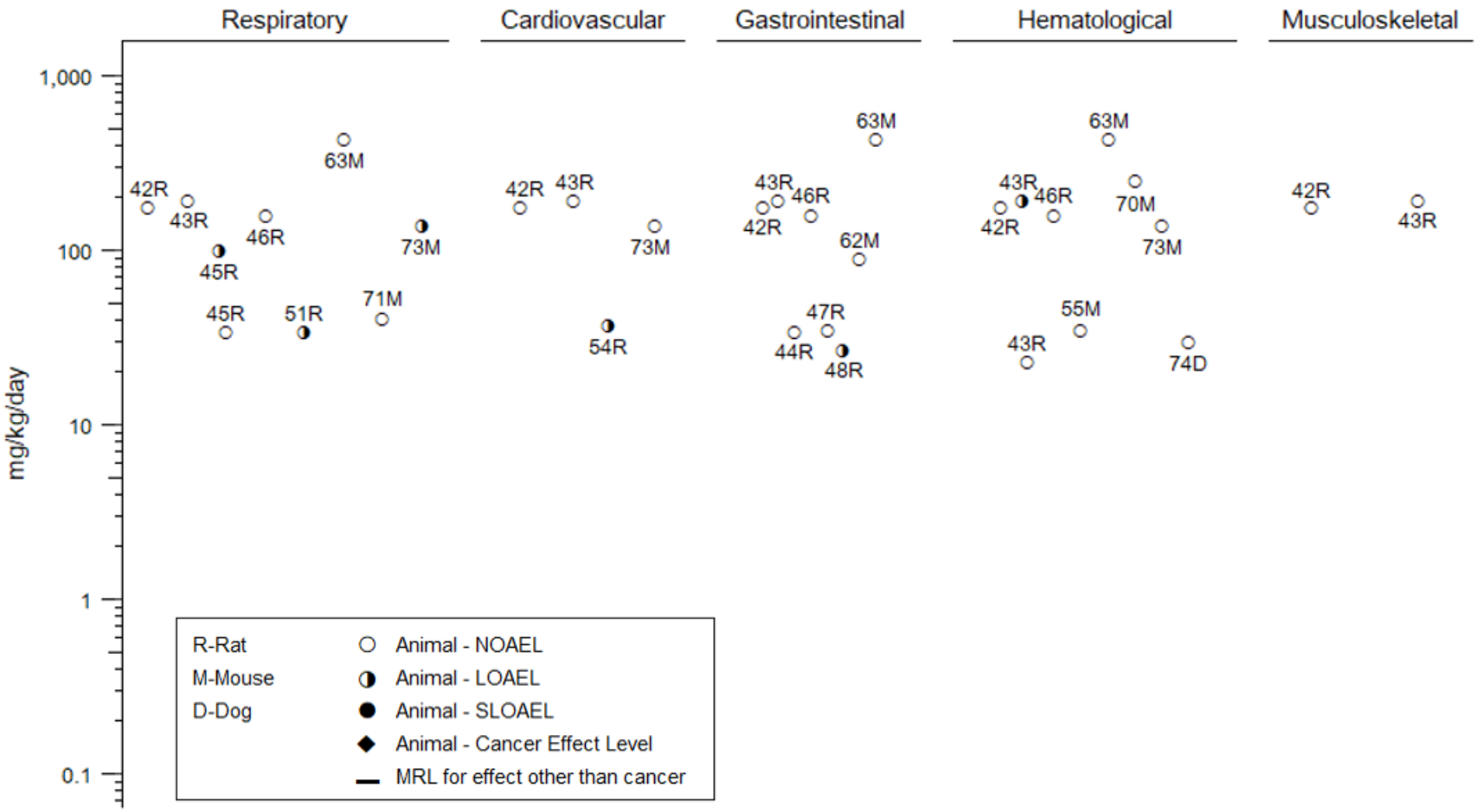

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

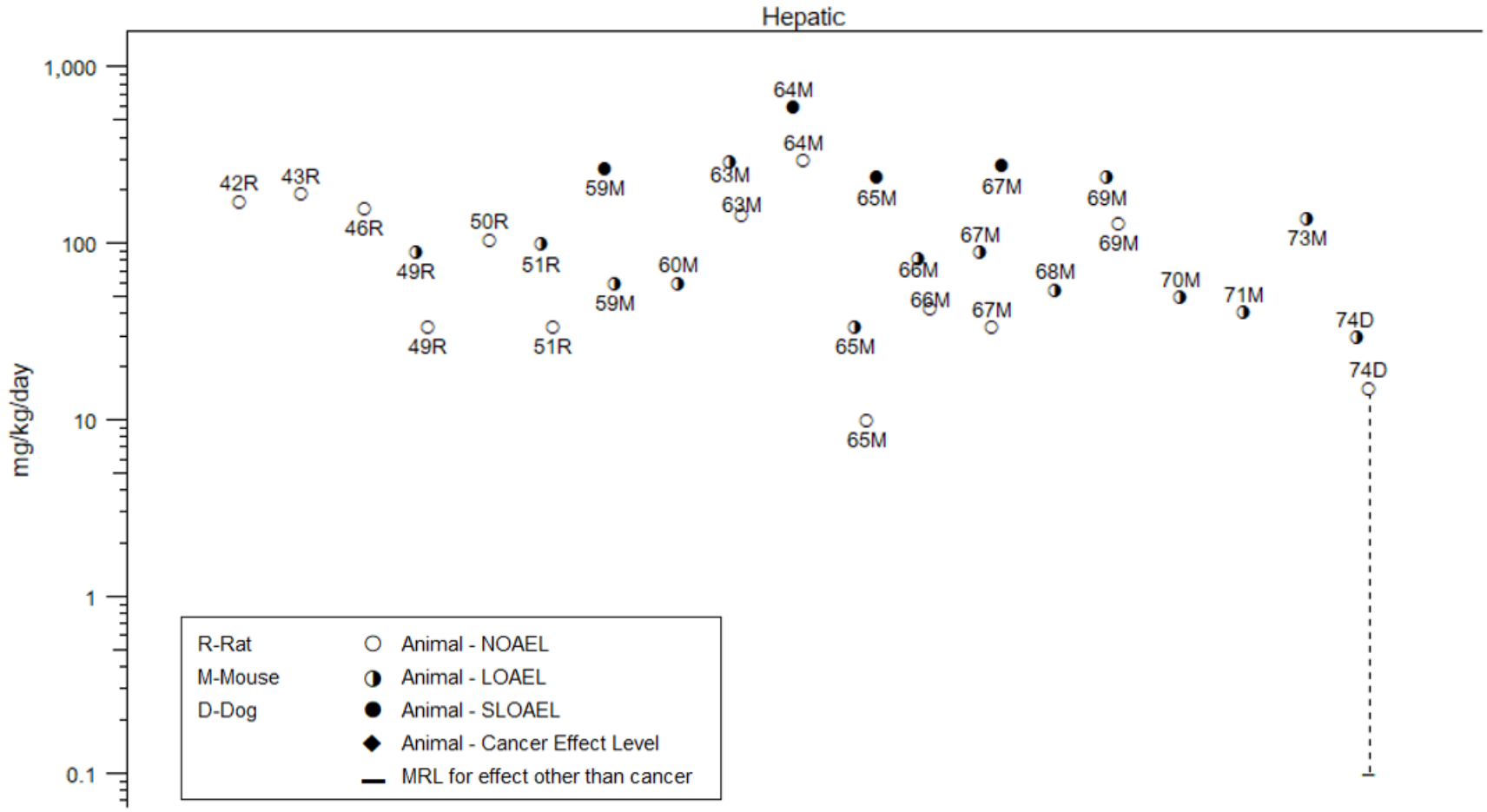

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

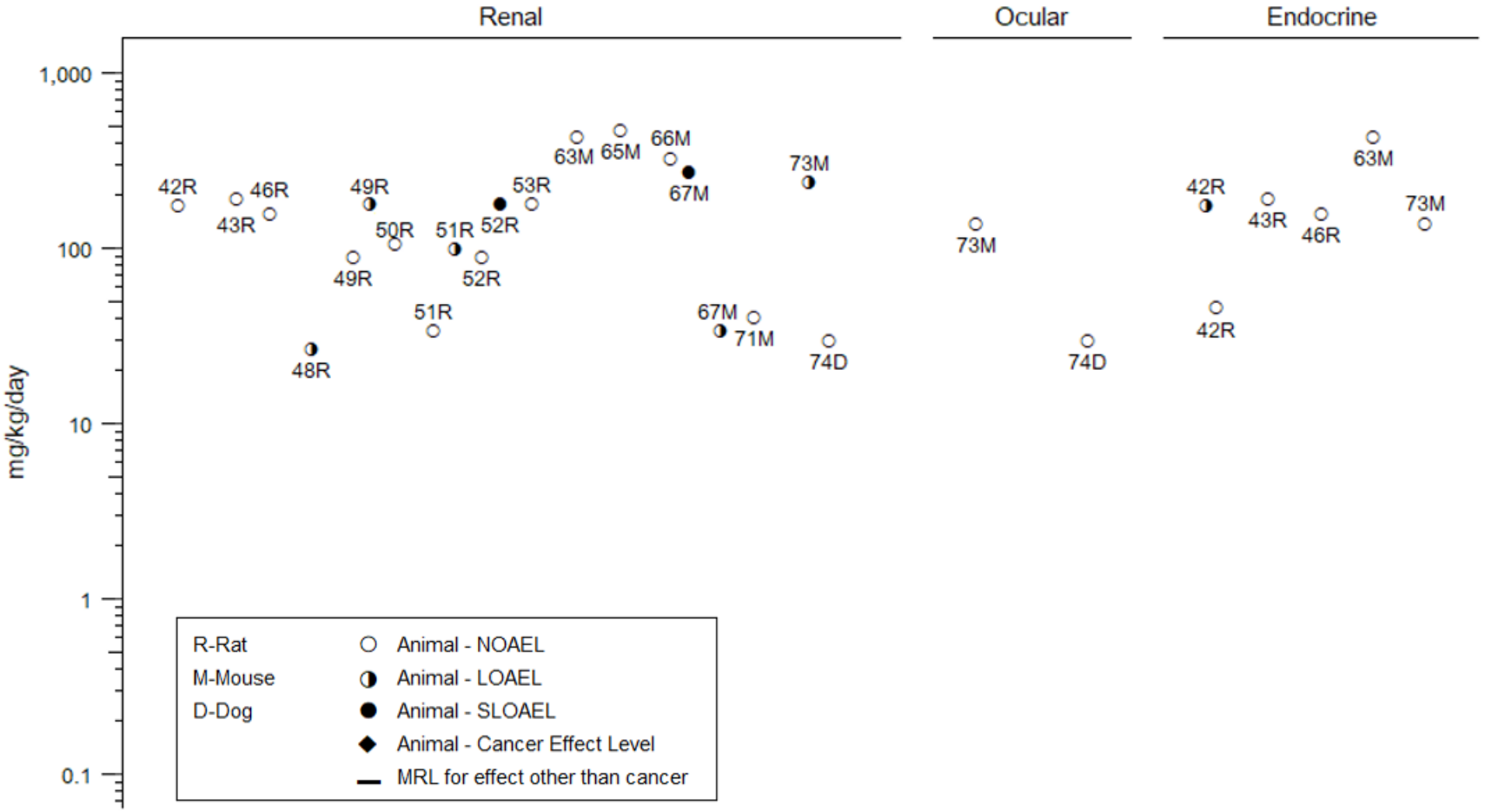

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

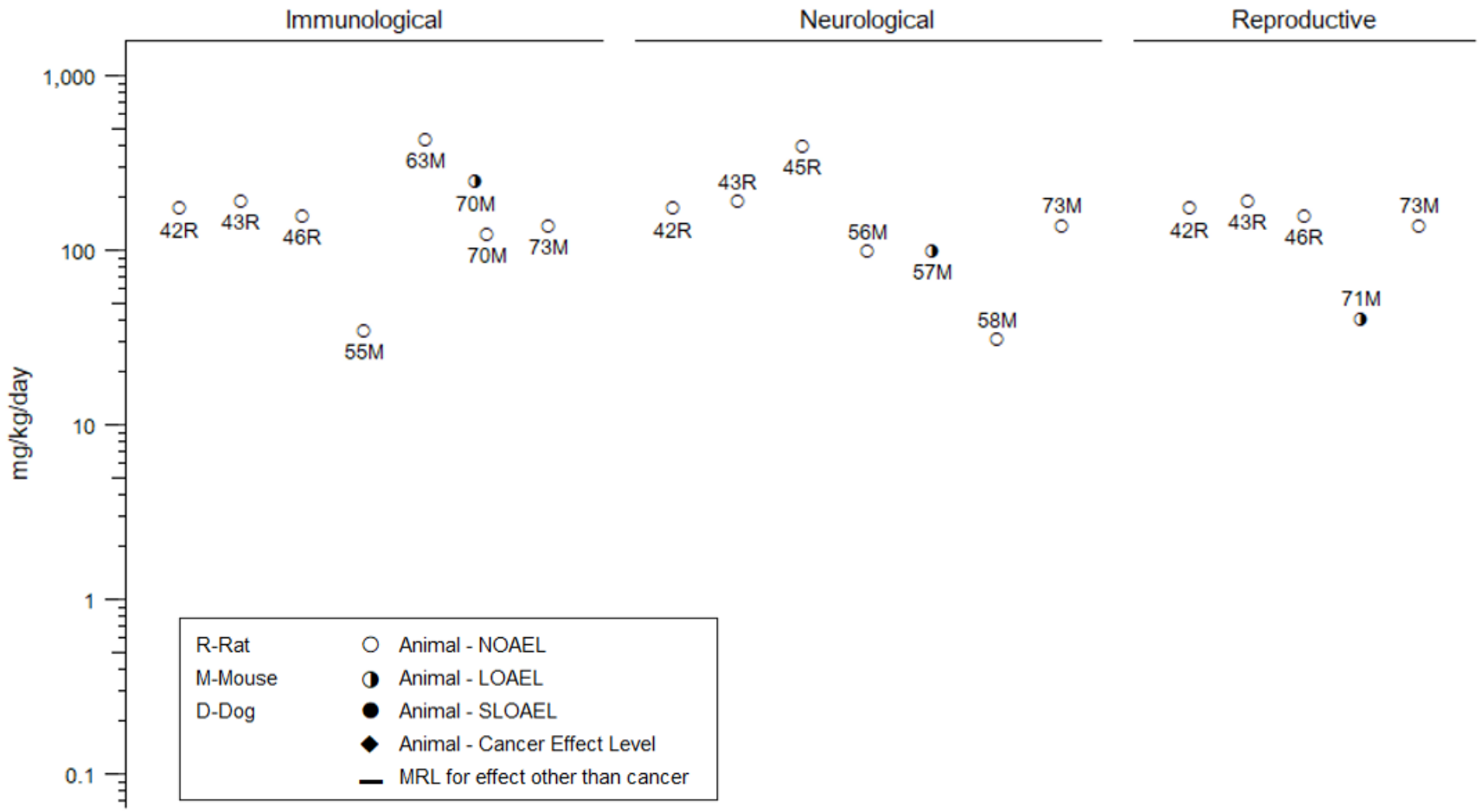

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

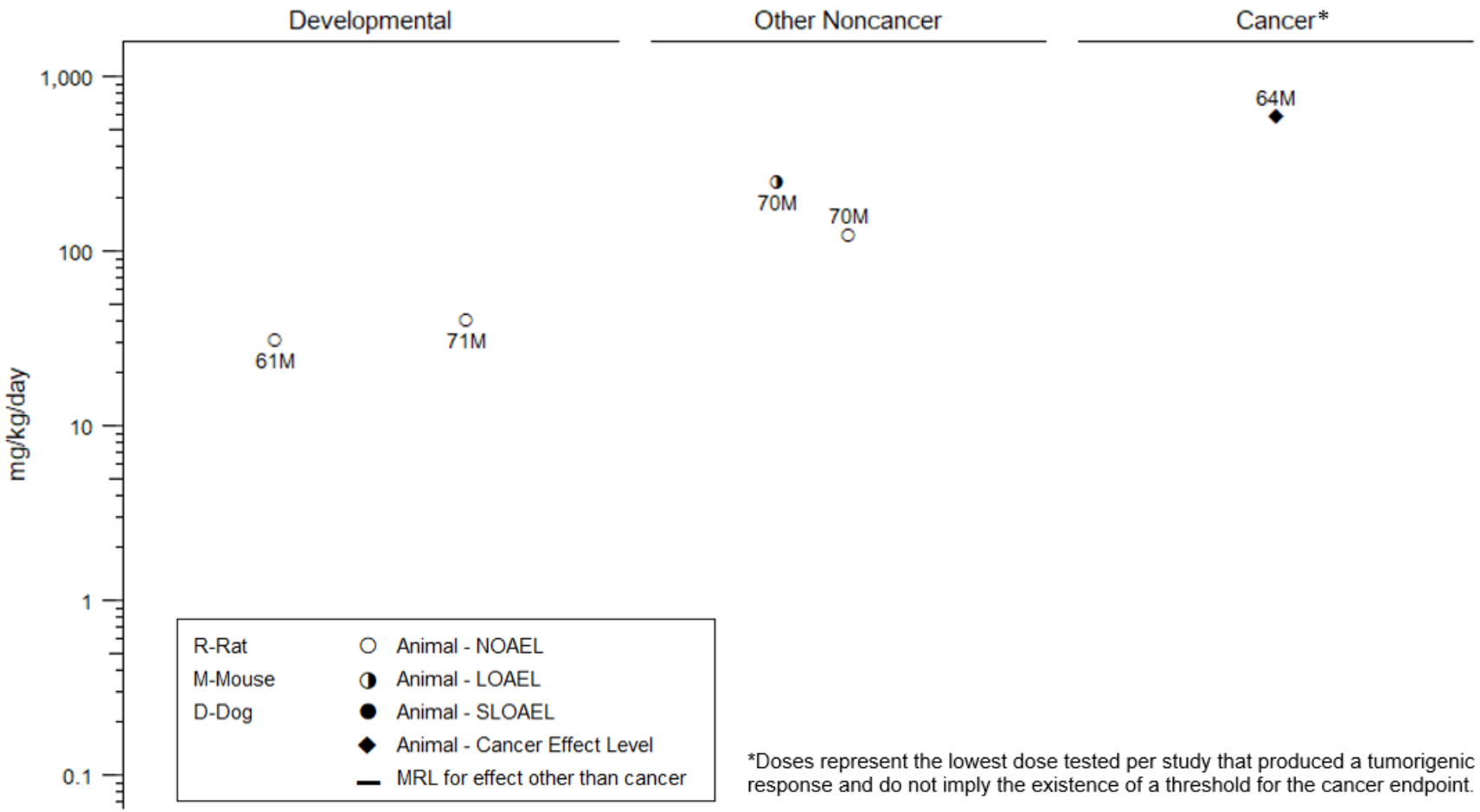

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Chronic (≥365 days)

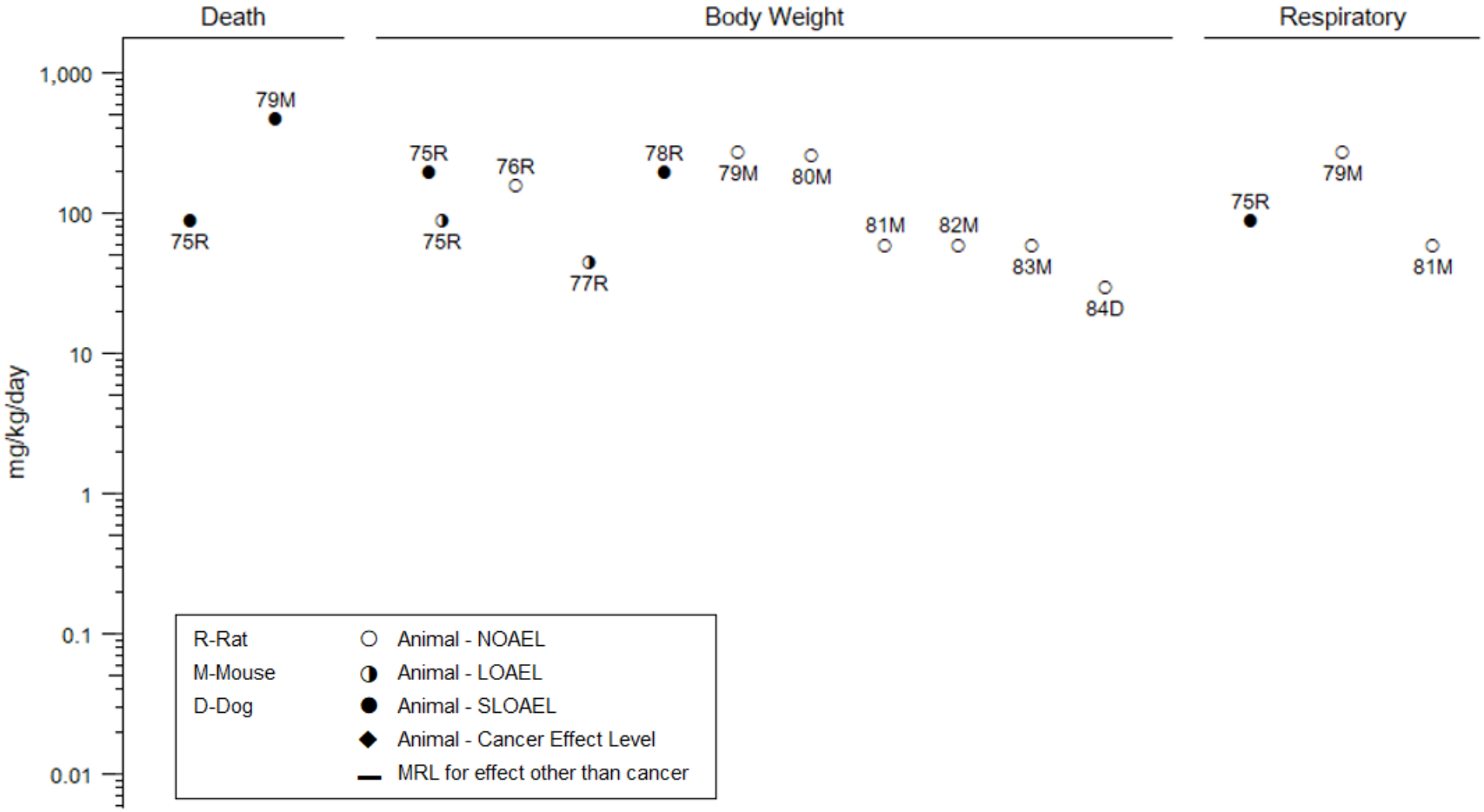

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Chronic (≥365 days)

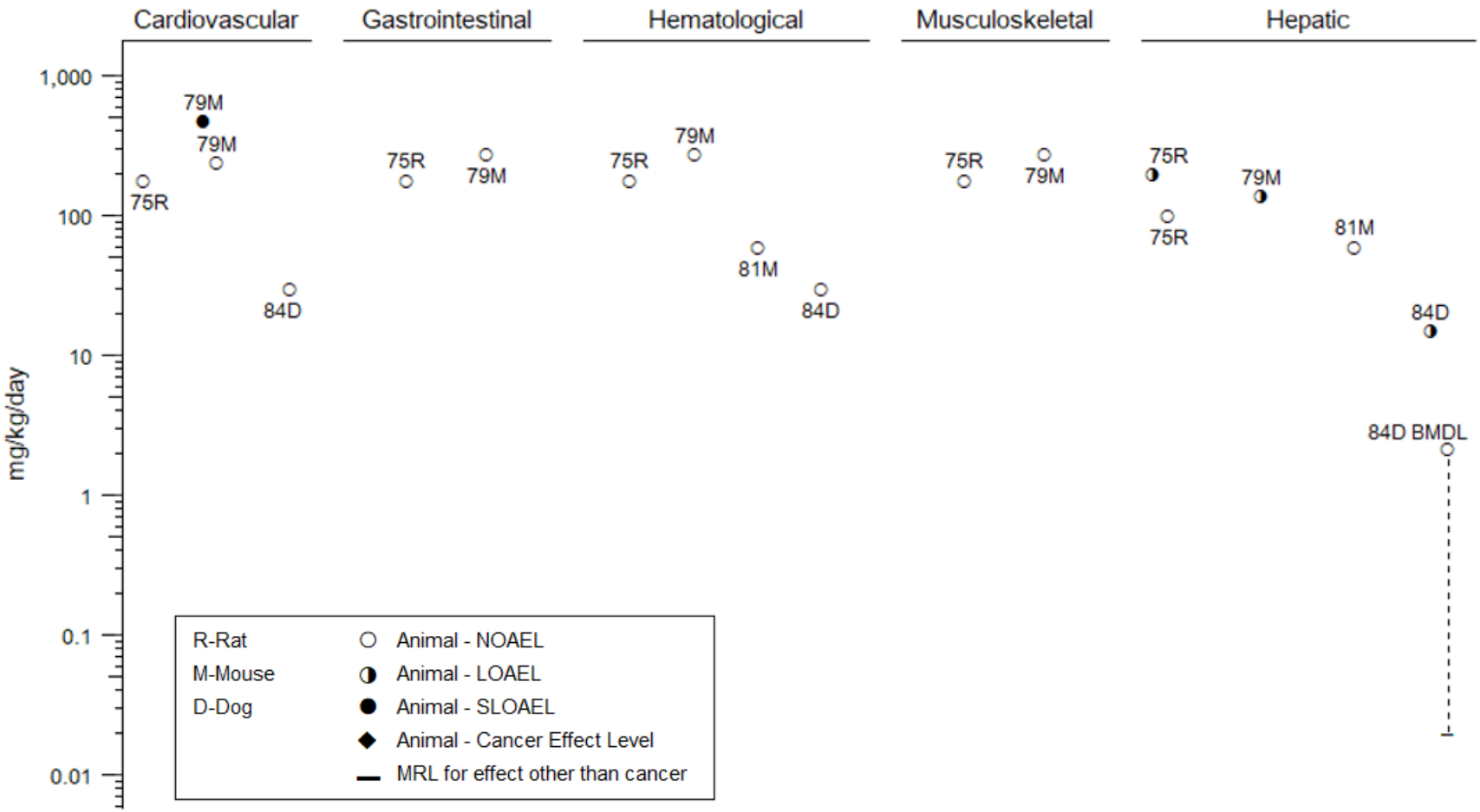

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Chronic (≥365 days)

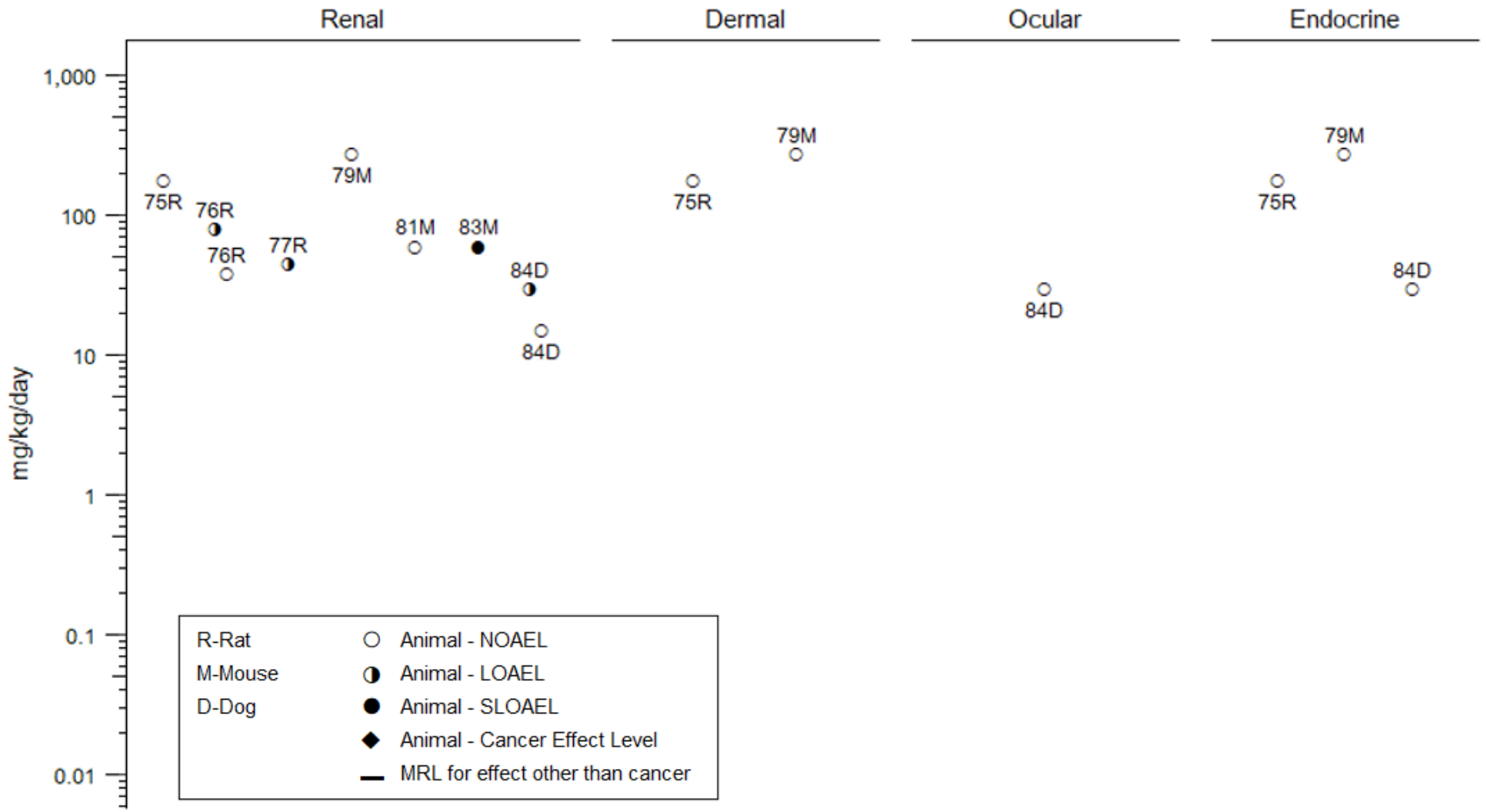

Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Chronic (≥365 days)

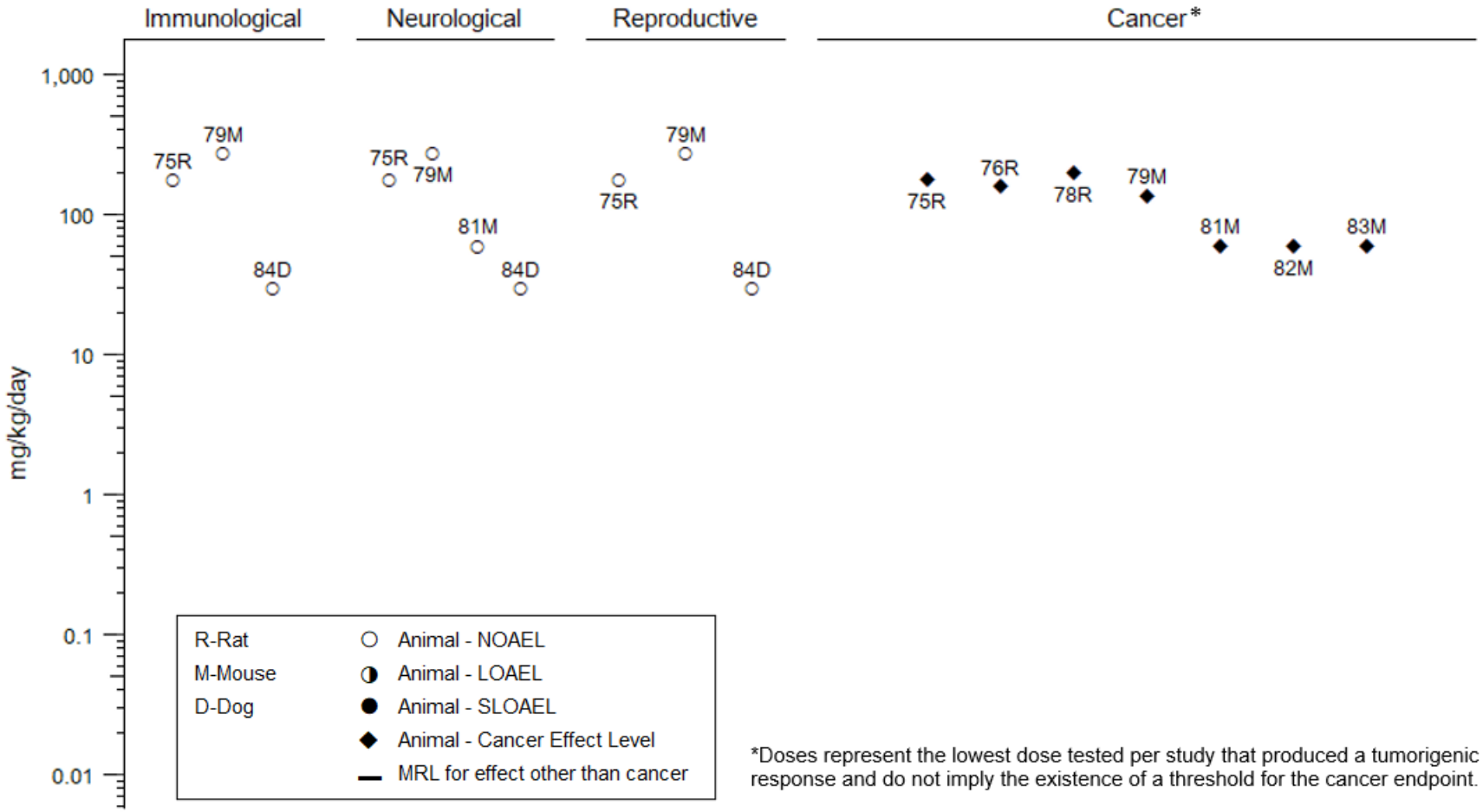

Table 2-3. Levels of Significant Exposure to Chloroform – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Smyth et al. 1962								
Rabbit (New Zealand) 5 M	24 hours	5 mg/kg	CS	Dermal		5 mg/kg		Slight skin irritation
Torkelson et al. 1976								
Rabbit (NS) 2 NS	24 hours	1,000, 2,000, 3,980 mg/kg	LE, CS, BW, GN, HP	Bd wt		1,000 mg/kg		Unspecified weight loss
				Hepatic	3,980 mg/kg			
				Renal		1,000 mg/kg		Degenerative tubular changes
				Dermal			1,000 mg/kg	Extensive necrosis

Bd wt or BW = body weight; CS = clinical signs; GN = gross necropsy; HP = histopathology; LE = lethality; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; NS = not specified

2.2 DEATH

Data from human and animal studies indicate that exposure to high levels of chloroform can be lethal via inhalation or oral exposure.

Most information on the exposure levels of chloroform leading to death in humans was obtained from clinical reports of patients exposed to chloroform as a method of anesthesia. It should be noted that when examining the ability of chloroform to cause death, these clinical reports need to be interpreted with caution because many of these patients had pre-existing health conditions that may have contributed to the cause of death. Therefore, chloroform toxicity may not have been the only factor involved in the death of the patient. Older clinical case reports suggest that concentrations of approximately 40,000 ppm, if continued for several minutes, could lead to death due to severe respiratory depression/failure or disturbances in cardiac rhythm (Featherstone 1947). Several cases were reported of death in women after childbirth when chloroform anesthesia had been used; however, actual exposure levels were not reported (Royston 1924; Townsend 1939). Death was attributed to acute hepatotoxicity. It should be noted that prolonged labor with starvation, dehydration, and exhaustion may have contributed to the chloroform-induced hepatotoxicity. No indication of increased mortality was found in a large case-review of 1,502 patients, ranging in age from 1 to 80 years, exposed to <22,500 ppm as anesthesia during surgery (Whitaker and Jones 1965). In most patients, the anesthesia did not last longer than 30 minutes; however, a few received chloroform for more than 2 hours.

There are numerous fatal human cases of forced or intentional inhalation of high concentrations of chloroform in non-clinical settings. While external exposure levels are unknown in these fatal cases, blood chloroform levels of 5–280 mg/L have been reported in suicides (Ago et al. 2011; Giusti and Chiarotti 1981), homicides (Ago et al. 2011; Farrow 1984; Flanagan and Pounder 2010; Kim et al. 1996; Risse et al. 2001; Vendura et al. 1996), and accidental deaths (Allan et al. 1988; Byard et al. 2000; Harada et al. 1997; Singer and Jones 2006). The cause(s) of death in these cases include acute heart failure, hypoxia/asphyxiation, and/or respiratory failure. In cases of forceable inhalation, hypoxia may have been due to both chloroform exposure as well as suffocation by a soaked cloth or rag pressed over the nose and mouth. Acute liver failure and rhabdomyolysis were the causes of death in a woman who repeatedly inhaled an unknown level of chloroform and abused alcohol over a 6-day period (Lionte 2010).

Death has also occurred in humans following accidental or intentional ingestion of chloroform (Kohr 1990; Piersol et al. 1933; Schroeder 1965). Fatal doses have been reported to be as low as 10 mL

(14.8 g), or approximately 212 mg/kg; however, individuals have recovered from oral exposure to doses as high as 2,410 mg/kg (Schroeder 1965). Death in humans after oral exposure to chloroform is usually caused by respiratory obstruction by the tongue due to jaw relaxation, central respiratory paralysis, acute cardiac failure, severe hepatic injury, or multisystem organ failure (Dettling et al. 2016; Piersol et al. 1933; Schroeder 1965). A fatal case report of a 13-year-old girl noted blood chloroform levels of 833.9 mg/L; however, the exposure route was unknown (Gaillard et al. 2006).

Sun et al. (2021a) identified an increased risk of all-cause mortality with increasing blood chloroform levels among 6,365 participants (>40 years of age) in the 1999–2012 National Health and Nutrition Examination Survey (NHANES). Following adjustment for covariates, the risk was increased by 31, 41, and 35% in the second (4.20−8.90 pg/mL), third (8.91–-18.0 pg/mL), and fourth (>18.0 pg/mL) quartiles of exposure, respectively, relative to the first (≤4.19 pg/mL) quartile. Chloroform blood levels were not associated with increased risk of specific causes of mortality (e.g., heart disease, cancer).

Levels of acute-duration inhalation exposure resulting in animal deaths are generally lower than those reported for human patients under anesthesia; however, the exposure durations are generally longer in the animal studies. Mice appear to be more susceptible than rats, with male mice being the most sensitive rodents. In rats, a 4-hour LC_{50} (lethal concentration, 50% kill) value of 9,770.6 ppm was determined (Lundberg et al. 1986). In another 4-hour exposure study, 5/6 rats exposed to 8,000 ppm died (Smyth et al. 1962). In mice, an LT_{50} (lethal time, 50% kill) of 560 minutes was determined at 4,500 ppm (Gehring 1968). In a series of experiments by Deringer et al. (1953), young male mice (2 months old) were less susceptible to acute toxicity than adult male mice. All adult male mice died after exposure to 983 ppm for 1 hour, while none of the young male mice died following similar exposure to up to 1,106 ppm. All young and adult male mice died following a 2-hour exposure to 942 or 963 ppm, respectively, or a 3-hour exposure to 692 or 786 ppm, respectively. Death was associated with renal toxicity in both adult and young male mice. No deaths were observed in similarly exposed female mice (Deringer et al. 1953).

In repeat-exposure studies in rats, one study reported increased mortality in male and female rats exposed to 2,000 ppm for up to 2 weeks (Kasai et al. 2002). In other studies in rats, mortality was not increased following exposure to concentrations up to 4,117 ppm for 8 days (EPA 1978), 400 ppm for 13 weeks (Kasai et al. 2002; Templin et al. 1996b), or 100 ppm for 2 years (Nagano et al. 2006; Yamamoto et al. 2002).

Increased mortality associated with renal toxicity in males and liver toxicity in females was observed in mice following repeated inhalation exposure. Increased mortality was observed in male mice exposed to 92 ppm for 4 days (Constan et al. 1999). In 2-week studies, increased mortality was observed in male mice at ≥30 ppm and in female mice at ≥1,000 ppm (Kasai et al. 2002; Templin et al. 1996c). In a 13-week study, mortality was observed in male mice at ≥12 ppm (Kasai et al. 2002). Due to high mortality, longer-duration inhalation studies in male mice utilized a step-up exposure paradigm to slowly increase exposure concentration from 5 to 90 ppm over the course of 6 weeks to prevent early mortality. Using this approach, no exposure-related mortalities were observed in male mice at time-weighted average (TWA) concentrations up to 55 ppm for 13 weeks (Templin et al. 1998) or 85.8 ppm for 104 weeks (Yamamoto et al. 2002). In female mice, no exposure-related mortalities were observed at concentrations up to 90 ppm for 3 weeks (Larson et al. 1996), 200 ppm for 13 weeks (Kasai et al. 2002), or a TWA concentration of 85.8 ppm for 104 weeks (Yamamoto et al. 2002).

In oral studies, LD_{50} (lethal dose, 50% kill) values for chloroform ranged from 908 to 2,180 mg/kg in adult rats (Chu et al. 1982b; Kimura et al. 1971; Smyth et al. 1962; Torkelson et al. 1976) and from 550 to 1,400 mg/kg in adult mice (Bowman et al. 1978; Ewaid et al. 2020). Kimura et al. (1971) reported increased susceptibility in neonatal rats (14 days old) compared to adult rats (LD_{50} values of 445 and 1,187 mg/kg, respectively). Decreased survival was observed in male rats exposed to 2,000 mg/kg/day for 3 days via gavage (Wada et al. 2015). In other acute-duration studies, treatment-related deaths were observed in mice exposed to a single gavage dose ≥750 mg/kg (Jones et al. 1958; Philip et al. 2006), drinking water doses ≥138 mg/kg/day for 4 days (Larson et al. 1994d), or gavage doses ≥250 mg/kg/day for 14 days (NTP 1988a). In pregnant animals, increased mortality was observed at gavage doses of 516 mg/kg/day in rats and ≥63 mg/kg/day in rabbits (Thompson et al. 1974).

In intermediate-duration studies in rats, increased mortality was observed at drinking water concentrations ≥175 mg/kg/day for 90 days (Chu et al. 1982a). Histopathological examination revealed atrophy of the liver and extensive squamous debris in the esophagus and gastric cardia, suggesting to the study authors that the rats had died of starvation. No exposure-related deaths were observed in rats similarly exposed to drinking water concentrations up to 193 mg/kg/day for 28 days (Chu et al. 1982b) or 160 mg/kg/day for 3–10 months (EPA 1980; Hooth et al. 2002;). In mice, increased mortality was observed at drinking water concentrations of 400 ppm for 60 days (Balster and Borzelleca 1982). However, another study did not observe increased mortality in mice exposed to drinking water doses up to 435 mg/kg/day for 90 days (EPA 1980). Exposure to chloroform via gavage in toothpaste for 6 weeks caused increased mortality in male mice at ≥150 mg/kg/day and in female mice at 425 mg/kg/day (Roe et al. 1979). In other gavage

studies (water or oil vehicle), no exposure-related deaths were observed in mice at doses up to 300 mg/kg/day for 21–30 days (Anand et al. 2006; Larson et al. 1994d), up to 2,376 mg/kg/day for 30 days (Eschenbrenner and Miller 1945), or up to 250 mg/kg/day for 90 days (Munson et al. 1982).

In chronic-duration studies, decreased survival was observed in rats exposed to doses ≥90 mg/kg/day via gavage in oil for 78 weeks (NCI 1976). In similarly exposed mice from the same study, survival was decreased in females at 477 mg/kg/day but was not affected at males at doses up to 277 mg/kg/day (highest dose tested in males). In drinking water studies, no treatment-related increase in mortality was observed at concentrations up to 160 mg/kg/day in rats (Jorgenson et al. 1985; Nagano et al. 2006) or 263 mg/kg/day in mice (Jorgenson et al. 1985). No exposure-related deaths were observed in dogs exposed to chloroform via capsule for 80 weeks (Heywood et al. 1979).

2.3 BODY WEIGHT

No data were located regarding body weight effects in humans after exposure to chloroform. Decreased body weight has frequently been reported in animals exposed to chloroform via inhalation or oral routes; however, there are some inconsistencies in the database. In many cases, body weight effects may be due in part to decreased food and/or water intake resulting from CNS depression. Additionally, there is some evidence of palatability issues when chloroform is administered via drinking water. These confounding factors may contribute to observed inconsistencies across studies and must be considered when interpreting the data.

In rats, body weight or body weight gain decreases were consistently observed following inhalation exposure to acute-duration concentrations ≥271 ppm (Larson et al. 1994c; Templin et al. 1996b). In intermediate-duration studies in rats, decreased body weight gains were observed in females at ≥30 ppm for 3 weeks (Templin et al. 1996b), males at ≥90 ppm for 3 or 6 weeks (Templin et al. 1996b), both sexes at ≥30 ppm for 13 weeks (Kasai et al. 2002; Templin et al. 1996b), and males at ≥50 ppm for 6 months (Templin et al. 1976). Effects were often severe (>20% decrease in body weight or body weight loss) at concentrations ≥271 ppm for all durations. However, no body weight effects were noted in male rats exposed to concentrations up to 100 ppm for 104 weeks (Nagano et al. 2006).

Body weight effects were observed less consistently in mice following inhalation exposure to chloroform. Templin et al. (1996c) reported body weight loss in male mice following exposure to concentrations ≥30 ppm for 4 or 14 days; however, another 4-day study did not observe body weight effects at

concentrations up to 92 ppm in male mice (Constan et al. 1999). No exposure-related decreases in body weight or body weight gain were observed in female mice exposed to concentrations up to 90 ppm for 4 days or 288 ppm for 7 days (Larson et al. 1994c; Templin et al. 1996c). Body weights were comparable to control in male and female mice exposed to 7 ppm for 5 days (de Oliveira et al. 2015). In intermediate-duration exposure studies, no body weight effects were noted in mice following exposure to concentrations up to approximately 90 ppm for 3 or 6 weeks (Larson et al. 1996; Templin et al. 1998) or 26 ppm for 7 weeks (Templin et al. 1998). Two 13-week studies in mice reported an absence of body weight effects in either sex at concentrations up to 88 ppm (Larson et al. 1996) or 200 ppm (Kasai et al. 2002). However, Templin et al. (1998) reported decreased body weight gain in male mice exposed to concentrations ≥5 ppm for 13 weeks; similarly exposed females in this study did not show decreased body weight gain at concentrations up to 90 ppm. In the only chronic-duration study, body weight decreases were observed in male and female mice at 85.8 ppm (Yamamoto et al. 2002).

In acute-duration oral studies, rodents were more sensitive to body weight effects following drinking water exposure compared to gavage, potentially due to concurrent decreases in water intake associated with unpalatability. Decreased water intake may influence body weight gain, even at levels not associated with overt dehydration (Vasilev et al. 2021). This is demonstrated most clearly in a series of 4-day drinking water and gavage studies in rats and mice by Larson et al. (1994b, 1994d, 1995a, 1995b). In gavage studies, only female rats showed decreased body weight following exposure to 400 mg/kg/day. No body weight effects were noted at gavage doses up to 180 mg/kg/day in male rats (highest dose tested), 200 mg/kg/day in female rats, or 477 mg/kg/day in male or female mice (highest dose tested). In contrast, decreased body weights along with decreased water intake were observed in 4-day drinking water studies in male rats exposed to 57.5 mg/kg/day and female mice exposed to 81 mg/kg/day, respectively, for 4 days (female rats and male mice were not evaluated in the drinking water studies).

In other acute-duration gavage studies in rats, decreased body weight or body weight gain was observed in males following exposure to 358.2 mg/kg once (Lilly et al. 1997) or 500 mg/kg/day for 3 days (Wada et al. 2015). Additionally, severe emaciation was reported for all rats (3/3) dosed with 1,000 mg/kg/day for 3 days (Wada et al. 2015). However, no body weight effects were noted in other gavage studies in rats at single doses up to 1,500 mg/kg (Chu et al. 1982b; Keegan et al. 1998; Larson et al. 1993; Templin et al. 1996a) or doses up to 179 mg/kg/day for 7 days (Potter et al. 1996). In mice, body weight losses of 20% were observed 7–14 days after a single gavage exposure to ≥700 mg/kg (Ewaid et al. 2020). No exposure-related changes in body weight were observed in mice exposed to gavage doses up to 477 mg/kg/day for 4 days (Larson et al. 1994b, 1994d). In 14-day gavage studies, body weight decreases

were observed in males at ≥250 mg/kg/day, but not in females at doses up to 500 mg/kg/day (Munson et al. 1982; NTP 1988a).

The apparent increase in sensitivity in acute studies via drinking water, compared to gavage exposure, was not clearly observed in longer-duration studies. In a series of 3-week studies by Larson at al. (1994b, 1995b), decreased body weights were observed in male rats at administered doses >100 mg/kg/day via drinking water or gavage administration, but not females at gavage doses up to 400 mg/kg/day (females not evaluated in the drinking water study). In mice, decreased body weights were observed in males exposed to 277 mg/kg/day via gavage, but not in female mice exposed to concentrations up to 477 mg/kg/day via gavage or 329 mg/kg/day via drinking water (Larson et al. 1994b, 1994d).

Body weight decreases were reported inconsistently in additional gavage studies. In intermediate-duration studies, no changes were observed in rats at doses up to 37 mg/kg/day for 4 weeks (Müller et al. 1997) or in mice at doses up to 477 mg/kg/day for 3 weeks (Melnick et al. 1998), 250 mg/kg/day for 90 days (Munson et al. 1982), 41 mg/kg/day for 105 days (NTP 1988a), or 240 mg/kg/day for 26 weeks (Sehata et al. 2002). However, some mouse studies reported decreased body weight or decreased body weight gain following intermediate-duration gavage exposure, including decreases in both sexes at ≥60 mg/kg/day for 6 weeks (Roe et al. 1979), in males at ≥130 mg/kg/day for 90 days (Bull et al. 1986), and in females at 270 mg/kg/day for 90 days (Bull et al. 1986). In chronic-duration studies, decreased body weights were observed in rats following gavage exposure to ≥90 mg/kg/day (NCI 1976), but no adverse effects on body weights were observed in mice at chronic doses up to 277 mg/kg/day in males or 477 mg/kg/day in females (Jorgenson et al. 1985; NCI 1976; Roe et al. 1979). No adverse effects were noted in dogs exposed to doses up to 30 mg/kg/day via capsule for up to 7.5 years (Heywood et al. 1979).

In intermediate- or chronic-duration drinking water studies in rats, doses ≥160 mg/kg/day for 13 weeks or ≥45 mg/kg/day for ≥2 years resulted in decreased body weights in rats (Chu et al. 1982a; EPA 1980;Nagano et al. 1006; Tumasonis et al. 1985, 1987). However, in a chronic-duration study that included water-matched controls for animals exposed to 160 mg/kg/day, body weight decreases were only observed in animals compared to *ad libitum* water controls (Jorgenson et al. 1985). No body weight effects were noted in rats at doses up to 193 mg/kg/day for 28 days (Chu et al. 1982b), 81 mg/kg/day for 13 weeks (Chu et al. 1982a; DeAngelo et al. 2002; EPA 1980), or 35 mg/kg/day for 26 weeks (Geter et al. 2004b). In mice, no adverse effects on body weight were noted following intermediate-duration exposure to drinking water doses up to 435 mg/kg/day (Auttachoat et al. 2009; DeAngelo et al. 2002; EPA 1980; Pereira 1994); no chronic-duration drinking water studies were identified in mice.

In pregnant animals, decreased maternal body weight gain was observed in rats following inhalation exposure to concentrations ≥119 ppm for 7 hours/day for 10 days during gestation (Baeder and Hofmann 1988; Schwetz et al. 1974). When exposure was only 1 hour/day for 8 days, decreased maternal body weight gain was not observed until 4,117 ppm (EPA 1978). In mice, decreased maternal body weight gain was observed after exposure to 97 ppm from gestation days (GDs) 1–7 or 8–15; however, the adversity of findings could not be determined because the magnitude of effect was not reported and findings were associated with decreased food and water intake (Murray et al. 1979). Exposure to 99 ppm on GDs 6–15 ppm did not result in significant decreases in maternal body weight gain (Murray et al. 1979). In oral studies, decreased maternal body weight gain was observed in rats and rabbits following gavage doses ≥50 mg/kg/day during gestation (Ruddick et al. 1983; Thompson et al. 1974).

In a dermal acute-duration lethality study, weight loss of an unspecified magnitude was reported in rabbits following exposure to doses ≥1,000 mg/kg for 24 hours under occluded conditions (Torkelson et al. 1976).

2.4 RESPIRATORY

In animals, the respiratory tract, particularly the nasal cavity, is a sensitive target of chloroform toxicity following both inhalation and oral exposure. Based upon systematic review (Appendix C), the respiratory system is a presumed target of chloroform toxicity based on inadequate evidence in human epidemiology studies and a high level of evidence in laboratory animal studies.

A limited number of human studies have evaluated potential associations between chloroform exposure and respiratory effects. A large case-review of 1,502 surgical patients undergoing chloroform anesthesia reported increased respiratory rates in 44% of patients (Whitaker and Jones 1965). This increase was found more frequently in patients with shorter duration of anesthesia (up to 1 hour). Respiratory depression was sometimes observed in patients that underwent longer and deeper anesthesia (often with co-administration of morphine or thiopentone). Chloroform exposure levels were not reported; however, the study authors indicate that none of the exposures exceeded 22,500 ppm. As discussed in Section 2.2 (Death), cases of fatal inhalation or oral exposure to chloroform often report respiratory arrest and/or asphyxiation, and have shown lung congestion and edema and erosion, hyperemia, and submucosal hemorrhage of the trachea and bronchi at autopsy (Ago et al. 2011; Featherstone 1947; Giusti and Chiarotti 1981; Harada et al. 1997; Piersol et al. 1933; Royston 1924; Schroeder 1965). Hypoxia and

increased respiratory rate followed by respiratory depression/failure have been reported in nonlethal cases of oral chloroform exposure (Cui et al. 2022; Jayaweera et al. 2017; Storms 1973).

One study evaluated potential respiratory effects of combined inhalation and dermal exposure to chloroform from swimming in an indoor chlorinated pool for 40 minutes (Font-Ribera et al. 2010). Median pool water and indoor air concentrations of chloroform were 16.7 µg/L and 21.4 µg/m^3 (4.38 ppb), respectively. The mean levels of chloroform in pre-swim and post-swim exhaled breath from 48 adult swimmers was 0.72 and 4.5 µg/m^3, respectively. Post-swim exhaled breath chloroform levels were not associated with measures of lung function or biomarkers of airway inflammation in exhaled breath. In a cross-sectional study using 2005–2012 NHANES data, Sun et al. (2022) did not identify an association between blood chloroform levels and risk of current asthma symptoms (chest wheezing or whistling in the past 12 months) or ever (lifetime) asthma (physician-diagnosed) in 2,359 adolescents (12–19 years of age). No data on actual exposure scenarios were reported by Sun et al. (2022); however, the study authors noted that humans are exposed to disinfection byproducts (including chloroform) in chlorinated water via ingestion and via dermal and inhalation routes during water use activities (e.g., showering, bathing, swimming).

In rats, nonneoplastic lesions in the nasal cavity and/or nasal bone proliferation were consistently reported after inhalation exposure to concentrations ≥10 ppm for acute durations (Kasai et al. 2002; Larson et al. 1994c; Mery et al. 1994; Templin et al. 1996b), intermediate durations (Kasai et al. 2002; Templin et al. 1996b), and chronic durations (Yamamoto et al. 2002). Findings after acute-duration exposures included complex morphological changes in the lamina propria of the ethmoid turbinates in areas lined by olfactory epithelium involving edema, atrophy of Bowman's (olfactory) glands, new bone formation, and proliferation of periosteal cells. With increasing duration and concentration, this progressed to atrophy of the ethmoid turbinates and overlying olfactory epithelium, necrosis of the olfactory epithelium, respiratory metaplasia of the olfactory epithelium, mineralization of the ethmoturbinate, and thickening of bone in the nasal septum.

Findings similar to the nasal lesions in rats were observed in mice following inhalation exposure to acute-duration concentrations ≥10 ppm (Constan et al. 1999; Larson et al. 1994c, 1996; Mery et al. 1994). However, inconsistencies were observed following intermediate-duration inhalation exposure in mice. One study reported thickening of nasal bones and eosinophilic changes in the olfactory and respiratory nasal epithelia after exposure to ≥12 ppm for 13 weeks (Kasai et al. 2002), while another reported no nasal effects in mice at concentrations up to 88 ppm for 3–13 weeks (Larson et al. 1996). In the only

chronic-duration study identified, thickening of the nasal bones was observed in mice ≥5.0 ppm, with atrophy and respiratory metaplasia of the olfactory epithelium in male mice at 85.8 ppm and in female mice at ≥5.0 ppm (Yamamoto et al. 2002).

Rats exposed to chloroform via gavage also developed dose-related nasal lesions generally similar to those described for inhalation exposure (early phases of new bone formation, periosteal hypercellularity, and degeneration followed by regeneration of the olfactory epithelium and superficial Bowman's glands in the ethmoid portion of the nasal passages lined by olfactory epithelium). The lowest LOAELs identified ranged from 34 to 100 mg/kg/day in the different studies, which ranged in duration from single dose to 3 weeks (Dorman et al. 1997; Larson et al. 1995b; Templin et al. 1996a). Despite the observed nasal lesions, no change in odor-cued avoidance behavior was seen in the rats, suggesting that olfactory function was not affected (Dorman et al. 1997).

Damage to the lower respiratory tract in animals was predominantly seen at lethal exposure levels. As was seen in human fatalities associated with chloroform exposure, lung inflammation and congestion were observed in rats that died following acute-duration inhalation exposure to ≥2,000 ppm (Kasai et al. 2002) or oral exposure to ≥1,120 mg/kg (Bowman et al. 1978). Following chronic-duration exposure to ≥90 mg/kg/day, increased mortality in rats was associated with wheezing and increased incidence and severity of inflammatory lesions in the lungs (NCI 1976).

Evidence for lower respiratory tract damage at nonlethal exposure levels in animals are limited. One inhalation study reported morphometric changes in the lungs of male and female mice exposed to 7 ppm for 5 days, including increased alveolar area and decreased volume density of alveolar septa (de Oliveira et al. 2015). Additional findings in this study included increased total leukocytes and macrophages in bronchioalveolar lavage fluid (BALF) of both sexes, increased lymphocytes and neutrophils in BALF of males, and increased relative lung weight in female mice. However, no histopathological changes to the lungs were observed following intermediate- or chronic-duration inhalation exposure to concentrations up to approximately 90 ppm in rats or mice (Larson et al. 1996; Yamamoto et al. 2002). In oral studies, one study reported increased incidence of bronchiolar epithelium (Clara cell) degeneration in the lungs of male and female mice treated with chloroform at 140–240 mg/kg/day by gavage in oil for 26 weeks relative to controls (Sehata et al. 2002). However, no histopathological changes to the lungs were observed following intermediate-duration exposure to doses up to 200 mg/kg/day in rats (Chu et al. 1982a, 1982b; EPA 1980), intermediate-duration exposure to doses up to 435 mg/kg/day in mice (EPA 1980; NTP 1988a), or chronic-duration exposure to doses up to 477 mg/kg/day in mice (NCI 1976).

Mechanisms of Respiratory Toxicity. The respiratory failure observed in patients under chloroform anesthesia was probably due to a direct effect of chloroform on the respiratory center of the CNS system. A decline of the systolic pressure in the cerebral vessels may also contribute to respiratory failure, as demonstrated in animals: when respiration had stopped under chloroform anesthesia, the animals (species not specified) breathed again if positioned head down (Featherstone 1947). Destruction of the surfactant monolayer may also contribute to severe respiratory effects, as it has been demonstrated that chloroform has a destructive influence on the pulmonary surfactant (Enhorning et al. 1986). This effect is probably due to the solubility of phospholipids in the surfactant monolayer that can cause collapse of the respiratory bronchiole due to the sudden increase in inhalation tension.

The mechanism of chloroform-induced nasal toxicity appears to involve metabolism to reactive intermediates. Studies using CYP2E1 knock-out mice and mice pretreated with the cytochrome P450 inhibitor, 1-aminobenzotriazole, showed that CYP2E1 metabolism is required for chloroform to produce nasal effects (either proliferation or lesions) (Constan et al. 1999). In animal studies, the occurrence of nasal lesions after both inhalation and gavage administration suggests a systemic mechanism of action for chloroform-induced nasal toxicity.

2.5 CARDIOVASCULAR

A limited number of human studies have evaluated potential associations between chloroform exposure and cardiovascular effects. One occupational study reported increased subjective complaints of palpitations in a group of workers exposed to chloroform at a geometric mean of 4.19 ppm for 1–15 years, compared to a small group of unexposed controls (Li et al. 1993). No additional cardiovascular endpoints were examined, and no confounders were considered in the analysis. Large case-reviews of surgical patients undergoing chloroform anesthesia have reported cardiac arrhythmia, bradycardia, and hypotension (Smith et al. 1973; Whitaker and Jones 1965). Chloroform exposure levels were not reported; however, the uppermost exposure levels were reportedly 20,000–22,500 ppm. As discussed in Section 2.2 (Death), some cases of fatal inhalation or oral exposure to chloroform have attributed death to acute heart failure (Ago et al. 2011; Harada et al. 1997; Royston 1924; Schroeder 1965). Cardiac effects have also been reported following near-fatal inhalation or oral exposures, including cardiac arrest, arrhythmia, tachycardia, and hypotension (Choi et al. 2006; Gosselink et al. 2012; Greene and White 2014; Hutchens and Kung 1985; Jayaweera et al. 2017; Storms 1973). In a cross-sectional study of 15,135 adults using 1999–2018 NHANES data, blood chloroform levels were not related to hypertension,

defined as self-reported physician's diagnosis of hypertension, use of antihypertensive medication, or systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg (Zhang et al. 2023).

No studies were located regarding cardiovascular function (e.g., blood pressure, heart rate) in animals following inhalation exposure to chloroform. No exposure-related changes in heart weight or histology were observed at intermediate-duration inhalation exposures up to 300 ppm in rats (Templin et al. 1996b) or 88 ppm in mice (Larson et al. 1996) or chronic-duration exposures up to approximately 90 ppm in rats or mice (Yamamoto et al. 2002).

Cardiovascular function was examined in rats following oral exposure by Müller et al. (1997), who observed decreased heart rate, increased blood pressure, and altered cardiac parameters (e.g., prolonged PR-interval and extended atrioventricular conduction and intraventricular extension times) in both conscious and urethane-anesthetized rats given chloroform as a single gavage dose of 149 mg/kg or as daily gavage doses of 37 mg/kg/day for 4 weeks. No other studies of cardiac function after oral exposure to chloroform were identified.

No exposure-related changes in heart weight or histology were observed at intermediate-duration oral exposures up 200 mg/kg/day in rats (Chu et al. 1982a, 1982b) or 240 mg/kg/day in mice (Sehata et al. 2002). In chronic-duration studies, cardiac atrial thrombosis was observed in 9/41 high-dose female mice exposed to 477 mg/kg/day for up to 78 weeks, which may have contributed to increased death rate in this group; conversely, thrombosis may have been secondary to concurrent hepatocellular carcinoma (NCI 1976). No histopathological changes were noted in the hearts of similarly exposed rats at doses up 200 mg/kg/day or male mice at doses up to 277 mg/kg/day (NCI 1976). In dogs, chronic-duration oral exposure to chloroform via capsule was not associated with histopathological changes in the heart at doses up to 30 mg/kg/day (Heywood et al. 1979).

Mechanisms of Cardiovascular Toxicity. While CNS depression may contribute to observed cardiovascular collapse in humans following exposure to high levels of chloroform, mechanistic data indicate chloroform may also have direct action on cardiovascular tissue and function. In guinea pig heart-lung preparations, chloroform caused structural damage of the transverse tubular system and is accompanied by increased storage of adenosine triphosphate (ATP) and phosphocreatine, resulting in a permanent contractile failure of the heart (Doring 1975). Damage is likely due to interference with the lipid arrangement of the transverse tubular walls (similar to the lipophilic membrane perturbation mechanism of action proposed for neurotoxicity, discussed in Section 2.15). In isolated rat hearts,

chloroform exposure caused bradycardia and ventricular fibrillation (Zhou et al. 2011). Additional *in vitro* studies also show that chloroform is cytotoxic to rat cardiomyocytes and may block intercellular communication via incorporation into the cell membrane near gap junctions (El-Shenawy and Abdel-Rahman 1993; Toraason et al. 1992). Chloroform also blocks cardiac ion channels transfected into transfected human embryonic kidney (HEK 293) cells or *Xenopus* oocytes, including the human *ether-à-go-go*-related gene (HERG) potassium channels, which is implicated in proarrhythmia in cardiac and noncardiac drugs (Scholz et al. 2006; Zhou et al. 2011). It is unknown if proposed cytotoxic and altered cellular communication mechanisms of toxicity are CYP2E1-mediated (reliant on metabolism to reactive metabolites).

2.6 GASTROINTESTINAL

Nausea and vomiting have been frequently observed side effects in patients exposed to high concentrations of chloroform via anesthesia (Royston 1924; Smith et al. 1973; Townsend 1939; Whitaker and Jones 1965). In small occupational hygiene studies, nausea and vomiting were reported in some workers exposed to concentrations ranging from 2 to 400 ppm for months or years (Bomski et al. 1967; Challen et al. 1958; Phoon et al. 1983). In both patients and workers exposed to chloroform via inhalation, observed effects are likely secondary to concurrent depression of the CNS system and/or toxic hepatitis. However, erosion of the stomach and upper jejunum were reported in a man who committed suicide via intentional inhalation of chloroform (Ago et al. 2011). Vomiting, gastric distress, pain, and severe damage to the lining of the gastrointestinal system have also been observed in case studies of patients who intentionally or accidentally ingested high doses of chloroform (Hakim et al. 1992; Jayaweera et al. 2017; Piersol et al. 1933; Schroeder 1965). Case reports of nonfatal inhalation and dermal exposure have also reported nausea and/or vomiting (Dettling et al. 2016; Vlad et al. 2014).

In animal inhalation studies, chloroform did not cause histopathological changes in the gastrointestinal system in rats or mice following intermediate-duration exposure to concentrations up to 300 or 88 ppm, respectively (Larson et al. 1996; Templin et al. 1996b), or following chronic-duration exposure to concentrations up to 90.1 or 85.8 ppm, respectively (Yamamoto et al. 2002).

In acute-duration oral exposure studies, gastric erosion was observed in pregnant rats exposed to 516 mg/kg/day via gavage for 10 days during gestation (Thompson et al. 1974). Gastrointestinal lesions were not reported in pregnant rabbits similarly treated with gavage doses up to 398 mg/kg/day; however, diarrhea was observed (Thompson et al. 1974). No lesions were observed in the glandular stomach of

male rats exposed to 500 mg/kg/day for 3 days via gavage (Wada et al. 2015). No additional acute-duration oral studies evaluated the gastrointestinal system or reported clinical signs of gastric distress.

In an intermediate-duration oral study in Eker rats (animal model of hereditary renal cancer), aberrant crypt foci (an early putative preneoplastic lesion of colon neoplasia) were observed in nearly all males exposed to ≥27 mg/kg/day via drinking water for 10 months; these findings were not observed in similarly exposed female rats at doses up to 158 mg/kg/day (McDorman et al. 2003b). The incidence of aberrant crypt foci in the colon was not increased relative to controls in male F344 rats or B6C3F1 mice exposed to 34 or 89 mg/kg/day, respectively, in drinking water for 13 weeks (DeAngelo et al. 2002) or in male F344 rats exposed to drinking water concentrations up to 35 mg/kg/day for 26 weeks (Geter et al. 2004b). In other oral studies, no histopathological changes in the gastrointestinal system were found in rats or mice following intermediate-duration exposure to drinking water doses up to 200 or 435 mg/kg/day, respectively (Chu et al. 1982a, 1982b; EPA 1980) or chronic-duration gavage doses up to 200 or 477 mg/kg/day, respectively (NCI 1976).

No increase in aberrant crypt foci formation was seen in the colon of rats that drank up to 35 mg/kg/day of chloroform for 26 weeks (Geter et al. 2004b).

2.7 HEMATOLOGICAL

Data pertaining to potential hematological effects in humans following exposure to chloroform are very limited. In a case-review of 58 surgical patients undergoing chloroform anesthesia (up to 20,000 ppm) by Smith et al. (1973), prothrombin time was measured as a test of liver function (prothrombin is formed in the liver). The study authors found a significant increase in prothrombin time in patients at both 4 and 24 hours post-anesthesia, relative to pre-treatment values, possibly reflecting hepatotoxicity of the chemical. Other hematological endpoints were not assessed in this case series.

Massive hemolysis was reported in a case of attempted suicide via inhalation of an unknown level of chloroform (Gosselink et al. 2012). Prolonged prothrombin time was noted in a woman who attempted suicide via ingestion of chloroform (Choi et al. 2006). In both attempted suicides, patients made a full recovery. In an oral case study of chronic-duration exposure, decreased erythrocytes and hemoglobin were observed in a subject who ingested approximately 21 mg/kg/day chloroform in cough medicine for 10 years (Wallace 1950). Levels of erythrocytes and hemoglobin returned to normal within 4–6 months of cessation of exposure and adjustment of diet and sleep habits.

In inhalation studies in animals, no exposure-related changes in hematological parameters were observed in rats exposed to concentrations up to 85 ppm for 6 months (Torkelson et al. 1976) or in rats or mice exposed to concentrations up to 90.1 or 85.8 ppm, respectively, for 104 weeks (Yamamoto et al. 2002).

In oral studies, there is limited and inconsistent evidence of changes in blood hematology in rats following exposure to chloroform. In acute-duration gavage studies, red cell parameters (hemoglobin, hematocrit, and/or red blood cell counts) were decreased in pregnant female rats at ≥100 mg/kg/day (Ruddick et al. 1983) and male and nonpregnant female rats at ≥546 mg/kg (Chu et al. 1982b). In nonpregnant females, a decrease in lymphocytes was also observed at ≥1,071 mg/kg (Chu et al. 1982b). However, evidence for hematological effects in rats following intermediate-duration oral exposure to chloroform is limited to decreased neutrophils in male rats exposed to 193 mg/kg/day via drinking water (Chu et al. 1982b) and increased cellular proliferation in the bone marrow in rats exposed to 410 mg/kg/day via oral administration in a toothpaste vehicle (Palmer et al. 1979). In other intermediate-duration studies, no adverse changes in hematological blood indices were noted in male or female rats at drinking water doses up to 150 mg/kg/day for 90 days (Chu et al. 1982a), and no histopathological changes in hematopoietic tissues were observed in rats exposed to drinking water doses up to 160 mg/kg/day for 13 weeks (EPA 1980). Similarly, no histopathological changes in hematopoietic tissues were observed in rats exposed to gavage doses up to 200 mg/kg/day for 104 weeks (NCI 1976).

In other species, there is no evidence of adverse hematological effects following oral exposure to chloroform. In mice, no changes in blood parameters were observed at acute- or intermediate-duration doses up to 250 mg/kg/day (Auttachoat et al. 2009; Munson et al. 1982; Sehata et al. 2002). After chronic-duration exposure of mice, no hematological effects were observed at 17 mg/kg/day, and the only observed change at 60 mg/kg/day was a decrease in hematocrit (Roe et al. 1979). Additionally, no histopathological changes in hematopoietic tissues were observed in mice exposed to drinking water doses up to 435 mg/kg/day for 13 weeks (EPA 1980) or gavage doses up to 200 mg/kg/day for 104 weeks (NCI 1976). In dogs, no adverse hematological effects were noted following oral exposure to 30 mg/kg/day via capsule for up to 7.5 years (Heywood et al. 1979).

2.8 MUSCULOSKELETAL

Human data pertaining to potential musculoskeletal effects associated with chloroform exposure are limited. As discussed in Section 2.2. (Death), rhabdomyolysis (destruction of striated muscle) was listed

as a cause of death, along with acute liver failure, in a woman that repeatedly inhaled an unknown level of chloroform and abused alcohol over a 6-day period (Lionte 2010). Rhabdomyolysis was also reported following inhalation exposure to unknown levels of chloroform following an occupational accident and an attempted suicide; in both cases, the patients made full recoveries (Gosselink et al. 2012; Meenakshisundaram et al. 2021). In a case report of accidental ingestion of approximately 2,410 mg/kg of chloroform, muscular relaxation of the jaw resulting in upper respiratory obstruction was observed, presumably secondary to an effect on the nervous system (Schroeder 1965).

In a cross-sectional study of 2005–2012 NHANES data evaluating potential associations between trihalomethanes and bone density, blood chloroform levels >16.30 pg/mL were associated with decreased lumbar spine bone mineral densities in 2,210 adolescents (12–19 years of age) (Sun et al. 2023a). The study authors stated that exposure to trihalomethanes is from water-use activities and suggested that chloroform levels in blood were due to exposure to disinfection byproducts in blood via water usage activities, including drinking, showering, and swimming. The concentration of chloroform in a single household tap water sample was determined but no additional exposure assessment was completed. The concentrations of chloroform in blood and water were significantly correlated.

As discussed in Section 2.4 (Respiratory), new nasal bone formation and/or periosteal hypercellularity were consistently reported in rodents following inhalation or gavage exposure to chloroform. These proliferative bone findings are likely in response to concurrent histopathological damage to the epithelial tissues lining the nasal cavity in both inhalation and oral studies. Therefore, these findings are considered respiratory effects, rather than musculoskeletal effects.

No exposure-related changes in skeletal muscle histology, non-nasal bone histology, or non-nasal bone cell proliferation were observed in rats or mice following intermediate-duration inhalation exposure to concentrations up to 300 or 88 ppm, respectively (Larson et al. 1996; Templin et al. 1996b), or chronic-duration inhalation exposure to concentrations up to 90.1 or 85.8 ppm, respectively (Yamamoto et al. 2002). In oral studies, no exposure-related, non-nasal musculoskeletal effects were observed at intermediate- or chronic-duration doses up to 200 mg/kg/day in rats (Chu et al. 1982a, 1982b; NCI 1976) or chronic-duration doses up to 477 mg/kg/day in mice (NCI 1976).

2.9 HEPATIC

Hepatotoxicity is one of the major toxic effects observed in both humans and animals after inhalation exposure to chloroform. Based upon systematic review (Appendix C), the liver is a known target of chloroform toxicity based on a low level of evidence from human epidemiological studies, a high level of evidence from animal studies, and other relevant data consisting of the extensive database of case reports and case series documenting hepatic effects of chloroform in exposed humans.

Data pertaining to hepatic effects in humans following exposure to chloroform have been reported in several epidemiological studies (Table 2-4) and numerous case reports. Hepatic effects have been reported in some surgical patients following chloroform-induced anesthesia. In a case series of 58 surgical patients undergoing anesthesia (maximum exposure level of 20,000 ppm), Smith et al. (1973) reported an increase in postsurgical levels of serum total bilirubin and lactate dehydrogenase (LDH), as well as bromosulfalein retention (measure of hepatic function), compared to pre-surgical levels. An increase in prothrombin time was also considered indicative of hepatotoxicity since prothrombin is produced in the liver. No post-surgical changes were observed in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), or alkaline phosphatase (ALP). Another large case series of surgical patients undergoing anesthesia (maximum exposure level of 25,000 ppm) reported jaundice in 1/1,502 cases; no other measures of hepatic function were discussed (Whitaker and Jones 1965). Several early case reports report delayed hepatotoxicity, characterized by liver enlargement and/or jaundice, in women exposed to chloroform via anesthesia during childbirth (Lunt 1953; Royston 1924; Townsend 1939). Centrilobular necrosis was found at autopsy in fatal cases (Royston 1924; Townsend 1939).

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Hepatic Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Surgical exposure			
Smith et al. 1973 Case series; 58 patients undergoing anesthesia for surgery; mean age of 35.68 years (Georgia)	Maximum inspired chloroform concentration: 20,000 ppm Mean (range) arterial blood chloroform level: 9.8 (7–16.2) mg/100 mL Average duration of surgery: 113 minutes	Measures of liver function	
		Bromosulfalein retention	↑
		ALP, ALT, AST	↔
		Total bilirubin	↑
		Total cholesterol	↔
		LDH	↑
		Prothrombin time	↑

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Hepatic Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Whitaker and Jones 1965 Case series; 1,502 patients undergoing anesthesia for surgery; 1–80+ years of age (South Africa)	Maximum inspired chloroform concentration: 25,000 ppm Duration of surgery: ≤30 minutes (n=1,164) 31–60 minutes (n=168) 61–120 minutes (n=146) >120 minutes (n=34)	Jaundice	↔
Occupational exposure			
Bomski et al. 1967 Cohort; 68 workers currently exposed to chloroform for 1–4 years (mean age 25 years), 39 workers previously exposed to chloroform, 23 unexposed workers with history of viral hepatitis (age 25–35 years), 165 unexposed workers without history of viral hepatitis (Poland)	Range of chloroform levels in production area: 2–205 ppm	Liver disease (enlarged liver, toxic hepatitis, fatty liver)	↑ (current exposure versus unexposed)
		Serum ALT, AST	↔
		Bromosulfalein retention	↑ (current exposure versus unexposed)
Challen et al. 1958 Cohort; 8 long-term workers (mean 5.4 service years; mean 50.5 years of age), 9 short-term workers (mean 15 service months; mean 42.9 years of age), and 5 unexposed controls (mean 51.4 years of age) (England)	Range of chloroform levels during current operations with ventilation system (ppm) Mixing room: 128–1,163 Cutting room: 23–71 Range of chloroform levels under historical conditions without ventilation; relevant for long-term workers (ppm) Cutting room: 77–237	Liver disease (jaundice, enlarged liver)	↔
		Liver function tests (serum bilirubin, thymol turbidity)	↔
Li et al. 1993 Cohort; 61 workers exposed to chloroform for 1–15 years (mean of 7.8 years) and 23 unexposed controls; mean age of 36.02 and 36.83 years, respectively (China)	Geometric mean chloroform level: 4.19 ppm	Hepatomegaly	↔
		Serum ALT	↔

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Hepatic Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Phoon et al. 1983 Case series; 31 workers from two factories exposed to chloroform for <6 months; no other known chemical exposure (Singapore)	Range of chloroform levels (ppm): 1st outbreak (n=13): >400 (upper LOD) 2nd outbreak (n=18) 14.4–50.4 Range of blood chloroform levels (mg/100 mL): 1st outbreak: 0.10–0.29 2nd outbreak: not measured	Toxic hepatitis with jaundice	↑ (two occupational outbreaks; no control group)
General population exposure			
Aiking et al. 1994 Cohort; 10 competitive swimmers who trained in indoor chlorinated pools for ≥10 hours/week for a mean of 8.3 years (mean age of 18.6 years), 8 competitive swimmers who trained in outdoor chlorinated pools for ≥10 hours/week for a mean of 12.1 years (mean age of 20.9 years), and 12 athletic controls (competitive korfball players, mean age of 24.3 years) (Netherlands)	Mean chloroform levels in pool water during training session (µg/L): Indoor: 24 Outdoor: 18.4 Mean blood chloroform after training session of unspecified duration (µg/L): Indoor: 0.89 Outdoor: <0.5 (LOD) Controls: <0.5 (LOD)	Measured at the end of the training session: ALT, AST, GGT	↔ (indoor versus control) ↔ (outdoor versus control)

[a]Unless otherwise noted, current exposure levels are reported.

↑ = association; ↔ = no association; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; LDH = lactate dehydrogenase; LOD = limit of detection

As discussed in Section 2.2 (Death), acute liver failure was listed as a cause of death, along with rhabdomyolysis, in a woman who repeatedly inhaled an unknown level of chloroform and abused alcohol over a 6-day period (Lionte 2010). Centrilobular liver steatosis was observed upon autopsy in another case study of death following intentional inhalation of high levels of chloroform (Giusti and Chiarotti 1981). Toxic hepatitis has also been reported in nonlethal cases of forced or intentional inhalation of high levels of chloroform (Dettling et al. 2016; Gosselink et al. 2012; Hutchens and Kung 1985; Kang et al. 2014; Minor et al. 2018). Toxic hepatitis was also reported in an occupational case series from a Korean automotive parts manufacturing plant with exposure levels nearly 5 times the acceptable occupational

limit of 10 ppm for 8 hours TWA (Hwang and Kim 2022). Additional case reports of adverse liver effects have been reported following high accidental occupational exposures to chloroform, including elevated serum ALT, AST, and bilirubin levels, and acute liver injury (Meenakshisundaram et al. 2021; Suehiro et al. 2023).

There is limited evidence of liver disease following occupational exposure to chloroform. In general, findings from occupational studies need to be interpreted with caution due to numerous study limitations, including poor exposure characterization, small subject numbers, and lack of control for confounding factors (e.g., co-exposures). For more details on study quality, please refer to Appendix C.

In a Polish cohort of workers exposed to chloroform as a solvent for 1–4 years, current chloroform exposure levels (ranging from 2 to 205 ppm in the production area) was associated with an increased risk of liver disease, compared to unexposed workers (Bomski et al. 1967). Liver disease was characterized by enlarged liver in 25% of workers, toxic hepatitis in 5.6% of workers, and fatty liver in 20.6% of workers; some of these workers also had jaundice and elevated ALT and AST activity levels. However, neither ALT nor AST levels were directly associated with chloroform exposure. Decreased liver function, assessed via bromosulfalein retention, was also observed in exposed workers, compared to unexposed. The study authors indicated that there were only trace amounts of other solvents in the production area. Phoon et al. (1983) described two outbreaks of toxic hepatitis (with jaundice) in Singapore associated with occupational chloroform exposure for <6 months. The first outbreak (13 cases) consisted of workers from a single department of a large factory that used chloroform as a degreaser for welding machines. Measured chloroform levels in the affected department were >400 ppm (the upper limit of detection). The second outbreak (19 cases) consisted of workers from a casing department of a different factory that used chloroform as an adhesive. Measured chloroform levels in this department ranged from 14.4 to 50.4 ppm. No associations between hepatomegaly or serum ALT levels were observed in a Chinese cohort exposed to chloroform for 1–15 years at a geometric mean of 4.19 ppm (Li et al. 1993). Challen et al. (1958) also reported no associations between chloroform exposure and liver disease using measures of liver function in short-term or long-term workers exposed to 22–1,163 ppm for a mean duration of 15 months or 5.4 years, respectively. However, this study had very small subject numbers (8 long-term workers, 9 short-term workers, 5 controls). Additional cases of hepatotoxicity have been linked to occupational chloroform exposure to 34.24–82.74 ppm for 40–45 days (Kang et al. 2014) or estimated levels of 17.7 ppm for 2 weeks (Lin et al. 2005).

Aiking et al. (1994) evaluated the potential adverse hepatic effects in a small group of competitive swimmers exposed to chloroform for >10 hours/week for ≥5 years while swimming in indoor or outdoor chlorinated swimming pools. While dermal exposure was a consideration, the focus was on inhalation exposure to volatilized chloroform; however, air concentrations were not reported. Mean blood chloroform concentrations post-training were 0.89 µg/L in the indoor training environment and below the level of detection (0.5 µg/L) in the outdoor training environment. No significant differences in liver enzyme function (ALT, AST, gamma-glutamyl transferase [GGT]) were seen between competitive swimmers from either group or controls (competitive korfball players; a Dutch game similar to basketball).

Numerous case reports of ingestion of chloroform indicate that the liver is also a primary target of chloroform toxicity in humans following oral exposure. As discussed in Section 2.2. (Death), fatty degeneration and extensive centrilobular necrosis were observed during the autopsy of a fatal case of chloroform ingestion (Piersol et al. 1933). Jaundice, liver enlargement, and elevated levels of ALT, AST, LDH, and bilirubin were observed prior to death. Elevated serum liver enzymes were observed in a man who drank a large quantity of chloroform prior to death due to multisystem organ failure (Dettling et al. 2016). Similar clinical signs of hepatotoxicity were noted in numerous nonlethal cases of chloroform poisoning within 1–7 days of ingestion (Choi et al. 2006; Dell'Aglio et al. 2010; Hakim et al. 1992; Jayaweera et al. 2017; Kim 2008; Rao et al. 1993; Schroeder 1965; Sridhar et al. 2011; Storms 1973). Most cases showed a full recovery within a couple of weeks. Rao et al. (1993) reported that biomarkers of liver regeneration are key determinants of a favorable prognosis following acute toxicity, including des-γ-carboxy prothrombin, α-fetoprotein, retinol binding protein, and 5-glutamyl-peptide:amino-acid 5-glutamyltransferase. Increased bromosulfalein retention indicated impaired liver function in an individual who ingested 21 mg/kg/day chloroform in a cough medicine for 10 years (Wallace 1950). The changes reversed to normal after exposure was discontinued.

In a dermal case study, hepatic steatosis, jaundice, and elevated serum transaminases (not specified) were observed in a man 3 days after spilling chloroform on his shirt (Vlad et al. 2014). His liver function tests and transabdominal ultrasound were normal 8 weeks post-exposure.

The liver is a clear target of toxicity for chloroform in animal studies. There is clear and consistent evidence of dose-dependent increases in occurrence and severity of hepatic effects in rodents following inhalation and oral exposure to chloroform. There is also some evidence of hepatotoxicity in dogs and rabbits following oral exposure to chloroform.

Histopathological lesions have been reported in rats and mice following acute-, intermediate-, and chronic-duration inhalation exposure to chloroform, with increased susceptibility in mice compared to rats. In reviewing the available database, most studies show that the occurrence and severity of lesions increased in a concentration- and/or duration-dependent manner, beginning with mild histopathological damage after lower, shorter exposures (e.g., lipid accumulation, cellular swelling and vacuolation, scattered necrosis, hepatocellular proliferation) and progressing to widespread and severe necrosis and degeneration with higher and/or longer duration exposures (Tables 2-5 and 2-6, for rats and mice, respectively). In rodents, hepatic damage was consistently observed at acute-duration exposures ≥100 ppm and intermediate- and chronic-duration exposures ≥85 ppm. Following acute-duration inhalation exposure, the lowest identified LOAELs in mice and rats were 10 and 100 ppm, respectively (Larson et al. 1994c). Following intermediate-duration inhalation exposure, the lowest identified LOAELs in mice and rats were 17 and 25 ppm, respectively (Templin et al. 1998; Torkelson et al. 1976). Only one chronic-duration inhalation study was available, which identified LOAELs of 85.8 and 90.1 ppm for hepatic lesions in mice and rats, respectively (Yamamoto et al. 2002).

Table 2-5. Non-Neoplastic Hepatic Lesions in Rats Following Inhalation Exposure to Chloroform

Duration	Concentration (ppm)	Histology	Lesion details	Reference
Acute-duration				
≤7 days 6 hours/day	≤30	↔		Larson et al. 1994c; Templin et al. 1996b
4 days 6 hours/day	90	↔		Templin et al. 1996b
7 days 6 hours/day	100	↑	Hepatocellular proliferation	Larson et al. 1994c
7 days 6 hours/day	271	↑	Swelling and mild centrilobular vacuolation, cell necrosis, hepatocellular proliferation	Larson et al. 1994c
4 days 6 hours/day	300	↑	Hepatocellular proliferation	Templin et al. 1996b
2 weeks 5 days/week 6 hours/day	≥500	↑	Vacuolation in the central area of the liver	Kasai et al. 2002
Intermediate-duration				
6 months 5 days/week 1–4 hours/day	25	↔		Torkelson et al. 1976

Table 2-5. Non-Neoplastic Hepatic Lesions in Rats Following Inhalation Exposure to Chloroform

Duration	Concentration (ppm)	Histology	Lesion details	Reference
6 months 5 days/week 7 hours/day	25–50	↑	Lobular degeneration, focal necrosis	Torkelson et al. 1976
6 months 5 days/week 7 hours/day	85	↑	Marked degeneration	Torkelson et al. 1976
13 weeks 5 days/week 6 hours/day	≤90	↔		Kasai et al. 2002; Templin et al. 1996b
3 weeks 7 days/week 6 hours/day	90	F: ↑ M: ↔	Hepatocellular vacuolation, cell necrosis	Templin et al. 1996b
13 weeks 7 days/week 6 hours/day	90	↑	Hepatocellular vacuolation and hepatocyte degeneration and/or necrosis	Templin et al. 1996b
13 weeks 5 days/week 6 hours/day	≥100	↑	Localized hepatocyte loss	Kasai et al. 2002
3–13 weeks 7 days/week 6 hours/day	300	↑	Hepatocellular vacuolation, cell necrosis, hepatocellular proliferation	Templin et al. 1996b
13 weeks 5 days/week 6 hours/day	300	↑	Hepatocellular vacuolation and proliferation; hepatocyte degeneration and single-cell necrosis	Templin et al. 1996b
Chronic-duration				
104 weeks 5 days/week 6 hours/day	≤30	↔		Yamamoto et al. 2002
104 weeks 5 days/week 6 hours/day	90	F: ↑ M: ↔	Vacuolated cell foci	Yamamoto et al. 2002

↑ = increase in histopathological lesions; ↔ = no change; F = females; M = males

Table 2-6. Non-Neoplastic Hepatic Lesions in Mice Following Inhalation Exposure to Chloroform

Duration	Concentration (ppm)	Histology	Lesion details	Reference
Acute-duration				
≤7 days 6 hours/day	≤5	↔		Larson et al. 1994c; Templin et al. 1996c
4 or 7 days 6 hours/day	10–90	↑	Mild-to-moderate diffuse lipid vacuolation of hepatocytes, scattered hepatocyte necrosis; hepatocellular proliferation	Larson et al. 1994c, 1996; Templin et al. 1996c
2 weeks 4–5 days/week 6 hours/day	30	↔		Templin et al. 1996c
2 weeks 4–5 days/week 6 hours/day	90	↑	Minimal swelling in midzonal hepatocytes	Templin et al. 1996c
4 days 6 hours/day	92	↑	Moderate-to-marked vacuolar degeneration, increased cell proliferation	Constan et al. 1999
7 days 6 hours/day	≥101	↑	Extensive necrosis and severe vacuolar degeneration	Larson et al. 1994c
Intermediate-duration				
3–13 weeks 5 or 7 days/week 6 hours/day	≤12	↔		Larson et al. 1996; Templin et al. 1998
7 weeks 5 days/week 6 hours/day	17, 26	M: ↑	Centrilobular hepatocellular swelling	Templin et al. 1998
13 weeks 5 days/week 6 hours/day	23, 30	↑	Centrilobular hepatocellular swelling	Templin et al. 1998
3–13 weeks 5 or 7 days/week 6 hours/day	30	↑	Centrilobular hepatocellular swelling, vacuolation; hepatocellular proliferation	Larson et al. 1996; Templin et al. 1998
13 weeks 5 days/week 6 hours/day	≤50	↔		Kasai et al. 2002
13 weeks 5 days/week 6 hours/day	55	M: ↑	Centrilobular hepatocellular swelling, vacuolation, and mild degenerative changes; hepatocellular proliferation	Templin et al. 1998
3 or 6 weeks 7 days/week 6 hours/day	88	↑	Mild degenerative changes, karyomegaly, hepatocyte vacuolation and swelling, hepatocellular proliferation	Larson et al. 1996
13 weeks 5 or 7 days/week 6 hours/day	88	↑	Moderate centrilobular hepatocyte swelling and vacuolation; hepatocellular proliferation	Larson et al. 1996

Table 2-6. Non-Neoplastic Hepatic Lesions in Mice Following Inhalation Exposure to Chloroform

Duration	Concentration (ppm)	Histology	Lesion details	Reference
3 weeks 5 days/week 6 hours/day	90	F: ↑	Hepatocellular proliferation	Templin et al. 1998
13 weeks 5 days/week 6 hours/day	90	F: ↑	Centrilobular to midzonal vacuolation and degeneration; hepatocellular proliferation	Templin et al. 1998
13 weeks 5 days/week 6 hours/day	100	F: ↑ M: ↔	Atypical cells	Kasai et al. 2002
13 weeks 5 days/week 6 hours/day	200	↑	Atypical cells and necrosis (females); hepatocellular swelling (males)	Kasai et al. 2002
Chronic-duration				
104 weeks 5 days/week 6 hours/day	≤29.1	↔		Yamamoto et al. 2002
104 weeks 5 days/week 6 hours/day	85	↑	Fatty change	Yamamoto et al. 2002

↑ = increase in histopathological lesions; ↔ = no change; F = females; M = males

Histopathological changes in rodents were often accompanied by, or preceded by, elevated liver weights. The lowest reported concentrations associated with increased liver weights in mice and rats were 3 and 90 ppm, respectively (Larson et al. 1994c; Templin et al. 1996b). Several additional studies in mice also reported increased liver weight at higher concentrations (Constan et al. 1999; Kasai et al. 2002; Larson et al. 1996; Templin et al. 1998). Some rodent inhalation studies also reported mild elevations in serum activities of AST, ALT, and/or ALP at concentrations associated with histopathological changes in the liver; however, biologically-relevant changes of approximately 2-fold or greater were only observed in mice exposed to 200 ppm for 13 weeks (Kasai et al. 2002).

Available oral data indicate that rats and mice exposed for acute- or intermediate-durations to chloroform via gavage are much more susceptible to hepatotoxicity, compared to rodents exposed via drinking water. This is most clearly demonstrated in a series of studies by Larson et al. (1994b, 1995a), which exposed rats and mice to chloroform via gavage or drinking water for 4 days or 3 weeks. Evidence of hepatotoxicity (elevated liver weight, histopathological changes, and/or serum biochemistry changes) was observed in rats and mice at gavage doses ≥34 mg/kg/day (Larson et al. 1994b, 1995a). In drinking water

studies, adverse hepatic effects were inconsistently observed, and limited to centrilobular hepatocyte eosinophilic cytoplasm in mice exposed to ≥53.5 mg/kg/day for 4 days and elevated relative liver weight in mice exposed to 82.5 mg/kg/day for 3 weeks (Larson et al. 1994b). The clear difference in susceptibility between gavage and drinking water studies is likely due to saturation of metabolic detoxification pathways with bolus administration (see ***Mechanisms of Hepatotoxicity*** below). Additionally, a slower dosing of chloroform over time via drinking water may allow for adaptive mechanisms to begin. In support, hepatotoxicity in female mice associated with a 3-day gavage exposure to 263 mg/kg/day was attenuated if mice were exposed to chloroform at doses up to 520 mg/kg/day in drinking water for 3 weeks prior to gavage exposure (Pereira and Grothaus 1997).

Findings from numerous additional studies report hepatotoxicity in rodents following gavage exposure, while the majority of drinking water studies do not observe adverse hepatic effects. In gavage studies, dose- and duration-related increases in histopathological damage in the liver have been consistently observed in rats and mice following acute- and intermediate-duration exposure. Similar to inhalation exposure, mice generally appear more susceptible to hepatotoxicity compared to rats. Findings in both species range from mild histopathological damage after lower level, shorter duration exposures (e.g., lipid accumulation, cellular swelling and vacuolation, scattered necrosis, hepatocellular proliferation) to widespread and severe necrosis and degeneration with higher level and/or longer duration exposures (Tables 2-7 and 2-8 for rats and mice, respectively).

In mice and rats, the lowest identified LOAELs for hepatic lesions following acute- or intermediate-duration gavage exposure were 34 and 90–100 mg/kg/day, respectively (Larson et al. 1994a, 1994b, 1995a, 1995b). Review of these data suggest some differences in strain susceptibility, with decreased sensitivity in Osborne-Mendel rats and BALB/c mice, compared to other rat and mouse strains. In chronic-duration gavage studies in ICI mice, one study reported no adverse hepatic effects at gavage doses up to 60 mg/kg/day for 80 weeks (Roe et al. 1979), while NCI (1976) reported nodular hyperplasia at all tested doses (≥138 mg/kg/day in males and ≥238 mg/kg/day in females) in B6C3F1 mice. The inconsistency in the mouse chronic-duration studies may be due to strain differences; no other identified study evaluated ICI mice. In rats, chronic-duration gavage exposure to 200 mg/kg/day was associated with necrosis of the hepatic parenchyma in female Osborne-Mendel rats, but not in males at doses up to 180 mg/kg/day (NCI 1976). As discussed above, review of acute- and intermediate-duration studies (Table 2-7) show that Osborne-Mendel rats appear to be less sensitive than Fischer 433 rats, which were more commonly assessed in shorter-duration studies.

Table 2-7. Non-Neoplastic Hepatic Lesions in Rats Following Gavage Exposure to Chloroform

Strain; duration	Dose (mg/kg/day)	Histology	Lesion details	Reference
Acute-duration				
Fischer 344 or Osborne-Mendel; 4 days	≤34	↔		Larson et al. 1993, 1995a, 1995b
Fischer 344; 4 days	90–100	↑	Hepatocellular proliferation, slight hepatocyte vacuolation, swollen hepatocytes, individual cell necrosis	Larson et al. 1995a, 1995b
Fischer 344; 1 day	≤180	↔		Larson et al. 1993; Miyagawa et al. 1998;
Fischer 344; 4 days	180	↑	Hepatocellular proliferation, swollen hepatocytes, individual cell necrosis, thickening of centrilobular hepatic cords	Larson et al. 1995a
Fischer 344; 21 days	200	↑	Slight hepatocyte vacuolation and hepatocellular proliferation	Larson et al. 1995b
Sprague-Dawley; 1 day	220	↑	Increased leukocyte adherence to sinusoidal wall, hepatocyte swelling, reduced perfusion of sinusoids and increased phagocytosis activity of Kupffer cells	Ito et al. 2000
Sprague-Dawley; 3 days	≥250	↑	Centrilobular hepatocellular enlargement, necrosis, and vacuolation; centrilobular inflammatory cell infiltration	Wada et al. 2015
Fischer 344; 4 days	400	↑	Mild-to-severe centrilobular hepatocyte degeneration and necrosis, diffuse centrilobular swelling	Larson et al. 1995b
Osborne-Mendel; 1 day	≤477	↔		Templin et al. 1996a
Fischer 344; 1 day	477–500	↑	Mild hepatocyte necrosis, vacuolation, hypertrophy, and proliferation	Larson et al. 1993; Templin et al. 1996a; Miyagawa et al. 1998
Sprague-Dawley; 10 days	516	↑	Acute toxic hepatitis	Thompson et al. 1974
Intermediate-duration				
Fischer 344; 3 weeks	≤90	↔		Larson et al. 1995a, 1995b
Fischer 344; 3 weeks	100–200	↑	Hepatocellular proliferation	Larson et al. 1995a, 1995b

Table 2-7. Non-Neoplastic Hepatic Lesions in Rats Following Gavage Exposure to Chloroform

Strain; duration	Dose (mg/kg/day)	Histology	Lesion details	Reference
Fischer 344; 3 weeks	400	↑	Slight-to-mild diffuse vacuolar change, centrilobular degeneration, hepatocellular proliferation	Larson et al. 1995b
Chronic-duration				
Osborne-Mendel rat; 78 weeks	≤180	↔		NCI 1976
Osborne-Mendel rat; 78 weeks	200	↑	Necrosis of hepatic parenchyma	NCI 1976

↑ = increase in histopathological lesions; ↔ = no change

Table 2-8. Non-Neoplastic Hepatic Lesions in Mice Following Gavage Exposure to Chloroform

Species; duration	Dose (mg/kg/day)	Histology	Lesion details	Reference
Acute-duration				
B6C3F1; 1 or 4 days	≤34	↔		Larson et al. 1993, 1994b
B6C3F1; 4 days	34	↑	Hepatocellular proliferation and mild hepatocellular swelling and vacuolation	Larson et al. 1994d
B6C3F1; 21 days	34	↑	Mild vacuolation of hepatocytes	Larson et al. 1994b
Swiss; 1 day	35	↑	Midzonal fatty changes	Jones et al. 1958
B6C3F1; 4 days	90–138	↑	Vacuolation and swelling of hepatocytes; hepatocellular proliferation and scattered degeneration	Larson et al. 1994b, 1994d
Swiss; 1 day	≤199	↔		Moore et al. 1982
B6C3F1; 1 day	238	↑	Small, randomly scattered foci of hepatocyte necrosis	Larson et al. 1993
B6C3F1; 4 days	238–277	↑	Moderate centrilobular vacuolar degeneration; scattered necrosis; hepatocellular proliferation	Larson et al. 1994b, 1994d
Swiss; 1 day	273	↑	Hepatocellular proliferation	Moore et al. 1982
BALB/c; 1 day	≤300	↔		Ewaid et al. 2020
Swiss; 1 day	350	↑	Severe centrilobular necrosis	Jones et al. 1958

Table 2-8. Non-Neoplastic Hepatic Lesions in Mice Following Gavage Exposure to Chloroform

Species; duration	Dose (mg/kg/day)	Histology	Lesion details	Reference
B6C3F1; 1 or 4 days	≥350	↑	Marked hepatocellular swelling, vacuolation, degeneration and necrosis, hepatocellular proliferation	Larson et al. 1993, 1994b
BALB/c; 1 day	≥700	↑	Centrilobular necrosis	Ewaid et al. 2020
Intermediate-duration				
B6C3F1; 3 weeks	≤34	↔		Larson et al. 1994b
B6C3F1; 3 weeks	34	↑	Mild vacuolation of hepatocytes	Larson et al. 1994b
CD-1; 105 days	41	↑	Hepatocellular degeneration	NTP 1988a
Swiss; 3 weeks	55	↑	Hepatocyte hydropic degeneration	Melnick et al. 1998
B6C3F1; 90 days	60	↑	Fatty changes	Bull et al. 1986
B6C3F1; 3 weeks	90	↑	Scattered necrosis, moderate-to-marked vacuolation and swelling of hepatocytes; hepatocellular proliferation	Larson et al. 1994b, 1994d
Swiss; 3 weeks	110	↑	Hepatocyte hydropic degeneration, hepatocellular proliferation	Melnick et al. 1998
Swiss; 54 days	130	↔		Mostafa et al. 2009
B6C3F1; 90 days	130	↑	Fatty changes, vacuolation, focal necrosis	Bull et al. 1986
B6C3F1; 3 weeks	138	↑	Hepatocellular swelling	Larson et al. 1994d
CB6F1; 26 weeks	140	↑	Hepatocellular vacuolation; hepatocellular proliferation	Sehata et al. 2002
B6C3F1 or Swiss; 3 weeks	238	↑	Hepatocyte degeneration, necrosis, and proliferation	Larson et al. 1994b; Melnick et al. 1998
Swiss; 54 days	238	↑	Marked cellular inflammatory infiltration (males), necrosis (females)	Mostafa et al. 2009
CB6F1; 26 weeks	240	↑	Hepatocellular vacuolation and swelling; hepatocellular foci; hepatocellular proliferation	Sehata et al. 2002
B6C3F1; 90 days	270	↑	Extensive disruption of hepatic architecture, including mild to moderate early cirrhosis	Bull et al. 1986
B6C3F1; 3 weeks	277	↑	Degeneration and necrosis	Larson et al. 1994d
Swiss; 54 days	277	↑	Marked cellular inflammatory infiltration and necrosis (males, females); polymorphic and hyperchromatic nuclei (females)	Mostafa et al. 2009
Strain A; 30 days	≤297	↔		Eschenbrenner and Miller 1945

Table 2-8. Non-Neoplastic Hepatic Lesions in Mice Following Gavage Exposure to Chloroform

Species; duration	Dose (mg/kg/day)	Histology	Lesion details	Reference
Swiss or B6C3F1; 3 weeks	477	↑	Marked hepatocellular swelling, vacuolation, degeneration, and necrosis; hepatocellular proliferation	Larson et al. 1994b; Melnick et al. 1998
Swiss; 54 days	477	↑	Polymorphic, hyperchromatic nuclei	Mostafa et al. 2009
Strain A; 30 days	≥594	↑	Cirrhosis	Eschenbrenner and Miller 1945
Chronic-duration				
ICI; 80 weeks	≤60	↔		Roe et al. 1979
B6C3F1; 78 weeks	≥138	↑	Nodular hyperplasia	NCI 1976

↑ = increase in histopathological lesions; ↔ = no change

Histopathological changes in rats and mice following gavage exposure were often accompanied by, or preceded by, elevated liver weights. The lowest reported concentrations associated with increased liver weights in rats was 34 mg/kg/day (Larson et al. 1995a) in mice was 41 mg/kg/day (NTP 1988a). Several additional studies in mice also reported increased liver weight at higher doses (Bull et al. 1986; Ewaid et al. 2020; Larson et al. 1994b, 1995b; Lipsky et al. 1993; Melnick et al. 1998; Munson et al. 1982; Sehata et al. 2002).

Consistent with human exposure cases, changes in hepatic clinical chemistry values were also observed in rodents following acute- and intermediate duration gavage exposure to chloroform; no chronic-duration gavage studies evaluated serum biochemistry. Observed changes in rats and mice included elevations in serum activities of AST, ALT, ALP, LDH, and/or sorbitol dehydrogenase (SDH) (Tables 2-9 and 2-10, respectively). The lowest identified dose associated with elevations of ≥2-fold in one or more serum hepatic enzyme activity levels following acute-duration gavage exposure in rats and mice was 90 mg/kg/day (Keegan et al. 1998; Larson et al. 1994b). In intermediate-duration gavage studies, the lowest identified doses associated with a ≥2-fold change in rats and mice were 180 and 90 mg/kg/day, respectively (Larson et al. 1994b, 1995b).

Table 2-9. Hepatic Clinical Chemistry in Rats Following Gavage Exposure to Chloroform

Strain, Duration	Dose (mg/kg/day)	ALT[a]	AST[a]	ALP[a]	LDH[a]	SDH[a]	Reference
Acute-duration							
Wistar; 1 day	12.5	↔	↔	–	–	–	Wang et al. 1997
Fischer 344; up 4 days	≤34	↔	↔	–	–	↔	Keegan et al. 1998; Larson et al. 1995a
Fischer 344; 1 day	60	↑ (55)[b]	↑ (40)[b]	–	–	↑ (80)[b]	Keegan et al. 1998
Fischer 344; 1 day	89.5	↔	↔	–	↔	↑ (47)[b]	Lilly et al. 1997
Fischer 344; 1 day	90	↑ (100)[b]	↑ (80)[b]	–	–	↑ (250)[b]	Keegan et al. 1998
Fischer 344; 4 days	90	↑ (1,220)	–	–	–	↑ (3,067)	Larson et al. 1995a
Fischer 344; 1 day	119	↑ (55)[b]	↑ (35)[b]	–	–	↑ (125)[b]	Keegan et al. 1998
Fischer 344; 1 day	119.4	↔	↔	–	↔	↑ (100)[b]	Lilly et al. 1997
Fischer 344; 1 day	≤150	–	↔	–	–	–	Miyagawa et al. 1998
Fischer 344; 1 day	179	↑ (220)[b]	↑ (180)[b]	–	–	↑ (300)[b]	Keegan et al. 1998
Fischer 344; 1 day	179.1	↑ (120)[b]	↑ (100)[b]	–	↑ (250)[b]	↑ (170)[b]	Lilly et al. 1997
Fischer 344; 1 day	≤180	↔	↔	–	–	↔	Larson et al. 1993
Fischer 344; 4 days	180	↑ (86)	–	–	–	↑ (156)	Larson et al. 1995a
Wistar: 1 day	200	↑ (388)	↑ (348)	–	–	–	Wang et al. 1997
Fischer 344; 1 day	238.8	↑ (340)[b]	↑ (260)[b]	–	↑ (350)[b]	↑ (230)[b]	Lilly et al. 1997
Fischer 344; 1 day	358.2	↑ (560)[b]	↑ (460)[b]	–	↑ (800)[b]	↑ (380)[b]	Lilly et al. 1997
Fischer 344; 1 day	477	↑ (1,120)	↑ (647)	–	–	↑ (1,029)	Larson et al. 1993
Fischer 344; 1 day	500	–	↑ (330)[b]	–	–	–	Miyagawa et al. 1998
Intermediate-duration							
Fischer 344; 3 weeks	≤90	↔	–	–	–	↔	Larson et al. 1995a

Table 2-9. Hepatic Clinical Chemistry in Rats Following Gavage Exposure to Chloroform

Strain, Duration	Dose (mg/kg/day)	ALT[a]	AST[a]	ALP[a]	LDH[a]	SDH[a]	Reference
Fischer 344; 3 weeks	180	↑ (243)	–	–	–	↑ (363)	Larson et al. 1995a

[a]Numbers in ( ) are percent change compared to control, calculated from quantitative data (unless otherwise noted).
[b]Percent change compared to control estimated from graphically reported data.

↑ = increased; ↔ = no change; – = not assessed; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; F = females; LDH = lactate dehydrogenase; M = males; SDH = sorbitol dehydrogenase

Table 2-10. Hepatic Clinical Chemistry in Mice Following Gavage Exposure to Chloroform

Strain; duration	Dose (mg/kg/day)	ALT[a]	AST[a]	ALP[a]	LDH[a]	SDH[a]	Reference
Acute-duration							
B6C3F1; 4 days	≤10	↔	–	–	–	↔	Larson et al. 1994b
B6C3F1; 4 days	90	↑ (145)	–	–	–	↔	Larson et al. 1994b
CD-1; 14 days	≤125	↔	↔	↔	↔	–	Munson et al. 1982
Swiss or B6C3F1; 1 day	≤238	↔	↔	–	–	–	Larson et al. 1993; Moore et al. 1982
B6C3F1; 4 days	238	↑ (900)	–	–	–	↑ (543)	Larson et al. 1994b
CD-1; 14 days	250	↑ (191–3,505)	F: ↑ (47)	↔	↔	–	Munson et al. 1982
Swiss; 1 day	273	↑ (122)	↔	–	–	–	Moore et al. 1982
B6C3F1; 1 day	350	↑ (NR)	↔	–	–	↑ (NR)	Larson et al. 1993
B6C3F1; 4 days	477	↑ (1,855)	–	–	–	↑ (1,186)	Larson et al. 1994b
Intermediate-duration							
B6C3F1; 3 weeks	≤10	↔	–	–	–	↔	Larson et al. 1994b
B6C3F1; 3 weeks	34	↑ (65)	–	–	–	↑ (48)	Larson et al. 1994b
Swiss; 3 weeks	55	↑ (50)[b]	–	–	–	↑ (15)[b]	Melnick et al. 1998
B6C3F1; 90 days	60	–	↔	–	↔	–	Bull et al. 1986
B6C3F1; 3 weeks	90	↑ (236)	–	–	–	↑ (144)	Larson et al. 1994b

Table 2-10. Hepatic Clinical Chemistry in Mice Following Gavage Exposure to Chloroform

Strain; duration	Dose (mg/kg/day)	ALT[a]	AST[a]	ALP[a]	LDH[a]	SDH[a]	Reference
Swiss; 3 weeks	110	↑ (50)[b]	–	–	–	↑ (30)[b]	Melnick et al. 1998
B6C3F1; 90 days	130	–	↑ (65–74)	–	↔	–	Bull et al. 1986
CB6F1; 26 weeks	140	↑ (312)	↑ (103)	↑ (15)	–	–	Sehata et al. 2002
B6C3F1; 3 weeks	238	↑ (4,378)	–	–	–	↑ (5,660)	Larson et al. 1994b
Swiss; 3 weeks	238	↑ (770)[b]	–	–	–	↑ (613)[b]	Melnick et al. 1998
CB6F1; 26 weeks	240	↑ (556)	↑ (141)	↑ (21)	–	–	Sehata et al. 2002
CD-1; 90 days	≤250	↔	↔	↔	↔	–	Munson et al. 1982
B6C3F1; 90 days	270	–	↔	–	↔	–	Bull et al. 1986
B6C3F1; 3 weeks	477	↑ (2,857)	–	–	–	↑ (5,340)	Larson et al. 1994b
Swiss; 3 weeks	477	↑ (2,660)[b]	–	–	–	↑ (2,023)[b]	Melnick et al. 1998

[a]Numbers in ( ) are percent change compared to control, calculated from quantitative data (unless otherwise noted).
[b]Percent change compared to control estimated from graphically reported data.

↑ = increased; ↔ = no change; – = not assessed; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; F = females; LDH = lactate dehydrogenase; M = males; NR = not reported; SDH = sorbitol dehydrogenase

In contrast to gavage studies, there is limited evidence for hepatic damage in rats or mice following acute- or intermediate-duration exposure via drinking water; no chronic drinking-water studies were identified. One acute-duration study in mice reported centrilobular hepatocyte eosinophilic cytoplasm following exposure to ≥53.5 mg/kg/day for 4 days (Larson et al. 1994b). No histopathological changes in the liver were observed in similarly exposed rats at drinking water doses up to 68.1 mg/kg/day (Larson et al. 1995a). In intermediate-duration studies, no histopathological changes in the liver were observed at drinking water doses up to 200 mg/kg/day in rats or 329 mg/kg/day in mice (Chu et al. 1982a, 1982b; Larson et al. 1994b, 1995a). One study reported fatty changes of the liver in mice exposed to drinking water doses ≥290 mg/kg/day for 90 days; this was not observed in rats or mice at doses up to 160 mg/kg/day (EPA 1980). No exposure-related changes in hepatic clinical chemistry were observed following acute-duration drinking water doses up to 68.1 mg/kg/day in rats (Larson et al. 1995a) or 105 mg/kg/day in mice (Larson et al. 1994b). Similarly, no adverse changes in hepatic clinical chemistry

were observed following intermediate-duration drinking water doses up to 200 mg/kg/day in rats (Chu et al. 1982a, 1982b; EPA 1980; Larson et al. 1995a) or 329 mg/kg/day in mice (Larson et al. 1994b).

Beagle dogs exposed to chloroform by capsule in toothpaste base daily for 7.5 years and subjected to periodic blood collection and clinical chemistry evaluation showed significant increases in serum ALT throughout the first year of the study at 30 mg/kg/day, with no increase at the lower dose of 15 mg/kg/day (Heywood et al. 1979). This continued during the chronic phase of the study until week 130 during the third year, after which serum ALT was significantly increased at both dose levels for the remainder of the study. Dogs were necropsied upon death during or at the end of the study (after a 19-week recovery period). At necropsy, livers showed a dose-dependent increase in incidence and severity of fatty cysts formed by vacuolated histiocytes.

Studies in rabbits include one gestational exposure study in pregnant does and a 24-hour dermal lethality study. Following exposure to ≥100 mg/kg/day for 13 days during gestation, acute toxic hepatitis was observed in does that died (Thompson et al. 1974). Of the two survivors at 100 mg/kg/day, one showed mild fatty changes. In the dermal acute-duration lethality study, no histopathological changes to the liver were observed in rabbits exposed to doses up to 3,980 mg/kg for 24 hours under occluded conditions (Torkelson et al. 1976).

Mechanisms of Hepatotoxicity. Available data pertaining to mechanisms underlying chloroform-induced effects clearly show that metabolism of chloroform is required for hepatotoxic effects in rodents. Supporting evidence includes increased hepatotoxicity with co-exposure to microsomal enzyme inducers, such as phenobarbital, and decreased hepatotoxicity with co-exposure to inhibitors of microsomal enzymes, such as SKF-525A (Brown et al. 1974a; Gopinath and Ford 1975). Additionally, hepatotoxicity was not observed following chloroform exposure in CYP2E1 knockout mice (Constan et al. 1999) or Liver-Cpr-null mice, which lack cytochrome P450 reductase only in the liver (Fang et al. 2008). These findings are supported in *in vitro* studies showing prevention of chloroform-induced cytotoxicity in rat and mouse hepatic cells following pretreatment with the cytochrome P450 inhibitor, 1-phenylimidazole (Ammann et al. 1998).

Once metabolized, however, the exact mode of action is unknown. Glutathione (GSH) depletion is observed at high exposure levels both *in vivo* and *in vitro* due to saturation of the detoxifying pathways, particularly when chloroform exposure is paired with the microsomal enzyme inducers (Ammann et al. 1998; Brown et al. 1974a; Wang et al. 1997). Brown et al. (1974a) also showed that both covalent

binding of chloroform metabolites and increased hepatotoxicity were increased with increasing GSH depletion. Based on this, the EPA (IRIS 2001) concluded that covalent binding of the chloroform metabolite, phosgene, to liver macromolecules is a likely mechanism underlying hepatic necrosis. GSH depletion is also associated with induction of oxidative stress and production of superoxide anion (Abbassi et al. 2010). Burke et al. (2007) proposed that cellular toxicity occurs in two distinct phases, a "metabolic phase" in which GSH is depleted, followed by an "oxidative phase" characterized by oxidative stress, mitochondrial permeability transition, and protein nitration. In support, reduced mitochondrial membrane potential is observed in mouse hepatocytes exposed to chloroform *in vitro* (Hartig et al. 2005).

Similar to the results from a human study by Rao et al. (1993), the rodent liver is capable of regenerative repair after oral or injection exposure to chloroform (Anand et al. 2003, 2005a, 2005b, 2006). This capacity for repair is a key determinant of the final outcome of the hepatotoxic effects associated with acute chloroform toxicity, as the capacity for repair can become overwhelmed at high doses resulting in potentially fatal liver injury (Anand and Mehendale 2004; Mehendale 1991, 2005). Mechanistic pathways involved in repair are varied, including various cellular signaling pathways (chemokines, cytokines, growth factors, nuclear receptors) that result in promitogenic gene expression and cell division. Initiation of this repair pathway via repeat, sublethal chloroform exposures in mice can be protective of acute lethal exposures by mitigating, in part, acute hepatotoxic effects (Philip et al. 2006), resulting in tolerance to low-dose repeat exposures (Anand et al. 2006).

2.10 RENAL

Renal toxicity is one of the major toxic effects observed in both humans and animals after inhalation exposure to chloroform. Based upon systematic review (Appendix C), the kidney is a presumed target of chloroform toxicity based on inadequate evidence in human epidemiological studies and a high level of evidence in laboratory animal studies.

Several case reports indicate that the kidney is a target of chloroform toxicity in humans following exposure to chloroform at high exposure levels via inhalation or oral routes. Renal damage, including fatty and hyaline degeneration of the renal tubule epithelium and casts of cell debris and hyaline material in the tubules, was reported in fatal exposure cases following exposure to chloroform via anesthesia during childbirth (Royston 1924) or ingestion (Piersol et al. 1933). Intentional exposure to high levels of chloroform via inhalation or ingestion have been associated with altered clinical chemistry (elevated

blood urea nitrogen [BUN] and creatinine) and/or urinalysis findings (oliguria, albuminuria, casts); full recovery was observed in nonfatal cases (Dettling et al. 2016; Gosselink et al. 2012; Piersol et al. 1933; Schroeder 1965; Sridhar et al. 2011). Numerous hyaline and granular casts and the presence of albumin were observed in the urine of one subject who ingested 21 mg/kg/day chloroform in cough medicine for 10 years (Wallace 1950). The urinalysis results reversed to normal after discontinuation of chloroform exposure.

Epidemiological data pertaining to renal toxicity in humans following exposure to chloroform is limited (Table 2-11). No associations between serum BUN levels were observed in a Chinese cohort exposed to chloroform for 1–15 years at a geometric mean of 4.19 ppm (Li et al. 1993). Similarly, Aiking et al. (1994) observed no exposure-related changes in serum creatinine or urinary β2-microglobulin levels between competitive swimmers exposed to chloroform for >10 hours/week for ≥5 years while swimming in indoor or outdoor chlorinated swimming pools, compared to controls (competitive korfball players; a Dutch game similar to basketball). While dermal exposure is a consideration, the focus was on inhalation exposure to volatilized chloroform. However, no air concentrations were reported. Mean blood chloroform concentrations post-training were 0.89 µg/L in the indoor training environment and below the level of detection (0.5 µg/L) in the outdoor training environment.

In a cross-sectional study of 2003–2010 NHANES data, Liu et al. (2023a) identified an inverse association between blood chloroform levels and albumin-to-creatinine ratio and the estimated glomerular filtration rate (eGFR) in 6,070 adults. The glomerular filtration rate was estimated using the Modification of Diet in Renal Disease study eGFR equation, which utilizes serum creatinine levels, age, and gender- and race-specific variables. Exposure route(s) were not evaluated in this study.

Table 2-11. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Renal Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Occupational exposure			
Li et al. 1993 Cohort; 61 workers exposed to chloroform for 1–15 years (mean of 7.8 years) and 23 unexposed controls; mean age of 36.02 and 36.83 years, respectively (China)	Geometric mean chloroform level: 4.19 ppm	Serum BUN	↔

Table 2-11. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Renal Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
General population exposure			
Aiking et al. 1994 Cohort; 10 competitive swimmers who trained in indoor chlorinated pools for ≥10 hours/week for a mean of 8.3 years (mean age of 18.6 years), 8 competitive swimmers who trained in outdoor chlorinated pools for ≥10 hours/week for a mean of 12.1 years (mean age of 20.9 years), and 12 athletic controls (competitive korfball players, mean age of 24.3 years) (Netherlands)	Mean chloroform levels in pool water during training session (µg/L): Indoor: 24 Outdoor: 18.4 Mean blood chloroform after training session of unspecified duration (µg/L) Indoor: 0.89 Outdoor: <0.5 (LOD) Controls: <0.5 (LOD)	Measured prior to training session: Urinary β2-microglobulin	↔ (indoor versus control) ↔ (outdoor versus control)
		Measured at the end of training session: Serum creatinine	↔ (indoor versus control) ↔ (outdoor versus control)
Liu et al. 2023a Cross-sectional; 6,070 adults, mean age 44.84 years (2003–2010 NHANES; United States)	Weighted median (interquartile range) blood chloroform (µg/L): 0.0089 (0.0042–0.018)	Albumin-to-creatinine ratio	↓ (Q2, Q3, Q4 versus Q1)
		Glomerular filtration rate (estimated)[b]	↓ (Q4 versus Q1)

[a]Unless otherwise noted, current exposure levels are reported.
[b]The glomerular filtration rate was estimated by investigators using the Modification of Diet in Renal Disease study equation, which utilizes serum creatinine levels, age, and gender- and race-specific variables.

↑ = association; ↓ = inverse association; ↔ = no association; BUN = blood urea nitrogen; LOD = limit of detection; NHANES = National Health and Nutrition Examination Survey; Q = quartile

The kidney is a clear target of toxicity for chloroform in animal studies. There is clear and consistent evidence of dose- and duration-dependent increases in occurrence and severity of kidney effects in rodents following inhalation and oral exposure to chloroform. There is also some evidence of renal toxicity in dogs and rabbits following oral exposure to chloroform, and renal toxicity in rabbits following dermal exposure.

Histopathological lesions have been reported in rats and mice following acute-, intermediate-, and chronic-duration inhalation exposure to chloroform. The main target of toxicity was the proximal convoluted tubule. In general, the occurrence and severity of lesions increased in a concentration-related manner, beginning with mild histopathological damage after lower level, shorter duration exposures (e.g.,

tubular dilation, single-cell necrosis, renal cell proliferation) and progressing to severe nephropathy characterized by widespread necrosis and degeneration with higher level and/or longer duration exposures (Tables 2-12 and 2-13 in rats and mice, respectively). In rodents, renal damage was consistently observed at acute- and intermediate-duration exposures ≥100 ppm and chronic-duration exposures ≥29.1 ppm. Following acute-duration inhalation exposure, the lowest identified LOAELs in mice and rats were approximately 30 ppm (Larson et al. 1994c; Templin et al. 1996c). Following intermediate-duration inhalation exposure, the lowest identified LOAELs in mice and rats were 10 and 25 ppm, respectively (Larson et al. 1996; Torkelson et al. 1976). A limited number of chronic-duration studies were identified, with renal lesions in rats and mice at concentrations ≥29.1 ppm (Nagano et al. 2006; Yamamoto et al. 2002). Male rodents, particularly mice, appear to be more susceptible to renal toxicity than females (Table 2-13).

Table 2-12. Non-Neoplastic Renal Lesions in Rats Following Inhalation Exposure to Chloroform

Duration	Concentration (ppm)	Histology	Lesion details	Reference
Acute-duration				
7 days 6 hours/day	≤10	↔		Larson et al. 1994c
7 days 6 hours/day	29.3	↑	Focal epithelial proliferation in the renal cortex	Larson et al. 1994c
4 days 6 hours/day	≤90	↔		Larson et al. 1996; Templin et al. 1996b
4 days 6 hours/day	100	↑	Focal epithelial proliferation in the renal cortex	Larson et al. 1994c
4 days 6 hours/day	271	↑	Focal epithelial proliferation in the renal cortex and outer medulla; regeneration of proximal tubule epithelium	Larson et al. 1994c
4 days 6 hours/day	300	↑	Minimal vacuolation of proximal convoluted tubule	Templin et al. 1996b
Intermediate-duration				
3–13 weeks 5 or 7 days/week 6 hours/day	≤10	↔		Templin et al. 1996b
6 months 5 days/week; 1–4 hours/day	25	↔		Torkelson et al. 1976
6 months 7 days/week 6 hours/day	25	M: ↑ F: ↔	Cloudy swelling of the renal tubular epithelium	Torkelson et al. 1976

Table 2-12. Non-Neoplastic Renal Lesions in Rats Following Inhalation Exposure to Chloroform

Duration	Concentration (ppm)	Histology	Lesion details	Reference
3–13 weeks; 7 days/week 6 hours/day	30	↑	Renal cell vacuolation in the proximal convoluted tubule	Templin et al. 1996b
6 months 5 days/week; 7 hours/day	≥50	↑	Cloudy swelling of the renal tubular epithelium	Torkelson et al. 1976
3–13 weeks; 7 days/week 6 hours/day	≥90	↑	Renal cell vacuolation in the proximal convoluted tubule	Templin et al. 1996b
13 weeks 5 days/week 6 hours/day	≤100	↔		Kasai et al. 2002; Templin et al. 1996b
13 weeks 5 days/week 6 hours/day	200	M: ↔ F: ↑	Vacuolic change in proximal tubules	Kasai et al. 2002
13 weeks 5 days/week 6 hours/day	300	↑	Scattered vacuolation and nuclear pyknosis in the proximal convoluted tubule	Templin et al. 1996b
13 weeks 7 days/week 6 hours/day	300	↑	Cell necrosis	Templin et al. 1996b
13 weeks 5 days/week 6 hours/day	400	M: ↑	Vacuolic change in proximal tubules	Kasai et al. 2002
Chronic-duration				
104 weeks 5 days/week 6 hours/day	≤25	↔		Nagano et al. 2006; Yamamoto et al. 2002
104 weeks 5 days/week 6 hours/day	≥30	↑	Nuclear enlargement of the proximal tubules, dilation of the tubular lumen, cytoplasmic basophilia	Nagano et al. 2006; Yamamoto et al. 2002

↑ = increase in histopathological lesions; ↔ = no change; – = not assessed; F= females; M = males

Table 2-13. Non-Neoplastic Renal Lesions in Mice Following Inhalation Exposure to Chloroform

Duration	Concentration (ppm)	Histology	Lesion details	Reference
Acute-duration				
4 days 6 hours/day	≤5	↔		Templin et al. 1996c

Table 2-13. Non-Neoplastic Renal Lesions in Mice Following Inhalation Exposure to Chloroform

Duration	Concentration (ppm)	Histology	Lesion details	Reference
4 days 6 hours/day	30	M: ↑ F: ↔	Mild-to-moderate proximal tubular necrosis and dilation; hyaline casts and tubular degeneration; cell proliferation	Templin et al. 1996c
2 weeks 4–5 days/week 6 hours/day	≥30	↑	Severe tubular necrosis and tubular degeneration	Templin et al. 1996c
4 days 6 hours/day	≤88	↔		Larson et al. 1996
4 days 6 hours/day	90	M: ↑ F: ↔	Moderate-to-severe necrosis	Templin et al. 1996c
4 days 6 hours/day	92	↑	Severe necrosis in proximal convoluted tubules, increased cell proliferation	Constan et al. 1999
7 days 6 hours/day	≤101	↔		Larson et al. 1994c; Mery et al. 1994
7 days 6 hours/day	288	↑	Proximal tubule epithelial regeneration, cellular proliferation in renal cortex and the medulla outer stripe	Larson et al. 1994c; Mery et al. 1994
Intermediate-duration				
7–13 weeks 5 days/week 6 hours/day	≤5	↔		Templin et al. 1998
3–13 week 7 days/week 6 hours/day	≤10	↔		Larson et al. 1996
13 week 5 days/week 6 hours/day	≥10	M: ↑ F: ↔	Renal cell proliferation	Larson et al. 1996
13 weeks 5 days/week 6 hours/day	12	M: ↑ F: ↔	Necrosis and cytoplasmic basophilia in the proximal tubules	Kasai et al. 2002
7 weeks 5 days/week 6 hours/day	≥17	M: ↑	Cellular proliferation and regenerative lesions in proximal convoluted tubule	Templin et al. 1998
13 weeks 5 days/week 6 hours/day	≥23	M: ↑ F: ↔	Cellular proliferation and regenerative lesions in proximal convoluted tubule	Templin et al. 1998
13 weeks 5 days/week 6 hours/day	≥25	M: ↑ F: ↔	Severe proximal tubular necrosis and degeneration	Kasai et al. 2002

Table 2-13. Non-Neoplastic Renal Lesions in Mice Following Inhalation Exposure to Chloroform

Duration	Concentration (ppm)	Histology	Lesion details	Reference
3 or 13 weeks 7 days/week 6 hours/day	≥30	M: ↑ F: ↔	Enlarged nuclei and renal cell proliferation in the proximal convoluted tubules; focal regeneration	Larson et al. 1996
6 weeks 7 days/week 6 hours/day	≤88	F: ↔		Larson et al. 1996
13 weeks 5 days/week 6 hours/day	88	M: ↑ F: ↔	Focal mineralization and regeneration	Larson et al. 1996
3 or 13 weeks 5 days/week 6 hours/day	≤90	F: ↔		Templin et al. 1998
Chronic-duration				
104 weeks 5 days/week 6 hours/day	5	↔		Yamamoto et al. 2002
104 weeks 5 days/week 6 hours/day	29.1	M: ↑ F: ↔	Renal tubular lesions	Yamamoto et al. 2002
104 weeks 5 days/week 6 hours/day	85.8	↑	Renal tubular lesions, cytoplasmic basophilia	Yamamoto et al. 2002

↑ = increase in histopathological lesions; ↔ = no change; – = not assessed; F= females; M = males

Histopathological changes in rodents were often accompanied by, or preceded by, elevated kidney weights. The lowest reported concentrations associated with increased kidney weights in rats and mice were 25 and 92 ppm, respectively (Constan et al. 1999; Torkelson et al. 1976). Additional studies in rats and mice also reported increased kidney weights at higher concentrations (Kasai et al. 2002; Templin et al. 1996b).

Changes in clinical chemistry or urinalysis parameter values were also observed in some rodents following intermediate- and chronic-duration inhalation exposure to chloroform; no acute-duration inhalation studies evaluated renal clinical chemistry. In rats, no exposure-related increases in serum BUN were observed at concentrations up to 85 ppm for 6 months (Torkelson et al. 1976) or 100 ppm for 104 weeks (Yamamoto et al. 2002). However, urinalysis findings indicative of impaired renal function (e.g., proteinuria, hematuria, glucosuria) were observed in rats exposed to ≥50 ppm for 13 weeks (Kasai

et al. 2002) or ≥30 ppm for 104 weeks (Nagano et al. 2006; Yamamoto et al. 2002). In mice, elevated serum BUN levels were observed following exposure to ≥50 ppm for 13 weeks (Kasai et al. 2002) or ≥29.1 ppm for 104 weeks (Yamamoto et al. 2002). Proteinuria was observed in male mice exposed to 12 ppm for 13 weeks; however, it was not observed in females similarly exposed up to 400 ppm (Kasai et al. 2002). No exposure-related changes in urinalysis were observed in mice exposed to concentrations up to 85.8 mg/kg/day for 104 weeks (Yamamoto et al. 2002).

Available oral data indicate that rats and mice exposed to chloroform for acute- or intermediate-durations are much more susceptible to renal toxicity via gavage administration, compared to rodents exposed via drinking water. This is most clearly demonstrated in a series of experiments by Larson et al. (1995a), which exposed rats to chloroform via gavage or drinking water for 4 days or 3 weeks. Mild-to-moderate degeneration of renal proximal tubules and tubule epithelial cell proliferation were observed in rats at gavage doses ≥34 mg/kg/day. However, no adverse renal effects were noted at drinking water doses up to 106 mg/kg/day. The clear difference in susceptibility between acute- and intermediate-duration gavage and drinking water studies is likely due to saturation of metabolic detoxification pathways with bolus administration (see ***Mechanisms of Renal Toxicity*** below). This pattern is not observed in chronic-duration studies, in which gavage studies only reported neoplastic renal lesions and drinking water studies reported both non-neoplastic and neoplastic lesions. As discussed below, while numerous additional acute-, intermediate-, and chronic-duration studies reported renal toxicity in rodents following gavage exposure, only chronic-duration (not acute- or intermediate-duration) drinking water studies observed adverse renal effects.

In gavage studies, dose- and duration-related increases in histopathological damage in the kidney have been consistently observed in rats and mice following acute- and intermediate-duration exposure. Similar to inhalation exposure, the main target of toxicity was the proximal convoluted tubule. In general, the occurrence and severity of lesions increased in a concentration-related manner, beginning with mild histopathological damage after lower level, shorter duration exposures (e.g., single-cell necrosis, renal cell regenerative proliferation) progressing to severe nephropathy characterized by widespread necrosis and degeneration with higher level and/or longer duration exposures (Tables 2-14 and 2-15 in rats and mice, respectively). In rats and mice, the lowest identified LOAELs for renal lesions following acute-duration gavage exposure were 10 and 34 mg/kg/day, respectively (Larson et al. 1994d; Moore et al. 1982). In intermediate-duration gavage exposure studies, the lowest identified LOAELs for rats and mice were 239 and 34 mg/kg/day, respectively (Larson et al. 1994b, 1994d, 1995b). As observed with

inhalation studies, there is some evidence that male mice may be more susceptible to renal toxicity than female mice (Table 2-15).

Table 2-14. Non-Neoplastic Renal Lesions in Rats Following Gavage Exposure to Chloroform

Strain; duration	Dose (mg/kg/day)	Histology	Lesion details	Reference
Acute-duration				
Fischer 344; 4 days	10	↔		Larson et al. 1995a
Osborne-Mendel; 1 day	10–34	↑	Regenerative cell proliferation in the epithelial cells of the proximal tubules of the renal cortex	Templin et al. 1996a
Fischer 344; 1 day	≤34	↔		Templin et al. 1996a
Fischer 344; 1 day	34	↑	Scattered necrosis of the renal proximal tubule	Larson et al. 1993
Fischer 344; 4 days	34	↑	Mild-to-moderate degeneration of renal proximal tubules and tubule epithelial cell proliferation	Larson et al. 1995a
Fischer 344 or Osborne-Mendel; 1 day	90	↑	Regenerative cell proliferation in the epithelial cells of the proximal tubules of the renal cortex	Templin et al. 1996a
Fischer 344; 4 days	90	↑	Mild-to-moderate degeneration of renal proximal tubules and tubule epithelial cell proliferation	Larson et al. 1995a
Fischer 344; 4 days	≤100	↔		Larson et al. 1995b
Fischer 344; 1 day	≤150	↔		Miyagawa et al. 1998
Fischer 344; 7 days	≤179	↔		Potter et al. 1996
Fischer 344 or Osborne-Mendel; 1 day	≥180	↑	Severe renal proximal tubule necrosis and/or vacuolation; regenerative cell proliferation in proximal tubule	Larson et al. 1993; Miyagawa et al. 1998; Templin et al. 1996a
Fischer 344; 4 days	≥180	↑	Necrosis, degeneration, and regeneration of proximal tubule epithelium; proliferation of proximal tubule epithelial cells in renal cortex	Larson et al. 1995a, 1995b
Sprague-Dawley; 10 days	516	↑	Acute toxic nephrosis	Thompson et al. 1974
Intermediate-duration				
Fischer 344; 3 or 4 weeks	≤90	↔		Larson et al. 1995a, 1995b; Lipsky et al. 1993

Table 2-14. Non-Neoplastic Renal Lesions in Rats Following Gavage Exposure to Chloroform

Strain; duration	Dose (mg/kg/day)	Histology	Lesion details	Reference
Fischer 344; 3 weeks	≥100	↑	Increased proliferation of proximal tubule epithelial cells in renal cortex	Larson et al. 1995b
Fischer 344; 3 weeks	180	↑	Progressive degeneration of the proximal tubules	Larson et al. 1995a
Fischer 344; 4 weeks	180	↑	Acute renal cell injury and necrosis; renal cell proliferation	Lipsky et al. 1993
Fischer 344; 3 weeks	400	↑	Tubular dilation and mineralization; increased proliferation of proximal tubule epithelial cells in renal cortex	Larson et al. 1995b
Chronic-duration				
Osborne-Mendel; 78 weeks	≤200	↔		NCI 1976

↑ = increase in histopathological lesions; ↔ = no change; – = not assessed; F= females; M = males

Table 2-15. Non-Neoplastic Renal Lesions in Mice Following Gavage Exposure to Chloroform

Strain; duration	Dose (mg/kg/day)	Histology	Lesion details	Reference
Acute-duration				
B6C3F1; 4 days	≥34	M: ↑	Extensive acute necrosis of the proximal convoluted tubule, regenerative cell proliferation in the renal cortex and medulla	Larson et al. 1994d
Swiss; 1 day	≤59.2	M: ↔		Moore et al. 1982
Swiss; 1 day	65.6	M: ↑	Occasional tubular necrosis and renal regenerative cell proliferation	Moore et al. 1982
Swiss; 1 day	≥199	M: ↑	Widespread tubular necrosis, renal regenerative cell proliferation	Moore et al. 1982
B6C3F1; 4 days	≤238	F: ↔		Larson et al. 1994b
BALB/c; 1 day	≤300	M: ↔		Ewaid et al. 2020
B6C3F1; 1 days	477	F: ↔		Larson et al. 1993
B6C3F1; 4 days	477	F: ↑	Renal regenerative cell proliferation	Larson et al. 1994b
BALB/c; 1 day	700–1,000	M: ↑	Hydropic degeneration	Ewaid et al. 2020

Table 2-15. Non-Neoplastic Renal Lesions in Mice Following Gavage Exposure to Chloroform

Strain; duration	Dose (mg/kg/day)	Histology	Lesion details	Reference
BALB/c; 1 day	1,500	M: ↑	Necrosis of proximal convoluted tubules	Ewaid et al. 2020
Intermediate-duration				
B6C3F1; 3 weeks	≤10	↔		Larson et al. 1994b
B6C3F1; 3 weeks	≥34	M: ↑	Regenerating proximal convoluted tubules	Larson et al. 1994b, 1994d
CD-1; 105 days	41	↔		NTP 1988a
CB6F1; 26 weeks	140	M: ↑ F: ↔	Increased renal cell proliferation	Sehata et al. 2002
CB6F1; 26 weeks	240	↑	Increased renal cell proliferation	Sehata et al. 2002
B6C3F1; 3 weeks	277	M: ↑	Severe degeneration and necrosis of the proximal tubules	Larson et al. 1994b, 1994d
B6C3F1; 3 weeks	≤477	F: ↔		Larson et al. 1994b
Chronic-duration				
ICI; 80 weeks	≤60	↔		Roe et al. 1979
B6C3F1; 78 weeks	≤477	↔		NCI 1976

↑ = increase in histopathological lesions; ↔ = no change; – = not assessed; F= females; M = males

No non-neoplastic renal lesions were reported in rats or mice exposed to chloroform via gavage for 78 weeks via gavage studies at doses up to 200 or 477 mg/kg/day, respectively (NCI 1976). Similarly, in ICI mice, no non-neoplastic lesions were observed at gavage doses up to 60 mg/kg/day for 80 weeks (Roe et al. 1979), although "moderate to severe kidney changes" (not further described) were noted for CBA and CF1 mice similarly exposed to 60 mg/kg/day for 80 weeks (Roe et al. 1979). The apparent inconsistency between chronic- and shorter-duration studies may be attributed to appearance of benign and/or malignant renal tumors in chronic studies (see Section 2.19 for more details). Observed tumors may obscure presence of nonneoplastic lesions or neoplastic kidney lesions may be a natural progression of nonneoplastic lesions following longer-duration exposure.

There is limited evidence of elevated kidney weights in rodents following gavage exposure to chloroform. Elevated kidney weights were observed in rats at acute- and intermediate-duration doses ≥546 and 238.8 mg/kg/day, respectively (Chu et al. 1982b; Lilly et al. 1997). In mice, elevated kidney weights were reported in males at single doses ≥199 mg/kg (Moore et al. 1982) but not females at doses up to 350 mg/kg (Larson et al. 1993).

A limited number of gavage studies evaluated renal clinical chemistry changes in blood or urine. No changes in BUN were observed in rats at acute-duration doses up to 180 mg/kg (Larson et al. 1993). However, changes in urinary levels of AST, LDH, and/or N-acetylglucosaminidase (NAG) were observed after single exposures to gavage doses ≥50 mg/kg (Lilly et al. 1997; Miyagawa et al. 1998). In acute-duration studies, plasma urea levels were elevated in mice exposed once to 273 mg/kg via gavage in corn oil, but not at doses up to 199 mg/kg via gavage in a toothpaste base (Moore et al. 1982). No exposure-related changes in BUN or creatinine were observed in male or female mice exposed to doses up to 250 mg/kg/day for 14 days (Munson et al. 1982) or 240 mg/kg/day for 26 weeks (Sehata et al. 2002).

In contrast to gavage studies, there is no evidence for renal damage in rats or mice following acute- or intermediate-duration exposure via drinking water. No histopathological changes in the kidney were observed in rats at acute- or intermediate-duration drinking water doses up to 68.1 or 200 mg/kg/day, respectively (Chu et al. 1982a, 1982b; EPA 1980; Larson et al. 1995a), or in mice at intermediate-duration drinking water doses up to 435 mg/kg/day (EPA 1980; Larson et al. 1994b). Additionally, no adverse changes in renal clinical chemistry were observed following intermediate-duration drinking water doses of 160 mg/kg/day in rats (EPA 1980).

However, histopathological changes in the kidney were observed in rats following chronic-duration exposure to drinking water doses ≥45 mg/kg/day, including renal tubule cell alterations (nuclear crowding, cytoplasmic vacuolation, cytoplasmic basophilia) and tubular dilation (Hard et al. 2000; Jorgenson et al. 1985; Nagano et al. 2006). Nagano et al. (2006) also reported an increased incidence of glycosuria (15%) in rats exposed to 45 mg/kg/day, compared to controls (0%). Additionally, when a rat strain susceptible to renal damage and tumor development (Eker rats) was exposed to chloroform via drinking water for 10 months, increased incidence of atypical tubules and hyperplasia were observed at ≥27 mg/kg/day in males and 158 mg/kg/day in females; these were the lowest tested doses in each sex (Hooth et al. 2002; McDorman et al. 2003a).

Beagle dogs exposed to chloroform by capsule in toothpaste base daily for up to 7.5 years showed increased fat deposition in the glomeruli at necropsy, performed at death or scheduled sacrifice after a 19-week recovery period (Heywood et al. 1979).

Studies in rabbits include one gavage exposure study in pregnant does and a 24-hour dermal study. Following exposure to ≥100 mg/kg/day for 13 days during gestation, acute toxic nephrosis was observed in does that died (Thompson et al. 1974). Of the two survivors at 100 mg/kg/day, one showed mild fatty

changes. In the dermal study, degenerative tubule changes were observed in rabbits sacrificed 2 weeks after exposure to ≥1,000 mg/kg for 24 hours under occluded conditions (Torkelson et al. 1976).

Mechanisms of Renal Toxicity. As discussed for nasal and hepatic toxicity, the mechanism of chloroform-induced renal toxicity appears to involve metabolism to reactive intermediates. Studies using CYP2E1 knock-out mice and mice pretreated with the cytochrome P450 inhibitor, 1-aminobenzotriazole, showed that CYP2E1 metabolism is required for chloroform to produce renal effects (either proliferation or lesions) (Constan et al. 1999). Reliance on CYP2E1 for toxicity also explains the apparent increased sensitivity in male rodents, particularly mice, compared to females. Several studies proposed that the greater susceptibility of male mice is due to the increased capacity to metabolize chloroform in male kidney tissue due to much higher levels of CYP2E1 activity associated with the influence of testosterone on CYP2E1 gene transcription (Deringer et al. 1953; Eschenbrenner and Miller 1945; Trevisan et al. 2012). Weir et al. (2005) tested this hypothesis directly and showed that coadministration of testosterone with gavage exposure to chloroform for 5 days resulted in renal toxicity in female mice comparable to that observed in exposed males. Similarly, in an acute-duration inhalation study, androgen treatment in female mice resulted in renal toxicity comparable to that observed in male mice, while castration of male mice resulted in renal toxicity comparable to that observed in chloroform exposed female mice (Culliford and Hewitt 1957).

Liu et al. (2013) conducted a series of studies in transgenic mice showing that cytochrome P450-mediated metabolic activation in the renal tubules plays an important role in renal toxicity. Four mouse strains were used with differing levels of the cytochrome P450 reductase (Cpr) gene: wild-type (normal Cpr), CL (low expression of Cpr throughout all tissues), XPT-CL (normal Cpr expression in the proximal tubule, but low levels elsewhere), and PTCN (Cpr gene is deleted specifically in the proximal tubule). As expected, gavage exposure to 200 mg/kg resulted in renal toxicity (elevated BUN and creatinine, renal tubule injury). Chloroform-induced renal effects were ameliorated in both PTCN and CL mice, compared to wild-type, but XPT-CL mice (with normal Cpr expression in the renal tubule) showed renal toxicity similar to effects observed in wild-type mice.

Once chloroform is metabolized, however, the exact mode of action for renal toxicity is unknown. It is likely that binding of phosgene to renal macromolecules could occur, as proposed for hepatotoxicity (IRIS 2001). Gap junction plaques were observed in the kidneys of rats exposed to chloroform via gavage for 3 days or 4 weeks, suggesting impaired intercellular communication (Mally and Chipman 2002). Jan et al. (2000) also proposed a potential role for increased cellular calcium based on

concentration-dependent increases in intracellular calcium concentrations in cultured canine kidney cells exposed to chloroform.

Although data are limited, the rodent kidney appears to be capable of regenerative repair following exposure to chloroform, as seen in the liver (Anand et al. 2006; Philip et al. 2006). Mechanistic pathways are likely similar to those proposed for the liver, which include various cellular signaling pathways (chemokines, cytokines, growth factors, nuclear receptors) that result in promitogenic gene expression and cell division (Anand and Mehendale 2004; Mehendale 1991, 2005). Initiation of this repair pathway via repeat, sublethal chloroform exposures in mice can be protective of acute-duration lethal exposures by mitigating, in part, acute renal toxicity (Philip et al. 2006), resulting in tolerance to low-dose repeat exposures (Anand et al. 2006).

2.11 DERMAL

Redness, swelling, and "mummification" of skin was reported in homicide cases associated with forced inhalation of chloroform via a cloth held to the nose and mouth (Risse et al. 2001). Similarly, redness, edema, blistering, and patchy desquamation of the skin of the face were observed in a woman following a suicide attempt in which she tied a plastic bag containing chloroform around her head (Greene and White 2014). In these cases, observed dermal effects are attributed to direct skin contact with liquid chloroform. In another nonfatal suicide attempt, dermatitis was observed on the face and upper back of a woman following ingestion of 20–30 mL of pure (99%) chloroform (Jayaweera et al. 2017). As with the inhalation case study, the dermatitis is attributed to direct contact with chloroform present in saliva and vomitus that pooled around the subject after she fell unconscious.

Damage to the horny outer layer of the skin (stratum corneum) was observed in three volunteers following repeated application of an unspecified concentration of chloroform to the forearm using a glass cylinder with an opening of 2 cm^2 for 15 minutes/day on 6 consecutive days (Malten et al. 1968). This damage to the barrier skin layer on the forearm resulted in increased water vapor loss for 30 days post-injury, which was more severe in the younger volunteers (<21 years of age), compared to the older volunteer (46 years old). Desquamation of the skin was also observed in a man following accidental dermal exposure via spilling chloroform on his shirt (Vlad et al. 2014). The initial skin reaction was redness without pain; within 3 days, this progressed to a partial thickness burn. In another study, topical application of aspirin dissolved in chloroform (approximately 43.3 mg/mL) was used to relieve pain in 42 patients with pain due to herpes zoster or postherpetic neuralgia (King 1993). The only reported side-

effect was an occasional burning sensation on the skin as the chloroform evaporated from the skin surface.

In inhalation studies in animals, no histopathological changes in the skin were observed in rats following intermediate-duration exposure to concentrations up to 300 ppm (Templin et al. 1996b), or in rats or mice following chronic-duration exposure to concentrations up to 90.1 or 85.8 ppm, respectively (Yamamoto et al. 2002). Following oral exposure, alopecia was noted in pregnant rats exposed to 126 mg/kg/day via gavage for 10 days during gestation (Thompson et al. 1974) and rough coats were reported in mice exposed to ≥100 mg/kg/day via gavage for 14 days (NTP 1988a). Histopathological examination of skin showed no effects of chloroform in rats or mice exposed to gavage doses up to 200 or 477 mg/kg/day, respectively, for 78 weeks (NCI 1976).

In dermal studies, uncovered application of 0.01 mL undiluted chloroform (~5 mg/kg) for 24 hours to the clipped skin of rabbits caused only slight irritation (Smyth et al. 1962), while extensive skin necrosis was observed in rabbits dermally exposed to ≥1,000 mg/kg chloroform for 24 hours under an impermeable plastic cuff (Torkelson et al. 1976).

2.12 OCULAR

No data were located regarding ocular effects in humans after exposure to chloroform.

In inhalation studies in animals, no histopathological changes in the eye were observed in rats or mice following intermediate-duration exposure to concentrations up to 300 or 88 ppm, respectively (Larson et al. 1996; Templin et al. 1996b) or following chronic-duration exposure to concentrations up to 90.1 or 85.8 ppm, respectively (Yamamoto et al. 2002).

One acute-duration oral study in mice reported excessive tearing in male rats prior to death at gavage doses ≥250 mg/kg/day for up to 14 days (NTP 1988a). No other acute-duration oral studies evaluated or reported ocular effects. In an intermediate-duration study in mice, no histopathological changes in the eye were observed at gavage doses up to 240 mg/kg/day (Sehata et al. 2002). In dogs, no ophthalmological changes were observed following exposure via capsule to doses up to 30 mg/kg/day for up to 7.5 years (Heywood et al. 1979).

2.13 ENDOCRINE

Data pertaining to potential endocrine effects in humans following exposure to chloroform are very limited. One population-based, cross-sectional study evaluated potential associations between blood chloroform levels and serum thyroid hormone and autoantibody levels in 2,233 adult men and women from the United States (Sun et al. 2021b). Using 2007−2008 NHANES data, increased serum free thyroxine (T4) levels were associated with increased levels of blood chloroform. No associations were found between blood chloroform levels and serum total T4, total or free triiodothyronine, thyroid releasing hormone, or thyroid autoantibodies TgAb or TPOAb. While the study authors suggested that blood trihalomethane levels (including chloroform) likely reflected exposure to chlorinated drinking water, no attempt was made to ascertain potential exposure histories for subjects in this study. It is noted that serum T4 levels were also associated with blood bromodichloromethane levels and total trihalomethane levels in this study.

In inhalation studies in animals, no histopathological changes were observed in endocrine organs of rats or mice following intermediate-duration exposure to concentrations up to 300 or 88 ppm, respectively (Larson et al. 1996; Templin et al. 1996b), or following chronic-duration exposure to concentrations up to 90.1 or 85.8 ppm, respectively (Yamamoto et al. 2002).

In one intermediate-duration drinking water study in Sprague-Dawley rats, an increased incidence and severity of thyroid lesions was observed in males exposed to 175 mg/kg/day for 90 days (Chu et al. 1982a). Lesions included reduced follicular size, colloid density, and increased epithelial height. Thyroid lesions were not observed in male rats exposed to concentrations up to 193 mg/kg/day for 28 days (Chu et al. 1982b) or female rats exposed to concentrations up to 200 mg/kg/day for 90 days (Chu et al. 1982a). In other drinking water studies, histopathological examination of the endocrine glands did not show adverse effects following exposure to doses up to 160 mg/kg/day in male Osborne-Mendel rats or 435 mg/kg/day in female B6C3F1 mice (EPA 1980). In gavage studies, no exposure-related histopathological changes were observed in endocrine glands in mice exposed to intermediate-duration doses up 240 mg/kg/day (Sehata et al. 2002) or in rats or mice at chronic-duration doses up to 200 or 477 mg/kg/day, respectively (NCI 1976). In dogs, no changes in organ weight or histology were observed in endocrine glands following exposure via capsule to doses up to 30 mg/kg/day for up to 7.5 years (Heywood et al. 1979).

2.14 IMMUNOLOGICAL

Bomski et al. (1967) observed enlarged spleens in a small percentage of workers occupationally exposed to chloroform at 2–205 ppm for 1–4 years in a pharmaceutical plant; splenomegaly was not observed in unexposed control workers. No other immune-related endpoints were evaluated in this study.

One study reported potential associations between increased levels of serum immune markers and exposure to chlorination byproducts while swimming in a chlorinated pool, including chloroform, bromodichloromethane, bromoform, and dibromochloromethane (Vlaanderen et al. 2017). While several cytokines and chemokines were significantly decreased in swimmers following 40 minutes in the pool, none of the changes were clearly associated with chloroform in exhaled breath (or any other chlorination byproduct). Dettling et al. (2016) presented case reports of systemic inflammatory response syndrome (SIRS) following exposure to high levels of chloroform. One case was associated with forced inhalation exposure (via soaked handkerchief) combined with injection exposure; a large increase in leukocyte count was observed within 1 day of exposure. The second case was a result of an attempted suicide via chloroform ingestion, with leukocyte counts continuously increasing over an 11-day period post exposure prior to death. In both cases, blood and urine cultures were negative for bacterial infections that could contribute to increased white cell counts.

In a population-based, cross-sectional study using 2005–2006 NHANES data, blood chloroform levels were associated with increased immunoglobulin E (IgE) allergen-specific antibodies for pets (dogs and cats) in 906 adolescents (12–19 years of age) (Sun et al. 2023b). The study authors indicated that exposure to chloroform occurs from exposure to disinfection byproducts from all sources; however, no specific exposure assessments were conducted for study participants. No associations were observed between blood chloroform (or other trihalomethane) levels and IgE allergen-specific antibodies for molds, dust mites, plants, cockroaches, rodents, or foods.

There is some evidence for impaired immune function in mice following inhalation exposure to chloroform. Mice exposed to 10.6 ppm for 3 hours/day for 5 days showed increased susceptibility to death following *Streptococcus zooepidemicus* infections; this increase in susceptibility was not observed following a single 3-hour exposure (Aranyi et al. 1986). However, impaired immune responses to *S. zooepidemicus* infection reported in mice in another 3-hour exposure study included decreased phagocytic activity of alveolar macrophages at 100 ppm, decreased bacterial clearance in the lung at

500 ppm, and increased susceptibility to infection-related death at 1,000 ppm (Selgrade and Gilmour 2010).

As discussed in Section 2.4 (Respiratory), exposure to 7 ppm of chloroform for 5 days (20-minute exposures 3 times daily) resulted in an inflammatory immune response in the lungs of mice, as evidenced by increases in total leukocytes and macrophages in the BALF of both males and females (de Oliveira et al. 2015). Additional changes in BALF observed in male mice included increases in lymphocytes and neutrophils. However, Ban et al. (2006) did not observe any changes in pulmonary inflammatory immune responses, including cell composition of BALF, in mice exposed to 20 ppm for 4 days (6 hours/day).

Munson et al. (1982) also reported altered immune function in mice following oral exposure to chloroform. Humoral immunity, as measured by primary IgM response to sheep red blood cells (sRBCs) in splenocytes, was significantly decreased in male and female mice exposed via gavage to ≥50 mg/kg/day for 14 days and in male mice exposed to 250 mg/kg/day for 90 days (Munson et al. 1982). Cell-mediated immunity, as measured by delayed-type hypersensitivity response to sRBCs, was significantly decreased in female mice exposed to 250 mg/kg/day for 90 days; this was not observed in males similarly exposed for 90 days or either sex similarly exposed for 14 days (Munson et al. 1982). No changes in hemagglutination titer were observed at either timepoint. In a comprehensive assessment of chloroform immunotoxicity, chloroform had no effect on immune function in female mice exposed for 28 days in drinking water to doses up to 35 mg/kg/day (Auttachoat et al. 2009). Assays included neutrophil myeloperoxidase activity, macrophage cytotoxic/cytostatic activity, natural killer (NK) cell activity, hemolytic plaque assay for detecting IgM antibody-forming cells (antibody-forming cell response to sRBC), quantitation of serum IgM antibody titers to T-dependent antigen (sRBCs), one-way mixed leukocyte response, flow cytometric enumeration of splenocyte immune cell subsets, and host resistance against *Listeria monocytogenes* infection (Auttachoat et al. 2009).

No additional animal studies evaluated the function of the immune system; however, several studies evaluated the weight and/or histology of immune organs. In inhalation studies, no exposure-related changes in immune organ weight and/or histology were observed in rats following acute-duration exposures up to 311 ppm (Baeder and Hofmann 1988), intermediate-duration exposures up to 300 ppm (Templin et al. 1996b; Torkelson et al. 1976), or chronic-duration exposures up to 90.1 ppm (Yamamoto et al. 2002). Similarly, no exposure-related changes in immune organ weight and/or histology were observed in mice at intermediate- or chronic-duration inhalation exposure concentrations up to 88 or 85 ppm, respectively (Larson et al. 1996; Yamamoto et al. 2002). In oral studies, no exposure-related

changes in immune organ weight and/or histology were observed in rats at intermediate- or chronic-duration doses up to 200 mg/kg/day (Chu et al. 1982a, 1982b; EPA 1980; NCI 1976), in mice at intermediate or chronic durations at doses up to 435 or 477 mg/kg/day, respectively (EPA 1980; NCI 1976; Sehata et al. 2002), or in dogs at chronic durations at doses up to 30 mg/kg/day (Heywood et al. 1979).

Mechanisms of Immunotoxicity. Limited information is available pertaining to potential mechanisms of chloroform-mediated changes observed in the immune system. Immunological effects may result from the ability of chloroform to dissociate antigen-antibody complexes, since it can cause dissociation of certain enzyme inhibitor complexes (Berger et al. 1983). *In vitro* treatment of serum with chloroform resulted in a loss of complement activity (Stefanovic et al. 1987). Findings from an *in vitro* study in human keratinocytes indicate that chloroform exposure may induce an inflammatory response via upregulation of thymic stromal lymphopoietin (TSLP), which is dependent upon early growth response 1 (Erg-1) protein expression mediated through the c-JUN N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) signaling pathways (Lee et al. 2015). Inflammatory responses mediated via upregulation of TSLP may exacerbate allergic skin diseases, such as atopic dermatitis.

2.15 NEUROLOGICAL

The CNS is a primary target for chloroform toxicity in humans and in animals at high exposure levels. Based upon systematic review (Appendix C), the nervous system is a known target of chloroform toxicity based on a low level of evidence in human epidemiological studies, high level of evidence in laboratory animal studies, and other relevant data including historical use of chloroform as a general anesthetic, case reports and case series documenting marked neurological effects of chloroform in exposed humans, and a plausible mechanism of action.

Chloroform was once widely used as an anesthetic during surgery in humans but is not currently used as a surgical inhalant anesthetic in modern-day medical practice. Based on historical evidence, increasing the concentration of chloroform gradually to 25,000 or 30,000 ppm during the first 2 or 3 minutes will induce deep anesthesia, which can be maintained at an exposure level of 20,000–25,000 ppm (Featherstone 1947; Smith et al. 1973; Whitaker and Jones 1965). Concentrations of ≈40,000 ppm, if continued for several minutes, could result in death (Featherstone 1947). Concentrations <1,500 ppm are insufficient to induce anesthesia, while concentrations of 1,500–2,000 ppm cause light anesthesia (Goodman and Gilman 1980). A case-series report indicates that the mean arterial blood chloroform at anesthetic levels is

9.8 mg/100 mL, with patients becoming responsive to stimuli with blood levels ≤5 mg/100 mL (Smith et al. 1973). It is common for the patient to be nauseous and/or vomit upon regaining consciousness (Featherstone 1947; Smith et al. 1973; Whitaker and Jones 1965). As discussed in other sections of this profile, inhalation overdose during chloroform-induced anesthesia or intentional inhalation of chloroform for recreational or suicidal/homicidal purposes has been associated with respiratory and cardiovascular effects secondary to depression of the CNS, including death due to respiratory and cardiac arrest.

Recreational inhalation of chloroform has also resulted in unconsciousness (Hutchens and Kung 1985). A case report of an individual addicted to chloroform inhalation for ≈12 years reported psychotic episodes, hallucinations and delusions, and convulsions (Heilbrunn et al. 1945). Withdrawal symptoms, consisting of pronounced ataxia and dysarthria, occurred following an abrupt discontinuation of chloroform use. Moderate, unspecified, degenerative changes were observed in the ganglion cells in the putamen and the cerebellum at autopsy. Death resulted from an unrelated disease.

Occupational data pertaining to neurotoxicity in humans following exposure to chloroform are very limited (Table 2-16). Workers exposed to low levels of chloroform (average of 2.76 ppm for one group of 14 workers and 6.04 ppm for another group of 46 workers) for 1–15 years (average 7.8 years) in factories in China experienced significant increases in dizziness, fatigue, somnolence, insomnia, increased dreaming, impaired memory, anorexia, depression, and anger relative to control workers "without obvious exposure to occupational hazards," based on self-reported symptoms and questionnaire (Li et al. 1993). In formal neurological testing, significant deficits in simple visual reaction time, symbol-digit substitution, digit span, visual retention, and pursuit aiming were seen in the high exposure group relative to controls. In the low-exposure group, the only difference from controls was in pursuit aiming.

In a small cohort of 17 workers exposed to chloroform levels ranging from 223 to 1,163 ppm in an English factory, lassitude and drowsiness were subjectively reported at work and in the evening after work, sometimes persisting through the weekend (Challen et al. 1958). Workers who had been employed long-term (mean of 5.4 service years) reported decreased concentration, slowness, depression, and irritability; these subjective complaints were not made by short-term workers (mean of 15 service months). It is unclear the extent to which co-exposures to other chemicals in these factories may have influenced these results.

A case study of occupational exposure to an unknown level of chloroform reported altered mental status, headache, and dizziness in a patient admitted to the emergency room (Meenakshisundaram et al. 2021).

The patient was a scientist working with high-density chloroform "all night;" about 2 hours after returning home, he vomited and lost consciousness.

Table 2-16. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Neurological Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Occupational exposure			
Challen et al. 1958 Cohort; 8 long-term workers (mean 5.4 service years; mean 50.5 years of age), 9 short-term workers (mean 15 service months; mean 42.9 years of age), and 5 unexposed controls (mean 51.4 years of age) (England)	Range of chloroform levels during current operations (with ventilation system) Mixing room: 128–1,163 ppm Cutting room: 23–71 ppm Range of chloroform levels under historical conditions (without ventilation; relevant for long-term workers) Cutting room: 77–237 ppm	Lassitude/drowsiness	↑ (long-term) ↑ (short-term)
		Decreased concentration/ slowness	↑ (long-term) ↔ (short-term)
		Depression/irritability	↑ (long-term) ↔ (short-term)
Li et al. 1993 Cohort; 61 workers exposed to chloroform for 1–15 years (mean employment of 7.8 years; mean age of 36.02 years) and 83 unexposed controls (23 in control group 1, mean age 36.83 years; 60 in control group 2, mean age of 36.07 years) (China)	Geometric mean chloroform level: 4.19 ppm Mean chloroform level (ppm): Low exposure (n=14): 2.76 High exposure (n=46): 6.04	Subjective symptoms (all exposed versus control 1) Headache Dizziness Fatigue Somnolence Insomnia Increased dreaming Impaired memory	 ↔ ↑ ↑ ↑ ↑ ↑ ↑
		Profile of mood states (all exposed versus control 2) Tension Depression Anger Vigor Fatigue Confusion	 ↔ ↑ ↑ ↔ ↑ ↔
		Neurobehavioral function (low and high exposed groups versus control 2) Visual reaction time Symbol-digit substitution Manual dexterity Digit span Visual retention 	 ↔ (low) ↑ (high) ↔ (low) ↓ (high) ↔ (low) ↔ (high) ↔ (low) ↓ (high) ↔ (low) ↓ (high)

Table 2-16. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Neurological Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
		Pursuit aiming	↓ (low) ↓ (high)

[a]Unless otherwise noted, current exposure levels are reported.

↑ = association; ↓ = inverse association; ↔ = no association

Data regarding neurological effects in humans after oral exposure to chloroform were obtained from clinical case reports. Unconsciousness occurred in cases immediately after intentional ingestion of chloroform (Choi et al. 2006; Dell'Aglio et al. 2010; Rao et al. 1993; Schroeder 1965;), which was followed by coma in some patients (Cui et al. 2022; Jayaweera et al. 2017; Kim 2008; Piersol et al. 1933; Storms 1973). Some cases reporting these effects estimated exposure levels of 2,410–3,755 mg/kg. In most cases, all reflexes were abolished, and pupil size varied. Most patients survived after regaining consciousness; however, one patient died in coma several days later due to extensive liver necrosis (Piersol et al. 1933). Mild cerebellar damage (instability of gait, intentional tremor) was observed in one patient, but reversed to normal in 2 weeks (Storms 1973).

In a dermal case study, nausea, vomiting, and malaise were observed in a man after spilling chloroform on his shirt (Vlad et al. 2014). Findings persisted for 3 days after exposure; at which time he was admitted to the hospital. He made a full recovery.

CNS depression is well-established in animals following inhalation and oral exposure to high levels of chloroform. There is minimal evidence for adverse effects in the nervous system below exposure levels associated with CNS depression.

CNS depression following acute-duration inhalation exposure has been reported in several species. In rats, narcosis is observed following 1-hour exposures to ≥2,233 ppm, with no evidence of decreased alertness at 942 ppm (EPA 1978). In mice, acute-duration exposure results in narcosis at concentrations ≥3,100 ppm (Gehring 1968; Lehmann and Flury 1943), with lethargy reported at 92 ppm (Constan et al. 1999). In cats, exposure to 7,200 ppm resulted in disturbed equilibrium within 5 minutes, light narcosis within 78 minutes, and deep narcosis after 93 minutes (Lehmann and Flury 1943).

Data pertaining to neurological effects following acute-duration inhalation to non-narcotic concentrations are limited. In male rats, alterations in motor activity in an open field during a single 30-minute exposure included increased total distance traveled and decreased vertical activity (rearing) at ≥3,206 ppm of chloroform (DHA 2022). Further, "stereotypic activity" was decreased at ≥401 ppm; however, this behavior was not clearly defined and the adversity is unclear. Male rats similarly exposed showed impaired motor coordination in the rotarod test during the 30-minute exposure at ≥3,206 ppm, including decreased duration of time on the rod and decreased distance traveled. These behavioral changes were not associated with alterations in post-exposure neurotransmitter levels in the brain at concentrations up to 6,411 ppm (DHA 2022). In another acute-duration study, olfactory nerve loss was reported in rats exposed to ≥10.4 ppm for 6 hours/day over a 7-day period (Larson et al. 1994c; Mery et al. 1994). This finding is likely in response to degeneration of the nasal olfactory epithelial tissue observed at the same exposure levels.

In intermediate- and chronic-duration inhalation studies, no clinical signs of neurotoxicity or histopathological changes in the nervous system were observed in rats or mice at concentrations up to 300 or 88 ppm for 13 weeks, respectively (Larson et al. 1996; Templin et al. 1996b), or 90.1 or 85.8 ppm for 104 weeks, respectively (Yamamoto et al. 2002). However, some longer-duration studies employed a stepwise exposure paradigm to gradually increase exposure over several weeks to prevent severe clinical signs of toxicity observed in acute-duration studies (Yamamoto et al. 2002).

In acute-duration oral studies, impaired motor coordination, ataxia, and anesthesia were observed in mice following single gavage exposures to doses ≥350 mg/kg (Balster and Borzelleca 1982; Bowman et al. 1978; Jones et al. 1958). Hemorrhaging in the brain was observed during gross pathological examinations of mice that died under chloroform anesthesia following doses ≥500 mg/kg/day (Bowman et al. 1978). Decreased spontaneous motor activity was noted in male rats exposed to 500 mg/kg/day via gavage for 3 days (Wada et al. 2015). Repeated exposure to gavage doses ≥250 mg/kg/day for 14 days resulted in hunched posture and inactivity in mice (NTP 1988a).

There is limited evidence of behavioral changes in mice at doses below those associated with CNS depression. Landauer et al. (1982) reported induction of conditioned taste aversion to a saccharin solution in mice when it was paired with gavage exposure to chloroform at 30 mg/kg/day for 10 days. Impaired operant conditioning was observed in mice after exposure to ≥100 mg/kg/day via gavage for 60 days, but not 30 days (Balster and Borzelleca 1982). No impairments in operant conditioning were observed following exposure to doses up to 31.1 mg/kg/day for 90 days (Balster and Borzelleca 1982). Adult

female rats trained with a coupled-tone or acetaldehyde odor-cued foot shock paradigm showed no behavioral changes after treatment with up to 400 mg/kg/day chloroform for 3 weeks (Dorman et al. 1997).

No histopathological changes were observed in the brains of rats or mice at intermediate-duration doses up to 200 or 240 mg/kg/day, respectively (Chu et al. 1982a, 1982b; Sehata et al. 2002), or chronic-duration doses up to 200 or 477 mg/kg/day, respectively (NCI 1976; Roe et al. 1979). In dogs, no histopathological changes in the brain were observed after exposure to doses up to 30 mg/kg/day via capsule for 7.5 years (Heywood et al. 1979).

Direct instillation of chloroform into the inner ear caused permanent damage to the cochlea in both guinea pigs and rats, resulting in both hearing and vestibular deficits (Hu and Schwarz 1987; Schwarz et al. 1988). No damage to hair cells or nerve fibers were observed (Schwarz et al. 1988).

Mechanisms of Neurotoxicity. The clinical effects of chloroform toxicity on the CNS are well documented. While the exact molecular mechanism of action is not well understood, the general consensus is that general anesthetics like chloroform are lipophilic membrane perturbants, which result in alterations in proteins that function as ion channels and/or neurotransmitter receptors (Harris and Groh 1985; Jenkins et al. 2001; Nakagawa et al. 2000). Anesthetics may affect calcium-dependent potassium conductance in the CNS (Caldwell and Harris 1985) as well as activation of phospholipase-linked potassium channels (Pavel et al. 2020), and the blockage of potassium conductance by chloroform and other anesthetics resulted in depolarization of squid axon (Haydon et al. 1988). While anesthetics may exert their effect via indirect alteration of protein function through disruption of lipid membrane properties, there is evidence of direct protein binding by chloroform (Johansson 1997; Nakagawa et al. 2000). For example, chloroform directly binds gamma-aminobutyric acid type a (GABA-a) receptors, which results in prolongation of synaptic inhibition (Jenkins et al. 2001).

Chloroform has also been shown to influence other neurotransmitter systems. *In vivo*, acute-duration oral exposure to 200 mg/kg resulted in decreased midbrain 5-hydroxyindoleacetic acid (5-HIAA) levels and increased hypothalamic dopamine concentrations (Kanada et al. 1994). In cortical slices, chloroform inhibited glutamate receptor responses (Carla and Moroni 1992).

2.16 REPRODUCTIVE

Several epidemiological studies have evaluated potential associations between chloroform levels in tap water and reproductive outcomes (Table 2-17). Some of these studies estimated total residential uptake of chloroform from tap water, including oral exposure from drinking as well as dermal and inhalation exposure from bathing, showering, and swimming activities. Findings from these studies should be interpreted with caution as the majority estimated intake based on community-level exposure levels. Additionally, none of the studies controlled for exposure to other known byproducts of water chlorination (e.g., chlorinated and brominated trihalomethanes), some of which have been associated with adverse reproductive outcomes in epidemiological and/or animal studies (Colman et al. 2011; Nieuwenhuijsen et al. 2000). Additionally, while some associations have been reported, no consistent findings have been found across studies.

Table 2-17. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Reproductive Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Pregnancy outcomes			
Costet et al. 2011 Prospective cohort of 3,074 women with nested case control study (105 cases of preterm birth and 2,969 controls) (France)	Estimated[b] chloroform levels in water distribution network serving maternal residences during third trimester (µg/L) Q1: <5 Q2: 5–<10 Q3: 10–<15 Q4: ≥15 Estimated[c] total maternal chloroform uptake during third trimester (µg/day) Q1: <0.068 Q2: 0.068–<0.133 Q3: 0.133–<0.237 Q4: ≥0.237	Preterm birth (<37 weeks gestation)	↔ (chloroform drinking water levels and maternal uptake)
King et al. 2000 Retrospective cohort study, 49,756 births with fetal weights ≥500 g; 214 cases of stillbirths were included in the cohort (Canada)	Chloroform levels in municipal tap water during pregnancy (µg/L) Q1: <50 Q2: 50–74 Q3: 75–99 Q4: ≥100	Stillbirth (all)	↑ (Q4 versus Q1)
		Asphyxia-related stillbirths	↑ (Q4 versus Q1)
		Unexplained stillbirths	↔

Table 2-17. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Reproductive Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Kramer et al. 1992 Case-control study, 342 prematurity cases and 1,710 controls; births occurred from January 1, 1989 to June 30, 1990 (Iowa)	Chloroform levels in municipal tap water in 1987 (µg/L) Group 1: undetectable Group 2: 1–9 Group 3: ≥10	Preterm delivery (<37 weeks of gestation)	↔
Rivera-Núñez et al. 2018 Prospective case-control study, 2,460 stillbirth cases (fetus ≥20 weeks of age or weight ≥350 g) and 24,460 live birth controls (Massachusetts)	Chloroform levels in municipal tap water during second trimester (µg/L) Q1: ≤6.2 Q2: >6.2–23.5 Q3:23.5–37.4 Q4: >37.4–54.0 Q5: >54–192.1	All stillbirths	↔
		Unexplained	↔
		Compression of umbilical cord	↔ (Q2 versus Q1) ↑ (Q3 and Q4 versus Q1) ↔ (Q5 versus Q1)
		Placental separation and hemorrhage	↔
		Prematurity	↔
Savitz et al. 2006 Prospective cohort study, 2,409 pregnant women, mean age of 28.3 years (three locations in the United States; one with high chlorinated DBPs, one with high brominated DBPs, and one with low DBPs)	Mean chloroform level in municipal tap water during periconceptional pregnancy window (µg/L) All sites: 23.9 High chlorinated DBP: 47.9 High brominated DBP: 12.4 Low DBP: 0.2	Pregnancy loss	↔ (all sites)
Villanueva et al. 2011 Prospective cohort study, 2,074 mother-child pairs, mean maternal age 29.9–31.7 years (Spain)	Estimated[d] median residential chloroform uptake during pregnancy from ingestion, inhalation, and dermal exposure to municipal tap water and swimming pool water across five locations (µg/day) Total: 0.03–0.44 Ingestion: 0.01–0.05 Shower/bath: 0.01–0.3 Swimming: 0.04–0.15	Preterm delivery (<37 weeks of gestation)	↔ (total residential) ↔ (ingestion) ↔ (showering/bathing) ↓ (swimming)
Wright et al. 2004 Retrospective population study; 196,000 singleton births, maternal age 12–53 years (Massachusetts)	Chloroform levels in municipal tap water during third trimester (µg/L) T1: 0–26 T2: >26–63 T3: >63–135	Preterm delivery (<37 weeks of gestation)	↔

Table 2-17. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Reproductive Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Zhu et al. 2022 Retrospective population study; 109,182 singleton births, mean maternal age 31.01 years (China)	Mean chloroform levels in tap water between 2016 and 2020: 8.17 (μg/L) *Trimester-specific exposure estimates not reported.*	Preterm delivery (<37 weeks of gestation)	↑ (1st trimester) ↓ (2nd trimester) ↑ (3rd trimester) ↑ (entire pregnancy)
		Premature rupture of membranes	↔ (each trimester) ↑ (entire pregnancy)
		Gestational diabetes	↔
		Gestational hypertension	↔
Menstrual cycle characteristics			
Windham et al. 2003 Prospective population study, 403 women,18–39 years of age (California)	Chloroform levels in municipal tap water (μg/L) Q4: ≥17	Cycle length	↔
		Follicular phase length	↔
		Luteal phase length	↔
Sperm parameters			
Chen et al. 2020 Cross-sectional study with 3-month follow-up, 1,199 healthy men, 22–45 years of age (China)	Chloroform levels in blood at initial visit (ng/L) T1: <12.3 T2: 12.3–19.0 T3: >19.0	Total count Initial Follow-up	 ↓ (T2 and T3 versus T1) ↓ (T2 and T3 versus T1)
		Concentration Initial Follow-up	 ↔ ↔
		Total motility Initial Follow-up	 ↓ (T3 versus T1) ↔
		Progressive motility Initial Follow-up	 ↓ (T3 versus T1) ↔
		Normal morphology Initial Follow-up	 ↔ ↔
Iszatt et al. 2013 Case-control study, 667 men with impaired sperm quality (count ≤20×10^6/mL) and motility ≤60%), and 959 controls, mean age 33.4 years (England and Wales)	Mean chloroform levels in municipal tap water (μg/L): Cases: 25.9 Controls: 27.3	Impaired sperm quality	↔
		Concentration	↔
		Motility	↔

Table 2-17. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Reproductive Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Zeng et al. 2013 Cross-sectional study, 401 men, mean age 30.5 years (China)	Chloroform levels in blood (ng/L): T1: <35.87 T2: 35.87–66.35 T3: >66.35 ng/L	Concentration	↔
		Count	↔
		Motility	↔
		Motion parameters	
		Straight-line velocity	↑ (T3 versus T1)
		Curvilinear velocity	↔
		Linearity	↔
Zeng et al. 2014 Prospective study, 324 fertile and sub-fertile men, mean age of 32.7 years (China)	Estimated[e] residential chloroform uptake from ingestion, inhalation, and dermal exposure to municipal tap water (µg/day) within 90 days of semen collection Ingestion Q1: <0.005 Q2: 0.555–0.011 Q3: 0.011–0.019 Q4: ≥0.019 Showering/bathing Q1: <0.064 Q2: 0.064–0.126 Q3: 0.126–0.246 Q4: ≥0.246	Concentration	
		Ingestion	↓ (Q4 versus Q1)
		Showering/ bathing	↔
		Count	
		Ingestion	↓ (Q3 versus Q1)
		Showering	↔
		Showering/ bathing	
		Motility	↔
		Motion parameters	
		Straight-line velocity	
		Ingestion	↑ (Q4 versus Q1)
		Showering/ bathing	↔
		Curvilinear velocity	
		Ingestion	↑ (Q4 versus Q1)
		Showering/ bathing	↑ (trend)
		Linearity	
		Ingestion	↔
		Showering/ bathing	↓ (Q3 versus Q1)
Serum hormone levels			
Wei et al. 2023 Cross-sectional; 2,633 women >20 years old (2013–2016 NHANES; United States)	Median chloroform levels in blood (ng/L): Low exposure (n=1,316): 10 High exposure (n=1,317): 20	Serum estradiol	↔
		Serum testosterone	↔
		Serum SHBG	↔

Table 2-17. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Reproductive Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Zeng et al. 2013 Cross-sectional study, 401 men, mean age 30.5 years (China)	Median chloroform levels in blood: 50.17 ng/L	Serum testosterone	↔

[a]Unless otherwise noted, current exposure levels are reported.
[b]Maternal environmental exposures levels were estimated by the study authors based on the time-weighted average of regulatory contaminant measurements for municipal drinking water networks in France serving maternal residences during the months of the women's pregnancies. Only data for the third trimester is shown above.
[c]Maternal total uptake via oral, inhalation, and dermal routes was estimated from maternal daily water intake from drinking water networks and bottled water, shower and bath habits, and swimming pool use using uptake factors from the literature.
[d]Maternal ingested dose from tap water was estimated by the study authors based on geographically representative chloroform levels in tap water and daily ingested volume of water (corrected for use of bottled water or water filtration). Dermal and inhalation uptake from bathing, showering, and swimming were modeled by the study authors using uptake factors from the literature. Intake values were estimated for this review from graphically presented data.
[e]Ingested dose from tap water was estimated by the study authors based on geographically representative chloroform levels in tap water and daily ingested volume of water and uptake factors obtained from the literature. Dermal and inhalation uptake from bathing and showering were estimated by multiplying estimated concentrations of chloroform in tap water by minutes/day spent showering or bathing and uptake factors obtained from the literature.

↑ = association; ↓ = inverse association; ↔ = no association; DBP = disinfection byproduct; NHANES = National Health and Nutrition Examination Survey; Q = quartile or quintile; SHBG = sex hormone-binding globulins; T = tertile

A few studies have evaluated potential associations between exposure to chloroform in chlorinated tap water and adverse birth outcomes. One large retrospective cohort study of 49,756 births from Canada observed an increased risk of stillbirth with estimated exposure to municipal tap water concentrations ≥100 µg/L during pregnancy (King et al. 2000). Specifically, associations were observed for asphyxia-related stillbirths. However, no clear associations were observed between maternal exposure to chloroform in tap water and risk of stillbirth in a large prospective study from Massachusetts containing 2,460 stillbirths and 24,460 live birth controls (Rivera-Núñez et al. 2018). Median chloroform levels in tap water were 29.3 µg/L, with a maximum concentration of 192.1 µg/L. In other cohort and case-control studies from the United States, chloroform levels in tap water were not associated with increased risk of pregnancy loss (Savitz et al. 2006) or preterm delivery (Kramer et al. 1992; Wright et al. 2004). A small increase in the risk of preterm birth was observed per unit increase in chloroform levels in municipal tap water during the first and third trimesters in a retrospective cohort of 109,182 live births from China; however, a decreased risk was observed per unit increase in chloroform levels in tap water during the second trimester (Zhu et al. 2022). Individual trimester chloroform levels were not associated with risk of

premature membrane rupture in this cohort; however, increasing chloroform levels over the entire pregnancy were associated with increased risk. Chloroform levels in tap water were not associated with increased risk of gestational diabetes or hypertension.

In a prospective Spanish pregnancy cohort, no association was observed between preterm birth and estimated total maternal residential uptake of chloroform from drinking, bathing, showering, or swimming (Villanueva et al. 2011). When different routes were evaluated, exposure via swimming was associated with a decreased risk of preterm birth. Preterm birth was not associated with chloroform levels in municipal drinking water or estimated maternal total intake (via oral, inhalation, and dermal routes) in a prospective French pregnancy cohort with a nested case-control study (Costet et al. 2011).

In a prospective cohort study of 403 Californian women aged 18–39 years of age, no associations were observed between chloroform levels in tap water and menstrual cycle characteristics (Windham et al. 2003). A population-based, cross-sectional study using NHANES (2013–2016) did not observe associations between blood chloroform levels and sex hormone levels (testosterone, estradiol) or sex hormone-binding globulins (SHBGs) in 2,633 adult women (Wei et al. 2023).

Decreased sperm quality has been associated with chloroform exposure in two studies in China. A cross-sectional study in 1,199 healthy Chinese men reported an inverse association between blood chloroform levels ≥12.3 ng/L and sperm count, total motility, and progressive motility (Chen et al. 2020). At a follow-up 3 months later, only total count was still inversely related to blood chloroform levels measured at the initial visit. No associations were observed for sperm concentration or morphology at either time point. In a prospective study from China, an inverse relationship was observed between sperm concentration and estimated total chloroform ingestion from tap water ≥0.019 µg/day; no association was observed with estimated intake from showering or bathing activities (Zeng et al. 2014). No exposure-related associations were observed between chloroform intake and sperm count or motility. When sperm motion parameters were evaluated, increased estimated chloroform intake via ingestion was associated with improved function (increased straight-line and curvilinear velocity). Another cross-sectional study from China also observed increased straight-line velocity with chloroform levels in blood >66.35 ng/L; however, no associations were observed between sperm concentration, count, or motility or serum testosterone and blood chloroform levels (Zeng et al. 2013). In a case-control study, chloroform levels in tap water (mean 25.9–27.3 µg/L) were not associated with sperm quality (Iszatt et al. 2013).

Inhalation exposure studies in pregnant rodents indicate that inhalation exposure to chloroform impacts pregnancy outcomes at high exposure concentrations, generally at doses associated with systemic maternal toxicity. In pregnant rats, exposure to ≥291 ppm for 7 hours/day for 10 days (GDs 6–15 or 7–16) resulted in increased incidence of resorption and decreased number of live fetuses/litter (Baeder and Hofmann 1988; Schwetz et al. 1974). Increased resorptions were also observed in pregnant rats exposed to 4,117 ppm for 1 hour/day on GDs 7–16 (EPA 1978). In mice exposed to chloroform for 7 hours/day during various gestational windows, decreased numbers of dams with implantation sites were observed at 97–99 ppm on GDs 1–7 or 6–15, and increased resorptions/litter were observed following exposure to 97 ppm on GDs 1–7 (Murray et al. 1979). However, no changes in number of implantation sites or resorptions/litter were observed following exposure to 97 ppm on GDs 8–15 (Murray et al. 1979). Decreased maternal body weight and/or decreased body weight gain were observed at concentrations associated with adverse pregnancy outcomes, with the exception of the mouse study by Murray et al. (1979) with exposure on GDs 6–15.

Similar effects were noted in pregnant animals following gavage exposure to high doses of chloroform during pregnancy. Increased resorptions were observed in rats following exposure to ≥316 mg/kg/day on GDs 6–15, and surviving rabbits exposed to ≥63 mg/kg/day on GDs 6–18 had no viable pregnancies (Thompson et al. 1974). In both rats and rabbits, pregnancy effects were observed at doses associated with systemic maternal toxicity (decreased maternal weight or decreased weight gain). In a 2-generation gavage study, no adverse effects on reproductive performance were observed in mice at doses up to 41 mg/kg/day (NTP 1988a). No exposure-related changes in female reproductive organs were noted; however, degeneration of the epididymal epithelium along with increased epididymal weights were noted in F1 adult males at 41 mg/kg/day (NTP 1988a).

Additional reproductive endpoints were evaluated in other studies that did not evaluate reproductive function (e.g., fertility, pregnancy outcomes, etc.). In male mice, exposure to ≥400 ppm for 5 days (4 hours/day) resulted in a 1.3–2% increase in the percentage of abnormal sperm evaluated 28 days after exposure began (Land et al. 1981). The biological adversity of these minimal changes is unclear, and no additional reproductive endpoints were examined in this study; therefore, this study was not included in the LSE table. In other inhalation studies, no histopathological changes in male or female reproductive organs were identified at intermediate-duration concentrations up to 300 ppm in rats (Templin et al. 1996b; Torkelson et al. 1976) or 88 ppm in mice (Larson et al. 1996). Similarly, no exposure-related changes in male or female reproductive organ histology were observed in rats or mice at chronic-duration exposure concentrations up to 90.1 or 85.8 ppm, respectively (Yamamoto et al. 2002).

In an acute-duration oral study, exposure to 179 mg/kg/day via gavage for 7 days resulted in a decrease in serum testosterone in male rats; no additional reproductive endpoints were examined in this study (Potter et al. 1996). Other studies in male rats did not observe exposure-related histopathological changes in male reproductive organs at doses up to 193 mg/kg/day via drinking water for 28 days (Chu et al. 1982b), 175 mg/kg/day via drinking water for 90 days (Chu et al. 1982a; EPA 1980), or 180 mg/kg/day via gavage for 78 weeks (NCI 1976). Similarly, no histopathological changes were noted in male reproductive organs in mice at gavage doses up to 140 mg/kg/day for 26 weeks (Sehata et al. 2002) or 277 mg/kg/day for 78 weeks (NCI 1976). In female rodents, no exposure-related changes in reproductive histology were observed at gavage doses up to 240 mg/kg/day for 26 weeks in mice (Sehata et al. 2002) or 200 or 477 mg/kg/day for 78 weeks in rats and mice, respectively (NCI 1976). In dogs, no histopathological changes were observed in male or female reproductive organs following exposure to doses up to 30 mg/kg/day via capsule for 7.5 years (Heywood et al. 1979).

Mechanisms of Reproductive Toxicity. Colman et al. (2011) proposed that exposure to trihalomethane drinking water disinfection byproducts, including chloroform, could result in adverse pregnancy outcomes via disruption of hormone levels during pregnancy. This proposed mechanism of action is supported specifically by data for bromodichloromethane from *in vivo* studies in rats and *in vitro* studies in human and rat tissues.

Liu et al. (2023b) proposed that reduced sperm quality associated with exposure to trihalomethanes, including chloroform, in some studies may be attributable to reductions in sperm mitochondrial deoxyribonucleic acid (DNA) telomere length. In support, an inverse association was observed between blood chloroform levels and sperm mitochondrial DNA telomere length in 958 sperm donors. The study authors proposed that oxidative damage may contribute to the observed association. Impaired sperm fertility may also be secondary to prostate gland damage. Wei et al. (2022) reported a positive association between blood chloroform and prostate-specific antigen (PSA) levels in 2,016 men recruited from the general population (NHANES 2001–2010). Since PSA is a key component in prostatic fluid, which mediates coagulation and liquefaction of semen, alterations in PSA levels could impact sperm fertility.

2.17 DEVELOPMENTAL

There is limited and inconsistent evidence for developmental effects from epidemiological studies. There is limited evidence for birth defects in animals following gestational exposure; however, several studies reported delayed ossification and impaired growth at exposure levels generally associated with maternal toxicity. Based upon systematic review (Appendix C), the developing organism is a suspected target of chloroform toxicity based on inadequate evidence in human epidemiological studies and a moderate level of evidence in laboratory animal studies. This is consistent with a systematic review by Williams et al. (2018), which concluded that chloroform is likely to cause developmental effects only at exposure levels associated with maternal toxicity based on weak epidemiological evidence in humans and consistent evidence in animal studies.

Swartz et al. (2015a, 2015b) evaluated potential associations between spina bifida and ambient outdoor chloroform levels during pregnancy in Texas from 1999 to 2004; no association was observed (Table 2-18). No other human studies evaluating potential associations between measured air levels of chloroform and developmental effects were identified.

Table 2-18. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Developmental Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Inhalation exposure via ambient outdoor air			
Swartz et al. 2015a, 2015b Case-control study, 533 cases and 3,695 controls, infants delivered between January 1, 1999 to December 31, 2004 (Texas)	Median levels of chloroform in ambient outdoor during pregnancy: 0.07 µg/m^3	Spina bifida	↔
Multiple potential exposure routes via tap water			
Bonou et al. 2017 Case-control study, 1,432 mother-child pairs (287 SGA age cases, 1,145 controls), >16 years of age (Canada)	Estimated[b] mean (SD) maternal chloroform uptake from ingestion, inhalation, and dermal exposure to municipal tap water during third trimester (µg/day) Cases: 135.4 (145.7) Controls: 133.6 (145.7)	SGA (<10th percentile)	↔

Table 2-18. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Developmental Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Botton et al. 2015 Prospective cohort study, 1,474 mother-child pairs, mean age 29.9–31.4 years (Spain)	Estimated[c] median residential chloroform uptake during 2nd trimester from ingestion, inhalation, and dermal exposure to municipal tap water and swimming pool water (µg/day) All Ingestion Gpuzkoa: 0.1 0.03 Sabadell: 0.2 0.01 Valencia: 0.05 0.01	Postnatal weight gain from birth through 6 months	Total residential uptake ↔ (all locations) Ingestion uptake only ↔ (Gpuzkoa) ↓ (Sabadell) ↔ (Valencia)
Cao et al. 2016 Prospective cohort study, 1,184 pregnant women, mean maternal age of 28.7 years (China)	Median chloroform levels in maternal blood collected at ≥35 weeks of gestation: 50.7 ng/L *Note: the study authors attributed blood levels to drinking water exposure*	Birth weight	↔
		Birth length	↔
		Gestational age at birth	↔
		SGA (<10th percentile)	↔
Costet et al. 2011 Prospective cohort of 3,094 women with nested case control study (171 cases of fetal growth restriction and 2,923 controls) (France)	Estimated[d] chloroform levels in water distribution network serving maternal residences during third trimester (µg/L) Q1: <5 Q2: 5–<10 Q3: 10–<15 Q4: ≥15 Estimated[e] total maternal chloroform uptake during third trimester (µg/day) Q1: <0.068 Q2: 0.068–<0.133 Q3: 0.133–<0.237 Q4: ≥0.237	Fetal growth restriction (<5th percentile of expected birth-weight distribution based on gestation age and sex, parity, and maternal weight and height)	↔ (chloroform drinking water levels and maternal uptake)
Dodds and King 2001 Retrospective cohort study, 48,845 women who delivered an infant between 1988 and 1995 (Canada)	Chloroform levels in municipal tap water during pregnancy (µg/L) Q1: <50 Q2: 50–74 Q3: 75–99 Q4: ≥100	Neural tube defects (n=77)	↔
		Cardiovascular anomalies (n=430)	↔
		Cleft defects (n=82)	↔
		Chromosomal abnormalities (n=96)	↔

Table 2-18. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Developmental Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Grazuleviciene et al. 2011 Prospective cohort study, 3,341 pregnant women that had live births, mean age 28.4 years (Lithuania)	Estimated[f] chloroform uptake from ingestion, inhalation, and dermal exposure to municipal tap water during pregnancy (µg/day) T1: 1.3–24.9 T2: 24.9–286.8 T3: 286.8–2132.8	Low birth weight (<2,500 g)	1st or 3rd trimester or entire pregnancy: ↑ (T2 versus T1) ↑ (T3 versus T1) 2nd trimester: ↔ (T2 versus T1) ↑ (T3 versus T1)
		SGA (<10th percentile)	↔
Grazuleviciene et al. 2013 Prospective cohort study, 3,074 pregnant women that had live births, mean age of 28.4 years (Lithuania)	Estimated[f] chloroform uptake from ingestion, inhalation, and dermal exposure to municipal tap water during first trimester (µg/day) T1: 2–26 T2: 26–288 T3: 288–2109	Heart anomalies	↔
		Musculoskeletal anomalies	↔
		Urogenital anomalies	↔
Hinckley et al. 2005 Retrospective cohort study, 48,119 pregnant women that had live births (Arizona)	Chloroform levels in municipal tap water during third trimester (µg/L) T1: <10 T2: 10–16 T3: ≥16	IUGR (<10th percentile of weight for gestational age)	↔
		Low birth weight (<2,500 g)	↔
Hoffman et al. 2008 Prospective cohort study, 1,854–1,958 pregnancies from two communities with drinking water containing either predominately chlorinated DBPs or brominated DBPs compounds (United States)	Mean measured drinking water chloroform concentration at chlorinated DBP site (µg/L) T1: 19.9–44.2 T2: 44.3–49.0 T3: 49.1–94.0 Mean measured drinking water chloroform concentration at brominated DBP site (µg/L) T1: 6.4–11.5 T2: 11.6–15.6 T3: 15.7–22.1	SGA (<10th percentile)	↔ (chlorinated and brominated sites)
		Birth weight	↔ (chlorinated and brominated sites)

Table 2-18. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Developmental Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Kaufman et al. 2018 Case-control study, 366 cases of craniofacial birth defects and 3,660 controls, live births between 22 and 44 gestational weeks (Massachusetts)	Median (IQR) levels of chloroform in municipal tap water during first trimester (µg/L) 35.4 (15.7–50.2)	Cleft palate	↔
		Cleft lip	↔
		Cleft lip and/or palate	↔
		Eye defects	↔
		Ear defects	↔
Kaufman et al. 2020 Case-control study, 187 cases and 1,870 controls, live births between 22 and 44 gestational weeks (Massachusetts)	Chloroform levels in municipal tap water during first trimester (µg/L) Q1: 0–18.2 Q2: >18.2–35.5 Q3: >35.5–51.4 Q4: >51.4–105.6 T1: 0–26.9 T2: >26.9–48.9 T3: 48.9–105.6	All musculoskeletal defects	↔ (Q2 versus Q1) ↑ (Q3 versus Q1) ↔ (Q4 versus Q1)
		Limb reduction defects (upper and lower)	↔
		Gastroschisis or omphalocele	↔
		Diaphragmatic hernia	↑ (T2 versus T1) ↑ (T3 versus T1)
Kramer et al. 1992 Retrospective case-control study, 187 IUGR cases and 935 controls; 159 low birth weight cases and 795 controls; births occurred from January 1, 1989 to June 30, 1990 (Iowa)	Chloroform levels in municipal tap water in 1987 (µg/L) Group 1: undetectable Group 2: 1–9 Group 3: ≥10	IUGR (<5th percentile of weight for gestational age)	↑ (Group 3 versus 1)
		Low birth weight (<2,500 g)	↔
Levallois et al. 2012 Case-control study, 571 SGA cases and 1,925 controls, maternal age range 25–34 years (Canada)	Chloroform levels in drinking water (µg/L) Q1: <15.96 Q2: 15.96–27.26 Q3: 27.27–51.07 Q4: >51.06 Estimated[g] maternal chloroform total intake (µg/day) Q1: <42.24 Q2: 42.24–80.21 Q3: 80.22–169.81 Q4: >169.81	SGA (<10th percentile)	↔ (chloroform drinking water levels and maternal intakes)

Table 2-18. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Developmental Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Liu et al. 2021 Longitudinal cohort, 1,516 singleton births, mothers recruited during first trimester between 2014 and 2017, maternal age range 18–40 years (China)	Maternal blood chloroform levels over entire pregnancy (ng/L) T1:<7 T2: 7–13 T3: >13 Median: 10.2 *Note: the study authors attributed blood levels to drinking water exposure*	Ultrasound fetal growth measurements (2nd and 3rd trimester)	
		Abdominal circumference	↓ (T2 versus T1) ↔ (T3 versus T1)
		Head circumference	↔
		Biparietal diameter	↔
		Femur length	↔
		Estimated fetal weight	↔
Porter et al. 2005 Retrospective population study, 15,315 singleton births with mean gestation age of 38.8 weeks (Maryland)	Mean (95% CI) chloroform levels in municipal tap water during pregnancy (ppb) 32.5 (32.5, 35.7)	IUGR (<10th percentile of weight for gestational age)	↔
Rivera-Núñez and Wright 2013 Retrospective cohort study, 672,120 births (Massachusetts)	Third-trimester chloroform levels in drinking water (µg/L) Reference: ≤5 Group 1: >5–21 Group 2: >21–36 Group 3: >36–52 Group 4: >52	Birth weight	↑ (Groups 1 and 4 versus reference) ↔ (Groups 2 and 3 versus reference)
		Risk of SGA (<10th percentile)	↓ (Groups 1 versus reference) ↔ (Groups 2, 3, and 4 versus reference)
Summerhayes et al. 2012 Retrospective case-control study, 314,982 live, singleton term births (New South Wales)	Mean (SD) levels of chloroform in municipal tap water (µg/L) 3rd trimester; pregnancy SGA: 33.7 (17.9); 34.0 (16.3) AGA: 33.2 (17.5); 33.6 (15.9)	SGA (<10th percentile)	↑ (3rd trimester and entire pregnancy)

Table 2-18. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Developmental Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Sun et al. 2020 Prospective cohort study, 1,660 mother-infant pairs, maternal age 18–40 years (China)	Maternal blood chloroform levels (ng/L) T1: 1.34–7.45 T2: 7.46–13.46 T3: >13.46 Median maternal blood chloroform levels, by trimester (ng/L) 1st (n=1,636): 10 2nd (n=1,337): 9.6 3rd (n=1,113): 11.2	SGA (<10th percentile)	↔ (1st trimester) ↑ (2nd trimester, T2 and T3 versus T1) ↑ (3rd trimester, T2 versus T1)
Villanueva et al. 2011 Prospective cohort study, 2,074 mother-child pairs, mean maternal age 29.9–31.7 years (Spain)	Estimated[c] median residential chloroform uptake during pregnancy from ingestion, inhalation, and dermal exposure to municipal tap water and swimming pool water across five locations (µg/day) Total: 0.03–0.44 Ingestion: 0.01–0.05 Shower/bath: 0.01–0.3 Swimming: 0.04–0.15	Birth weight	↔ (total residential) ↔ (ingestion) ↔ (showering/bathing) ↑ (swimming, Asturias only)
		SGA (<10th percentile)	↔ (total residential)
		Low birth weight (<2,500 g)	↔ (total residential)
Villanueva et al. 2018 Prospective cohort study, 1,855 mother-child pairs, mean maternal age 31 years (Spain)	Estimated[c] median residential chloroform uptake during pregnancy from ingestion, inhalation, and dermal exposure to municipal tap water and swimming pool water (µg/day) Total (all routes): 0.1 Ingestion: 0.01	Cognitive development	
		Bayley Scales of Infant Development (14 months)	↔ (ingestion) ↔ (total)
		McCarthy Scales of Children's Abilities (4–5 years)	↔ (ingestion) ↔ (total)
Wright et al. 2004 Retrospective population study, 196,000 singleton births, maternal age 12–53 years (Massachusetts)	Chloroform levels in municipal tap water during third trimester (µg/L) T1: 0–26 T2: >26–63 T3: >63–135	SGA (<10th percentile)	↑ (T2 and T3 versus T1)
		Body weight	↓ (T2 and T3 versus T1)
		Gestational age at birth	↔

Table 2-18. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Developmental Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Zaganjor et al. 2020 Case-control study, 191 hypospadias cases and 678 controls (United States)	Chloroform levels in tap water (µg/L) Low: <19.7 Moderate: ≥19.7–<35.0 High: ≥35.0 Estimated[h] maternal ingestion of chloroform (µg/day) Low: <7.5 Moderate: ≥7.5–<28.5 High: ≥28.5 Estimated[i] maternal total uptake of chloroform via all routes (µg/day) Low: <1.43 Moderate: ≥1.43–<2.99 High: ≥2.99	Hypospadias	↔ (water concentration) ↔ (maternal ingestion levels) ↔ (total maternal uptake)
Zhu et al. 2022 Retrospective population study, 109,182 singleton births, mean maternal age 31.01 years (China)	Mean chloroform levels in tap water between 2016 and 2020: 8.17 (µg/L) *Trimester-specific exposure estimates not reported.*	Low birth weight (<2,500 g)	↑ (1st trimester) ↓ (2nd trimester) ↑ (3rd trimester) ↑ (entire pregnancy)
		Risk of SGA (<10th percentile)	↔ (each trimester) ↔ (entire pregnancy)

[a]Unless otherwise noted, current exposure levels are reported.
[b]Maternal ingested dose from tap water were estimated by the study authors based on analysis of chloroform concentration in the water distribution system serving their residence and daily ingested volume of water (after adjustment for home water treatment devices and other handling). Intakes from inhalation and dermal absorption were estimated by the study authors using a PBPK model.
[c]Maternal ingested dose from tap water were estimated by the study authors based on geographically representative chloroform levels in tap water and daily ingested volume of water (corrected for use of bottled water or water filtration). Dermal and inhalation uptake from bathing, showering, and swimming were modeled by the study authors using uptake factors from the literature. Intake values estimated for this review from graphically presented data.
[d]Maternal environmental exposures levels were estimated by the study authors based on the time-weighted average of regulatory contaminant measurements for municipal drinking water networks in France serving maternal residences during the months of the women's pregnancies. Only data for the third trimester is shown above.
[e]Maternal total uptake via oral, inhalation, and dermal routes was estimated from maternal daily water intake from drinking water networks and bottled water, shower and bath habits, and swimming pool use using uptake factors from the literature.
[f]Maternal ingested dose from tap water were estimated by the study authors based on residential exposure index (using geocoded maternal address at birth and measured levels for water zones from all sampling sites for each distribution system) and water-use questionnaire data. Dermal and inhalation uptake from bathing and showering were modeled by the study authors using uptake factors from the literature.
[g]Maternal total uptake was estimated from maternal daily water intake and estimated chlorination by-product concentrations in tap water plus dermal and inhalation exposures, which were calculated from a PBTK model incorporating terms for increased body weight and surface area during pregnancy.

Table 2-18. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Developmental Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result

[h]Maternal ingested dose from tap water were estimated by the study authors based on household chloroform concentrations measured during the exposure assessment, water intake habits at home and at work during the 4-month periconceptional period, and reported changes in water intake habits during pregnancy.
[i]Maternal ingested dose from tap water were estimated by the study authors as described in footnote e. The number of average weekly showers/baths and duration of these activities were obtained from maternal interviews. Dermal and inhalation uptake from bathing and showering were modeled by the study authors using uptake factors from the literature.

↑ = association; ↓ = inverse association; ↔ = no association; AGA= appropriate-for-gestational-age; CI = confidence interval; DBP = disinfection byproduct; IUGR = intrauterine growth retardation; PBPK = physiologically based pharmacokinetic; PBTK = physiologically based toxicokinetic; Q = quartile; SD = standard deviation; SGA= small-for-gestational-age; T = tertile

Numerous studies have evaluated potential associations between chloroform levels in tap water and developmental effects (Table 2-18). Many of these studies estimated total residential uptake of chloroform from tap water, including oral exposure from drinking as well as dermal and inhalation exposure from bathing and showering activities. A few also included estimated uptake from swimming in chlorinated pools. Findings from these studies should be interpreted with caution as the majority estimated intake based on community-level exposure levels. Additionally, very few studies controlled for exposure to other known trihalomethane byproducts of water chlorination, several of which have been shown to cause developmental effects in animals (Colman et al. 2011; Williams et al. 2018). Additionally, while some associations have been reported, no consistent findings have been found across studies.

A few epidemiological studies evaluated potential associations between birth defects and chloroform exposure from municipal tap water (Table 2-18). In a prospective cohort study of 3,341 pregnancies from Lithuania, no associations were observed between total estimated maternal intake from tap water and heart, musculoskeletal, or urogenital anomalies; estimated daily maternal intake levels in the middle tertile ranged from 0.026 to 0.288 µg/day (Grazuleviciene et al. 2013). In a retrospective cohort study from Canada of 48,845 deliveries, no associations were observed between estimated levels in tap water during pregnancy (median of 75 µg/L) and neural tube defects, cardiovascular anomalies, cleft defects, or chromosomal abnormalities (Dodds and King 2001). In case-control studies from Massachusetts, no clear associations were found between chloroform levels in tap water (median levels of 35 µg/L) and increased risk of craniofacial birth defects (cleft palate/lip, eye, or ear defects), musculoskeletal defects, limb reduction defects, or gastroschisis or omphalocele (Kaufman et al. 2018, 2020). However, chloroform

levels in municipal tap were associated with a 6–7-fold increase in the risk of diaphragmatic hernia at tap water concentrations >26.9 µg/L (Kaufman et al. 2020). In a case-control study of women from 10 U.S. states, there was no association between chloroform levels in tap water (median levels of 19.7 µg/L) and risk of hypospadias (Zaganjor et al. 2020). Additionally, no associations were observed between risk of hypospadias and estimated maternal ingestion or total chloroform intake (including potential inhalation and dermal exposure from bathing/showering activities).

Several epidemiological studies evaluated potential associations between birth outcomes (birth weight or length, gestational age at birth, small for gestational age) and chloroform exposure from municipal tap water (Table 2-18). In these studies, low birth weight is defined as <2,500 g in full-term (>37 weeks gestation) delivery, and small for gestational age is defined as birth weight <10^{th} percentile for gestational age. Some studies evaluated intrauterine growth retardation (IUGR) or fetal growth restriction; however, some studies defined IUGR as birth weight <5^{th} percentile for gestational age, while other studies use a definition equivalent to small for gestational age (birth weight <10^{th} percentile for gestational age). To facilitate comparisons across studies in the following text, evaluation of birth weight <10^{th} percentile for gestational age is referred to as small for gestational age and birth weight <5^{th} percentile is referred to as IUGR. Table 2-18 contains endpoints evaluated and definitions as reported by the study authors.

In studies that estimated total residential uptake, one prospective study of 3,074 pregnant women from Lithuania reported an increased risk of low birth weight with estimated maternal intakes ≥24.9 µg/day during the first trimester (Grazuleviciene et al. 2013). However, when adjusted for gestational age, there was no association between chloroform exposure and risk of small for gestational age. Other prospective studies did not observe associations between decreased birth weight, risk of small for gestational age and/or IUGR and estimated maternal residential uptake of chloroform in 1,432 Canadian women (Bonou et al. 2017), 2,074 Spanish women (Villanueva et al. 2011), or 3,094 French women (Costet et al. 2011). In these studies, central estimates of maternal intakes were 133.6–135.4 µg/day in Canadian women, less than ~0.5 µg/day (estimated from graphical presentation) in Spanish women, and 0.133 µg/day in French women. A case-control study in Canadian women also did not observe an association between estimated maternal total chloroform uptake and small for gestational age; the estimated median maternal uptake was approximately 80 µg/day (Levallois et al. 2012).

In studies that evaluated potential associations between chloroform levels in municipal tap water and birth outcomes, retrospective studies reported associations between increased chloroform levels in tap water and increased risk of small for gestational age in 314,982 live, singleton births in New South Wales

(Summerhayes et al. 2012) or 196,000 live, singleton births in Massachusetts (Wright et al. 2004). Wright et al. (2004) also observed an inverse association between chloroform levels in tap water and birth weight; no association was observed between chloroform levels and gestational age at birth. Median chloroform water levels in these studies ranged from 25 to 34 µg/L. Another retrospective study from Iowa reported an increased risk of IUGR with exposure to chloroform levels ≥10 µg/L in tap water prior to pregnancy (tap water exposure during pregnancy was not reported); however, no association was observed between chloroform exposure and risk of low birth weight (Kramer et al. 1992). A small increase in the risk of low birth weight was observed per unit increase in chloroform levels in municipal tap water during the first and third trimesters in a retrospective cohort of 109,182 live births from China; however, a decreased risk was observed per unit increase in chloroform levels in municipal tap water during the second trimester (Zhu et al. 2022). In a retrospective cohort of 672,120 births from Massachusetts, an association was observed between chloroform levels >52 µg/L in drinking water and increased birth weight (8–12 g) among term births (Rivera-Núñez and Wright 2013). Drinking water levels of chloroform were not associated with the risk of small for gestation age (Rivera-Núñez and Wright 2013).

Other available cohort studies from the United States did not observe associations between chloroform levels in tap water and risk of low birth weight or small for gestational age, including a prospective study of 1,854–1,958 live births (Hoffman et al. 2008) and larger retrospective studies of 15,315–48,119 live births (Hinckley et al. 2005; Porter et al. 2005). Chloroform levels in municipal water supplies to maternal residences in France were not associated with IUGR in a prospective cohort of 3,094 women (Costet et al. 2011). Median chloroform levels in tap water from these cohort studies ranged from 10 to 50.2 µg/L. In a case-control study, Levallois et al. (2012) did not identify associations between the risk of small for gestation age and chloroform levels in drinking water. Mean chloroform levels in cases and controls were 43.3 and 41.1 µg/L, respectively.

A meta-analysis of 11 studies identified an association between chloroform levels in maternal drinking water and a slight increase in the risk of small for gestational age (odds ratio [OR]: 1.05, 95% confidence interval [CI]: 1.01–1.08) (Summerhayes et al. 2021).

Two studies from China evaluated potential effects of water disinfection byproducts, using maternal blood chloroform levels as the biomarker of exposure. In a cohort study of 1,516 singleton pregnancies, no exposure-related associations were observed between maternal blood levels (median of 10.2 ng/L) and intrauterine measures of fetal growth (abdominal or head circumference, biparietal diameter, femur

length, estimated fetal weight) during the 2nd or 3rd trimesters (Liu et al. 2021). Similarly, no associations were observed between third-trimester maternal chloroform levels (median 50.7 ng/L) measured in 1,184 pregnant women and birth weight, birth length, gestational age at delivery, or risk of small for gestational age (Cao et al. 2016). Sun et al. (2020) also used maternal blood chloroform levels as a biomarker of exposure in Chinese women to evaluate potential associations between water disinfection byproducts and small for gestational age. In this prospective cohort of 1,660 women, maternal blood levels during the 2nd trimester (median of 9.6 ng/L) were associated with increased risk of small for gestational age. No exposure-related associations were observed with maternal blood levels collected during the 1st or 3rd trimesters (medians of 10 and 11.2 ng/L, respectively).

One prospective study of 1,474 mother-child pairs evaluated potential associations between postnatal growth during the first 6 months after birth and estimated total maternal intake of chloroform via multi-route exposure to tap water and chlorinated pool water during the 2nd trimester (Botton et al. 2015). Three geographically distinct areas in Spain were assessed. Estimated median chloroform intake levels via all routes and ingestion only in these regions were 0.1 and 0.03 µg/day, respectively, in Gpuzkoa; 0.2 and 0.01 µg/day, respectively, in Sabadell; and 0.05 and 0.01 µg/day, respectively, in Valencia. No associations were observed between total residential uptake of chloroform and postnatal growth; however, in Sabadell only, increased estimated chloroform ingestion was associated with decreased postnatal weight gain through 6 months.

In a prospective study of 1,855 mother-child pairs from Spain, Villanueva et al. (2018) did not observe any associations between cognitive development in offspring at 14 months (Bayley Scales of Infant Development) and 4–5 years (McCarthy Scales of Children's Abilities) and estimated maternal residential chloroform uptake during pregnancy via ingestion or all routes (ingestion, bathing/showering, swimming).

As observed in human studies, there is inconsistent evidence of birth defects or adverse birth outcomes in laboratory animals following exposure to chloroform. Schwetz et al. (1974) reported delayed ossification and wavy ribs in rat fetuses following maternal exposure to ≥30 ppm on GDs 6–15, with missing ribs and acaudate fetuses with imperforate anus at ≥95 ppm. Delayed ossification was also observed in fetal mice following maternal exposure to 97–99 ppm on GDs 1–7, 6–15, or 8–15 for 7 hours/day (Murray et al. 1979). Increased incidence of cleft palate was also observed at 97 ppm in fetuses exposed on GDs 8–15 (Murray et al. 1979). However, no fetal variations or malformations were observed in rat fetuses

following maternal exposure to concentrations up to 311 ppm for 7 hours/day on GDs 7–16 (Baeder and Hofmann 1988) or 4,117 ppm for 1 hour/day on GDs 7–14 (EPA 1978).

Decreased fetal growth (decreased weight and/or length) was observed following maternal inhalation exposure to chloroform during gestation. With exposure for 7 hours/day for 10 days on GDs 6–15 or 7–16, fetal rats showed decreased weight and crown-rump length at maternal exposures ≥291 and 311 ppm, respectively (Baeder and Hofmann 1988; Schwetz et al. 1974). When exposure was only 1 hour/day on GDs 7–14, decreased fetal rat weight was only observed with maternal exposure to 4,117 ppm (EPA 1978). Similarly, decreased fetal mouse weights and crown-rump lengths were observed following maternal exposure to 97 ppm on GDs 1–7 or 8–15 for 7 hours/day, but not following maternal exposure to 99 ppm on GDs 6–15 for 7 hours/day (Murray et al. 1979). In all rat and mouse studies, fetal growth effects were only noted at concentrations associated with decreased maternal body weight. However, Schwetz et al. (1974) still noted an effect when findings were compared to a "starved" control group included to match the anorexia observed in dams exposed to 291 ppm.

In oral gestational exposure studies in rats, delayed ossification was observed following maternal exposure to 400 mg/kg/day on GDs 6–15 (Ruddick et al. 1983). No variations or malformations were noted in rats at maternal doses up to 316 mg/kg/day on GDs 6–15 (Ruddick et al. 1983; Thompson et al. 1974). Decreased fetal growth was also observed in rats following maternal gavage exposure to chloroform during gestation. Maternal gavage exposure on GDs 6–15 consistently resulted in decreased fetal body weights; however, the LOAEL varied among studies. Ruddick et al. (1983) observed a 19% decrease at 400 mg/kg/day, with no changes at ≤200 mg/kg/day; Experiment 1 by Thompson et al. (1974) observed an 8% decrease at 126 mg/kg/day, with no changes at ≤50 mg/kg/day; and Experiment 2 by Thompson et al. (1974) observed an unspecified decrease at 316 mg/kg/day, with no changes at ≤300 mg/kg/day. In all gestation-only experiments, fetal body weight effects occurred at doses associated with decreased maternal body weight. In a 2-generation study in mice, no effects on pup weight or survival were seen at gavage doses up to 41 mg/kg/day (NTP 1988a).

In rabbits, delayed ossification and decreased fetal body weight were observed following maternal exposure to ≥20 mg/kg/day on GDs 6–18 (Thompson et al. 1974). When total daily doses were split into two smaller divided doses per day, no developmental effects were observed at maternal doses up to 25 mg/kg/day (Thompson et al. 1974).

In a study designed to test for neurobehavioral effects using a battery of tests in offspring of mice exposed to 31.1 mg/kg/day via gavage from 21 days prior to mating through lactation, no consistent effects were seen that could be attributed to chloroform (Burkhalter and Balster 1979).

One study in rats evaluated potential effects on the developing endocrine system of male rats following maternal exposure to extremely low drinking water concentrations (75 µL/L) from 2 weeks prior to mating through parturition or lactation (Lim et al. 2004). Exposed offspring showed significant alterations in glucose homeostasis on postnatal day (PND) 1; effects did not persist at postnatal weeks (PNWs) 4 or 26 in pups exposed through parturition or lactation. The adversity of transient effects in glucose homeostasis are unclear. Body weights in male offspring were decreased by 14% at weaning in both groups; body weights recovered by PNW 26 in offspring only exposed through parturition but persisted in offspring exposed through lactation. While body weight effects are considered biologically relevant, accurate estimation of dose intake is precluded due to lack of maternal body weight or water intake data. The approximate dose is 0.01 mg/kg/day using the midpoint of reported pre-exposure female rat weight and allometrically determined drinking water intake values based on that pre-exposure body weight (EPA 1988c). However, it is noted that use of the allometric equation based on nonpregnant animals is not appropriate since both weight gain and water consumption will be greater in pregnant and lactating rats. Due to uncertainty in the exposure estimate, as well as lack of additional very low dose studies to corroborate findings, this study is not included in the LSE tables.

2.18 OTHER NONCANCER

One occupational study reported higher rates of abnormally low serum prealbumin (<28 mg/dL) and transferrin (<240 mg/dL) levels among workers exposed to chloroform at a geometric mean of 4.19 ppm for 1–15 years; when stratified by exposure, the findings were more common in workers exposed to a mean level of 6.04 ppm compared to those exposed to a mean level of 2.76 ppm (Li et al. 1993). These findings may be indicative of malnutrition associated with concurrent anorexia reported in these workers.

In a case-control study of multiple chemical sensitivity, both detection rate and levels of serum chloroform were higher in cases compared to controls (Baines et al. 2004). The underlying reason for elevated chloroform levels is unknown, but the study authors suggest that it could be due to increased exposure (e.g., via chlorinated drinking water) and/or impaired detoxification or excretion of chloroform following exposure. However, neither drinking water levels/habits nor toxicokinetics were evaluated in this study.

A study from Russia indicates that increased exposure to chloroform via drinking water may increase risk of childhood metabolic disorders (Luzhetskiy et al. 2015). In this study, the rates of metabolic disorders (excessive nutrition and obesity) were elevated in a group of 212 children (mean age 6.33 years) from an area that had been exposed to drinking water containing chloroform at levels nearly 3 times the maximum allowable concentration (150–170 µg/L) compared to a group of 146 referent children (mean age 6.07 years) exposed to drinking water containing acceptable levels of chloroform (0.3–0.4 µg/L). Blood chloroform analysis confirmed excess chloroform levels in children from the exposed area (0.69 µg/L) compared to the referents (0.29 µg/L).

Decreased glucose levels were observed in female, but not male, mice exposed to ≥125 mg/kg/day for 14 days (Munson et al. 1982). Conversely, elevated glucose levels were reported in both male and female mice exposed to 250 mg/kg/day for 90 days via gavage (Munson et al. 1982). No additional data were identified pertaining to other noncancer effects in animals following exposure to chloroform.

2.19 CANCER

Potential associations between occupational or general population exposure to chloroform and development of cancer have been evaluated in numerous epidemiological studies (Table 2-19). While some associations have been reported, no consistent findings were observed across studies or cancer type. Additionally, reported associations are confounded by co-exposure to other chemicals in occupational settings and/or drinking water. In animal studies, hepatic and/or renal tumors have been reported in rodents following exposure to high levels of chloroform via inhalation or oral exposure.

Table 2-19. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Cancer Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Occupational exposure			
Callahan et al. 2018 Case-control; 1,189 cases and 982 controls, 20–74 years of age (Iowa, California, Washington, Michigan)	Probability of exposure based on occupational history (unexposed, <50%, ≥50%)	Non-Hodgkin's lymphoma	↔

Table 2-19. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Cancer Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Christensen et al. 2013 Case-control study; 3,730 cases and 533 controls, males 35–70 years of age (Canada)	Qualitative exposure based on occupational history (unexposed, any exposure, substantial exposure)	Pancreatic cancer (n=116)	↑ (substantial exposure versus unexposed)
		Bladder cancer (n=484)	↔
		Prostate cancer (n=449)	↔
		Colon cancer (n=496)	↔
		Stomach cancer (n=251)	↔
		Rectum cancer (n=248)	↔
		Non-Hodgkin's lymphoma (n=215)	↔
		Kidney cancer (n=177)	↔
		Melanoma (n=103)	↔
		Esophagus cancer (n=99)	↔
		Liver cancer (n=48)	↔
Gold et al. 2011 Case-control study; 181 cases and 481 controls, 35–74 years of age (Washington, Michigan)	Job-exposure matrix (duration, cumulative exposure with and without 10-year lag)	Multiple myeloma	↔
Infante-Rivard et al. 2005 Case-control study; 790 cases and 790 controls; cases were 0–14 years of age at diagnosis (Canada)	Maternal occupational and home exposure before and during pregnancy estimated based on questionnaire (unexposed, possible, probable, definite exposure)	Childhood acute lymphoblastic leukemia	↔
Neta et al. 2012 Case-control study; 489 glioma cases, 197 meningioma cases, and 799 controls, 18–90 years of age (Arizona, Massachusetts and Pennsylvania)	Job-exposure matrix (ever/never, duration, cumulative, average weekly, and highest exposure)	Glioma	↔
		Meningioma	↔
Purdue et al. 2017 Case-control study; 1,217 cases and 1,235 controls, 20–79 years of age (Michigan, Illinois)	Job-exposure matrix (exposure duration, average weekly exposure, and cumulative hours)	Kidney cancer	↔

Table 2-19. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Cancer Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Ruder et al. 2013 Case-control study; 798 cases and 1,175 controls, 18–80 years of age (Iowa, Michigan, Minnesota and Wisconsin)	Job-exposure matrix (ever/never, cumulative exposure) Mean cumulative exposure to chloroform (ppm-years) Cases: 45.6 Controls: 58.2	Glioma	Ever versus never: ↓ (women) ↔ (men) Cumulative: ↓ (all)
General population exposure			
Bove et al. 2007 Case-control study, 129 cases and 256 controls, white men, 35–90 years of age (New York)	Chloroform levels in drinking water (µg/L) Q1: 0.00–17.14 Q2: 17.42–25.72 Q3: 26.15–38.61[b] Q4: 38.46–192.52	Urinary bladder cancer	↑ (Q4 versus Q1)
Cantor et al. 1978 Retrospective ecological study, 923 counties with 76 drinking water supplies (United States)	Estimated[c] range of chloroform levels in drinking water: 0.003–4.0 µM/L	Cancer mortality: Pancreatic Prostate Kidney Bladder	 ↔ ↔ ↔ ↔
Do et al. 2005 Case-control study, 486 cases and 3,596 controls, 30–75 years of age (Canada)	Mean chloroform in drinking water (µg/L) Cases: 19.5 Controls: 19.3	Pancreatic cancer	↔
Donat-Vargas et al. 2023 Case-control study, 697 cases (including 590 low- to medium-grade tumor cases and 97 high-grade tumor cases) and 927 controls, 20–80 years of age (Spain)	Mean chloroform in residential drinking water (µg/L) Cases: 21.4 Controls: 20.7 T1: <18.7[d] T2:18.4–25.5 T3: 25.5 Calculated mean adult lifetime waterborne ingested chloroform levels (µg/day) Cases: 15.4 Controls: 15.1 T1: <5.4 T2: 5.4–19.1 T3: >19.1	Prostate cancer	↔ (lifetime ingestion) ↑ (drinking water levels, T2 or T3 versus T1)

Table 2-19. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Cancer Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Donat-Vargas et al. 2024 Case-control study, 170 cases (105 Rai stage 0, 61 Rai stage I-IV, 4 unknown) and 1,442 controls, 20–85 years of age (Spain)	Mean chloroform in residential drinking water (µg/L) Cases: 17.9 Controls: 18.5 T1: <17.3 T2: 17.3-22.3 T3: >22.3 Calculated mean adult lifetime waterborne ingested chloroform levels (µg/day) Cases: 14 Controls: 11.6	Chronic lymphocytic leukemia (CLL)	↔ (lifetime ingestion) ↓ (drinking water levels, T3 versus T1 [all cases]) ↓ (per 10 µg/L drinking water, Stage I–IV) ↓ (per 10 µg/L drinking water, males)
Doyle et al. 1997 Prospective cohort study (1986–1993); 41,836 postmenopausal women (Iowa)	Geometric mean chloroform in municipal drinking water (µg/L) Ground water source: 0.231 Surface water source: 46.117 Chloroform levels in drinking water in 1986/1987 (µg/L) Q1: <LOD Q2: 1–2 Q3: 3–13 Q4: 14–287	Total cancer incidence combined (n=983)	↑ (Q3 versus Q1) ↑ (Q4 versus Q1)
		Colon cancer (n=178)	↑ (Q4 versus Q1)
		Upper digestive organ cancer (n=32)	↔
		Rectum/anus cancer (n=78)	↔
		Kidney cancer (n=30)	↔
		Bladder cancer (n=42)	↔
		Lung cancer (n=143)	↔
		Melanoma (n=44)	↑ (Q4 versus Q1)
		Non-Hodgkin's lymphoma (n=98)	↔
		Ovarian cancer (n=50)	↔
		Endometrium cancer (n=133)	↔
		Breast cancer (n=561)	↔
Font-Ribera et al. 2018 Case-control study; 1,003 cases and 1,458 controls, women 20–85 years of age (Spain)	Chloroform levels in drinking water (µg/L) Q1: ≤7.6 Q2: >7.6–18.8 Q3: >18.8–24.3 Q4: >24.3	Breast cancer	↑ (Q4 versus Q1)

Table 2-19. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Cancer Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Heck et al. 2013, 2014 Case-control study; 69 cases of neuroblastoma and 12,257 controls, 69 cases of acute lymphoblastic leukemia and 2,994 controls; 46 cases of acute myeloid leukemia and 19,209 controls; children <6 years of age (California)	Chloroform levels in ambient outdoor air between 1997 and 2007 (ppbv) Mean (SD): 0.034 (0.013) IQR: 0.016	Neuroblastoma	↔
		Acute lymphoblastic leukemia	↔
		Acute myeloid leukemia	↔
Jones et al. 2019 Prospective cohort study (1986–2010); 41,836 postmenopausal women (Iowa) *Note: follow-up to Doyle et al. 1997*	Mean chloroform levels in drinking water (µg/L) Q1: 0.60 Q2 0.60–1.85 Q3: 1.86–8.41 Q4: >8.41	Colon cancer (n=612)	↔
		Rectal cancer (n=155)	↑ (Q3 versus Q1) ↑ (continuous; per 1-unit change in ln-transformed drinking water level)
Medgyesi et al. 2022 Prospective cohort study (1986–2014); 10,544 postmenopausal women (Iowa) *Note: follow-up to Doyle et al. 1997*	Mean concentration of chloroform in drinking water (µg/L) Q1: <0.6 Q2: 0.6–<1.85 Q3: 1.85–<8.41 Q4: 8.41–185.6	Epithelial endometrial cancer	↑ (All women; Q3 or Q4 versus Q1) ↑ (No HRT use; Q3 versus Q1) ↑ (Ever HRT use; Q4 versus Q1)
Min and Min 2016 Prospective population study, 933 adults, 20–59 years of age (1999–2004 NHANES; United States)	Chloroform levels in blood (pg/mL) T1: ≤8.63 T2: >8.63–20.40 T3: >20.41	Total cancer mortality (through 2011)	↔
Gao et al. 2014 Case-control study; 105 cases and 105 controls, <15 years of age (China)	Median concentration of chloroform in indoor air from child's bedroom (µg/m^3) Cases: 2.1 Controls: 1.6	Childhood acute leukemia	↑ (cases versus controls) ↑ (concentration)
Salas et al. 2013 Case-control study; 686 cases and 750 controls, 20–80 years of age (Spain)	Median estimated lifetime exposure to chloroform in drinking water: 15 µg/L	Bladder cancer	↔

Table 2-19. Results of Epidemiological Studies Evaluating Exposure to Chloroform and Cancer Effects

Reference, study type, and population	Measure of exposure[a]	Outcome evaluated	Result
Villanueva et al. 2017 Case-control study; 2,047 cases and 3,718 controls, 20–85 years of age (Spain and Italy)	Estimated lifetime exposure to chloroform in drinking water (µg/L) Q1: <6 Q2: 6–17.4 Q3: 17.4–23.4 Q4: >23.4	Colorectal cancer	↓ (Q2 versus Q1) ↓ (Q3 versus Q1) ↓ (Q4 versus Q1)
Villanueva et al. 2021 Case-control study; 198 cases and 205 controls 48–85 years of age (Spain)	Median concentration of chloroform in drinking water (µg/L) Recent: 11.6 Long-term: 16.5 Estimated ingested levels of chloroform (µg/day) Recent: 2.1 Long-term: 4.4 *Recent = within 3 years of diagnosis; long-term = from age 18 until 2 years prior to diagnosis*	Colorectal cancer	↓ (recent, water levels) ↔ (recent exposures, ingestion) ↔ (long-term, water levels or ingestion)

[a]Unless otherwise noted, current exposure levels are reported.
[b]As reported in Table 4 of study report; there appears to be a typographical error in the primary report.
[c]Estimated from graphically reported data.
[d]As reported in Table 5 of study report; there appears to be a typographical error in the primary report. It is likely that either this value should be 18.4 µg/L or the lower value for the second tertile should be 18.7 µg/L.

↑ = association; ↓ = inverse association; ↔ = no association; HRT = hormone replacement therapy; IQR = interquartile range; LOD = limit of detection; NHANES = National Health and Nutrition Examination Survey; Q = quartile; SD = standard deviation; T = tertile

Several case-control studies have evaluated potential associations between occupational exposure to chloroform and cancer. All of the studies used questionnaires and/or job-exposure matrices to estimate the probability of exposure to various chemicals, including chloroform. One study reported an increased risk of pancreatic cancer in individuals with "substantial" exposure to chloroform, compared to unexposed individuals; however, this was based on a very small number of individuals with pancreatic cancer in the substantial exposure group (n=2) (Christensen et al. 2013). None of the other cancer types evaluated were associated with expected occupational exposure to chloroform based on job history, including non-Hodgkin's lymphoma, melanoma, or cancer of the esophagus, stomach, colon, rectum, liver, kidney, bladder, or prostate (Christensen et al. 2013). One case-control study reported a decreased risk of glioma in individuals with occupational exposure to chloroform (Ruder et al. 2013), while another

reported no association with glioma or meningioma (Neta et al. 2012). In other case-control studies, no associations were observed between occupational exposure to chloroform and non-Hodgkin's lymphoma (Callahan et al. 2018), multiple myeloma (Gold et al. 2011), or kidney cancer (Purdue et al. 2017). Infante-Rivard et al. (2005) did not observe an association between maternal occupational or domestic exposure to chloroform in the years preceding and during pregnancy and childhood acute lymphoblastic leukemia.

In a prospective general population study from the United States (Iowa Women's Health Study Cohort), Doyle et al. (1997) observed an association between total cancer incidence and higher levels of chloroform in drinking water in a large cohort of postmenopausal women followed from 1986 through 1993 (n=41,836). When individual cancer types were analyzed, colon cancer and melanoma were associated with chloroform in drinking water, showing increased risk in the highest quartile of exposure (≥14 µg/L) compared to the lowest (less than the limit of detection). This association held when adjusted for various confounding factors, including age, education, smoking status, pack-years of smoking, physical activity, all fruit and vegetable intake, total energy intake, body mass index, and waist-to-hip-ratio. However, analyses were not adjusted for other potential water contaminants, including other trihalomethane chlorination byproducts. No associations were observed between chloroform levels in drinking water and the other types of cancer evaluated, including non-Hodgkin's lymphoma or cancer of the lung, upper digestive organ, rectum/anus, kidney, bladder, breast, endometrium, or ovary (Doyle et al. 1997).

There was no association between colon cancer and chloroform in drinking water in the follow-up of the Iowa Women's Health Study Cohort followed through 2010 (Jones et al. 2019). However, increasing levels of chloroform in drinking water were associated with increased risk of rectal cancer in this population after adjusting for age, physical activity, smoking status, and nitrate levels. Other cancer types were not evaluated by Jones et al. (2019). Another follow-up of this cohort evaluated endometrial cancer incidence for the period 1986–2014 (Medgyesi et al. 2022). The risk of endometrial cancer was elevated in the two highest quartiles of chloroform exposure (≥1.85 µg/L) compared to the lowest (<0.6 µg/L) after adjustment for several confounders (e.g., age, body mass index, menopause age, oral contraceptive use, parity, smoking status, and nitrate levels). When use of hormone-replacement therapy (HRT) was considered, the risk of endometrial cancer was elevated only in postmenopausal women with a history of current or ever HRT use in the highest quartile (≥8.41 µg/L) of chloroform exposure.

In a small prospective study (n=998), no association was observed between blood levels of chloroform collected during the 1999–2004 Third NHANES and total cancer mortality through 2011 (Min and Min 2016). In a cross-sectional study of NHANES data from 2001 to 2010, a significant association was observed between blood levels of chloroform and PSA, an early biomarker of prostate cancer; however, the incidence of prostate cancer was not evaluated in this study (Wei et al. 2022). In a retrospective ecological study, no associations were observed between mortality associated with pancreatic, kidney, bladder, or prostate cancer and levels of chloroform in drinking water measured in 76 drinking water supplies from 923 counties in the United States (Cantor et al. 1978).

Several case-control studies in the general population have also evaluated potential associations between cancer and chlorination byproducts, including chloroform, in drinking water. One study found an increased risk of urinary bladder cancer in men exposed to ≥38.46 µg/L chloroform in their drinking water, compared to ≤17.14 µg/L (Bove et al. 2007). No association was observed in a second case-control study of bladder cancer in men and women with median chloroform exposure levels in drinking water of 15 µg/L (Salas et al. 2013). An increased risk of prostate cancer was associated with chloroform levels >18.4 µg/L in the Multicase-Control Study in Spain (MCC-Spain) after controlling for several confounders, including other disinfection byproducts (Donat-Vargas et al. 2023). However, when the study authors calculated mean lifetime waterborne ingested chloroform levels for study participants, no associations were observed between estimated lifetime exposure and risk of prostate cancer. A second report from the MCC-Spain study identified a decreased risk of chronic lymphocytic leukemia (CLL) with chloroform levels >22.3 µg/L in residential tap water following covariate adjustment (Donat-Vargas et al. 2024).

Other findings from case-control studies include an association between breast cancer and levels of chloroform in drinking water >24.3 µg/L (Font-Ribera et al. 2018), decreased risk of colorectal cancer with levels of chloroform in drinking water ≥6 µg/L (Villanueva et al. 2017) or per 10 µg/L increase in chloroform in drinking water during the 3-year period prior to diagnosis (Villanueva et al. 2021), and no association between pancreatic cancer and levels of chloroform in drinking water (Do et al. 2005).

One case-control study in children from China reported an increased median bedroom air concentration of chloroform in cases of childhood acute leukemia, compared to controls (Gao et al. 2014). When cases and controls were combined for analysis, risk of childhood acute leukemia increased with increased indoor air levels of chloroform. This association held after adjustment for parental education levels,

parental occupations, parental smoking histories, annual household income, season of indoor detection (summer or not), and outdoor sources of pollution; however, findings were not adjusted for other indoor pollutants that also showed associations with childhood acute leukemia (e.g., styrene, methyl ethyl ketone, methyl isobutyl ketone). In a case-control study in children from California, no associations were observed between ambient atmospheric air levels of chloroform during pregnancy or the first year of life and risk of neuroblastoma, acute lymphoblastic leukemia, or acute myeloid leukemia (Heck et al. 2013, 2014).

A meta-analysis of nine studies (6,142 cases) did not identify associations between chloroform exposure via residential drinking water and risk of all cancer (relative risk [RR]: 1.16, 95% CI: 0.83–1.62) (Shi et al. 2024). Similarly, associations between chloroform levels in drinking water and specific cancer types, including bladder cancer (RR: 1.50; 95% CI: 0.72–3.14; three studies, 857 cases) or colorectal cancer (RR: 0.97, 95% CI: 0.46–1.91; five studies, 2,860 cases) were not identified (Shi et al. 2024).

In a 2-year inhalation cancer bioassay of chloroform in F344 rats and BDF1 mice, renal adenomas and carcinomas were increased in male mice at ≥30 ppm (Yamamoto et al. 2002). Renal tumors were not observed in rats or female mice, and significant induction of tumors was not observed in any other target tissue in either species. In a follow-up study by the same researchers, Nagano et al. (2006) confirmed that 2-year inhalation exposure to chloroform at concentrations up to 100 ppm did not induce renal tumors in male F344 rats. However, combined exposure via both inhalation (100 ppm) and oral routes (1,000 ppm in drinking water, providing approximately 45 mg/kg/day) for up to 104 weeks resulted in increased renal adenomas and carcinomas, compared to unexposed controls or male rats exposed only via a single route. Since the combined inhalation plus oral dose was higher than either single-route dose tested, the study design is inadequate to determine if findings with multi-route exposure are additive or synergistic.

Kidney and liver tumors have been observed in rodents following oral exposure to chloroform. In a 78-week gavage study in Osbourne-Mendel rats and B6C3F1, exposure-related tumors included renal carcinomas and adenomas in male rats at 180 mg/kg/day and hepatocellular carcinomas in male and female mice at ≥138 and 238 mg/kg/day, respectively (Dunnick and Melnick 1993; NCI 1976). Liver tumors (described as hepatomas) were also seen in all Strain A mice (NCI-maintained colony with low spontaneous hepatoma incidence) exposed to ≥594 mg/kg/day via gavage for 30 days (Eschenbrenner and Miller 1945). In drinking water studies, increased incidences of renal tubular cell adenoma or adenocarcinoma were observed in male Osbourne-Mendel rats at 160 mg/kg/day; however, no exposure-related tumors were observed in female B6C3F1 mice at doses up to 263 mg/kg/day (Jorgenson et al.

1985). Female rats and male mice were not evaluated in the drinking water study by Jorgenson et al. (1985). In a second drinking water cancer bioassay, lifetime exposure to 200 mg/kg/days did not induce liver tumors in male or female Wistar rats including neoplastic nodules in female rats and hepatic adenofibrosis (similar to cholangiocarcinoma) in both male and female rats (Tumasonis et al. 1985, 1987). No exposure-related tumors were observed in B6C3F1 mice following exposure to drinking water doses up to 257 mg/kg/day for 52 weeks (Klaunig et al. 1986). In A/J mice (prone to tumor development), no exposure-related induction of lung tumors was observed following gavage exposure to 1,800 mg/kg/day for 8 weeks (Stoner et al. 1986).

In a series of studies using chloroform administered in a water-miscible toothpaste base, Roe et al. (1979) found increased incidences of kidney tumors in male ICI Swiss mice exposed to 60 mg/kg/day for 80 weeks in three separate experiments. The observed kidney tumors included benign cortical adenomas and potentially malignant hypernephromas. Male ICI mice treated with 17 mg/kg/d did not develop kidney tumors, and neither did female ICI mice at either dose. One of the experiments included male mice from three other strains (C57BL, CBA, CF/1), none of which showed an increase in kidney tumors. In one of the experiments, a separate group of male ICI mice were treated with 60 mg/kg/day chloroform by gavage in oil rather than toothpaste. These mice showed a larger increase in kidney tumors than the corresponding male ICI mice treated with chloroform in toothpaste. No statistically significant increase in tumor incidence was observed in the liver or other tissues in any of these mice. Similar studies conducted in male and female Sprague-Dawley rats for 80 weeks and male and female beagle dogs for 7.5 years found no significant tumor increases in kidney, liver, or other tissues (Heywood et al. 1979; Palmer et al. 1979).

A few oral studies evaluated the potential for chloroform to be a tumor initiator or promoter when administered before or after known carcinogens, respectively. In F344 rats, chloroform acted as a promoter of diethyl nitrosamine-induced preneoplastic foci in the liver following exposure to 800 mg/kg/day via gavage for 20 days or ≥25 mg/kg/day for 11 weeks (2 days/week) (Deml and Oesterle 1985). However, another study reported a dose-dependent reduction in diethyl nitrosamine-induced preneoplastic foci in the liver of F344 rats following exposure to chloroform at drinking water doses up to 98 mg/kg/day for 12 weeks (Reddy et al. 1992). In Sprague-Dawley rats, chloroform did not promote diethyl nitrosamine-induced preneoplastic foci in the liver following exposure to 252 mg/kg/day for 8 weeks or ethyl nitrosourea-induced liver tumors in Swiss mice following exposure to 342 mg/kg/day for 47 weeks (Herren-Freund and Pereira 1987). A single exposure to chloroform at doses up to 263 mg/kg

did not act as a tumor initiator in Sprague-Dawley rats subsequently exposed to phenobarbital in drinking water for 11 weeks (Herren-Freund and Pereira 1987).

IARC (1999) determined that chloroform is possibly carcinogenic to humans based on sufficient evidence in experimental animals and inadequate evidence in humans. EPA (IRIS 2001) determined that chloroform is likely to be carcinogenic to humans by all routes of exposure under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues; it is not likely to be carcinogenic to humans by any route of exposure at dose levels that do not cause cytotoxicity and cell regeneration (see IRIS 2001 for further information). This weight-of-evidence determination is based on findings in animal studies. HHS (NTP 2016) determined that chloroform is reasonably anticipated to be a human carcinogen based on sufficient evidence from animal studies.

Mechanisms of Carcinogenicity. The mode of action for chloroform carcinogenicity has been extensively reviewed (Borgert et al. 2015; Boobis 2009; de Castro Medeiros et al. 2019; IARC 1999; IRIS 2001; Meek et al. 2002, 2003). The consensus from the scientific community is that the cancer mode of action for chloroform is cytotoxicity followed by regenerative hyperplasia. Cytotoxicity is attributed to tissue-reactive metabolites (e.g., phosgene) formed during metabolism of chloroform via the CYP2E1 pathway. This pathway predominates at low exposure levels, and target tissues that form tumors (liver, kidney) that are capable of metabolizing chloroform via this pathway. This repeated cytotoxicity, followed by cell proliferation, increases the risk of spontaneous DNA mutation and subsequent tumor formation. In support, numerous rodent studies showed cell proliferation in the liver and kidney following inhalation or oral exposures (Sections 2.9 and 2.10). This proposed pathway is more plausible than a direct mutagenic mode of action via DNA reactivity based on evidence that chloroform is not a strong mutagen or DNA binding agent (Section 2.20). Additionally, growth stimulation in the absence of cytotoxicity (as opposed to regenerative proliferation) is an unlikely mode of action due to lack of evidence for direct hyperplasia, apoptosis inhibition, or receptor activation (Boobis 2009).

IRIS (2001) and Boobis (2009) presented the following key events in the cancer mode of action for chloroform:

1. Oxidative metabolism of chloroform to the reactive metabolite phosgene by CYP2E1 in target tissue (liver, kidney).
2. Repeated/sustained cytotoxicity in hepatocytes and/or renal proximal tubule epithelial cells.
3. Regenerative cell proliferation in the liver and kidney.

4. Development of liver and kidney tumors due to increase in spontaneous cell mutation (due to increased cell division) and/or clonal expansion of cells initiated during the regenerative cell process.

This proposed mode of action is consistent with a nonlinear carcinogenic dose-response (Borgert et al. 2015; IRIS 2001; Meek et al. 2002, 2003).

2.20 GENOTOXICITY

The majority of the available data indicates that chloroform has a low genotoxic potential. A few studies indicate that chloroform may be a weak mutagen and DNA damaging agent at relatively high concentrations. Additionally, there is limited evidence that chloroform may cause clastogenic and epigenetic changes in mammalian cells. *In vitro* and *in vivo* studies of the genotoxic effects of chloroform are summarized in Tables 2-20 and 2-21, respectively.

Table 2-20. Genotoxicity of Chloroform *In Vitro*

Species (test system)	Endpoint	Results		Reference
		Activation		
		With	Without	
Prokaryotic organisms				
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	Araki et al. 2004
S. typhimurium TA98, TA100, TA1535	Reverse mutation	–	–	Gocke et al. 1981
S. typhimurium TA98, TA100, RSJ100	Reverse mutation	–	–	Kargalioglu et al. 2002
S. typhimurium TA98 and TA100	Reverse mutation	+	+	Khallef et al. 2018
S. typhimurium TA100	Reverse mutation	–	–	Kundu et al. 2004
S. typhimurium TA100	Reverse mutation	–	–	Le Curieux et al. 1995
S. typhimurium TA1535 (+GST)[a]	Reverse mutation	Not tested	(+)	Pegram et al. 1997
S. typhimurium TA1535	Reverse mutation	Not tested	–	Pegram et al. 1997
S. typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	Simmon et al. 1977
S. typhimurium TA1535, TA1538	Reverse mutation	–	–	Uehleke et al. 1977
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	Van Abbé et al. 1982
S. typhimurium TA98, TA1535, TA1537	Reverse mutation	–	(+)	Varma et al. 1988
S. typhimurium TA100	Reverse mutation	(+)	(+)	Varma et al. 1988

Table 2-20. Genotoxicity of Chloroform *In Vitro*

Species (test system)	Endpoint	Results		Reference
		Activation		
		With	Without	
S. typhimurium TA97, TA98, TA100, TA102	Reverse mutation	+ (TA98, TA100) – (TA97, TA102)	+ (TA97, TA100, TA102) – (TA98)	Zhang et al. 2021
S. typhimurium TA100, TA1535, TA97, TA98	Reverse mutation	–	–	NTP 2018b
Escherichia coli WP2*uvr*A/pKM101	Reverse mutation	–	–	Araki et al. 2004
E. coli WP2/pKM1010	Reverse mutation	–	–	Araki et al. 2004
E. coli WP2/pKM1010 (+GSH)[b]	Reverse mutation	Not tested	+	Araki et al. 2004
E. coli WP2P and WP2*uvr*A-p	Reverse mutation	–	–	Kirkland et al. 1981
E. coli PQ37	DNA damage	–	–	Le Curieux et al. 1995
Non-mammalian eukaryotic cells				
Saccharomyces cerevisiae	Reverse mutation	–	(+)	De Serres et al. 1981
S. cerevisiae	Recombination	Not tested	+	Brennan and Schiestl 1998
Schizosaccharomyces pombe	Recombination	(+)	–	Callen et al. 1980
Aspergillus nidulans	Aneuploidia	Not tested	+	Crebelli et al. 1988, 1995
Mammalian cells				
L5178Y mouse lymphoma cells	Forward mutation	+	–	Mitchell et al. 1988
L5178Y mouse lymphoma cells	Forward mutation	+	–	Myhr and Caspary 1988
Chinese hamster lung fibroblasts	Mutation at 8-azaquinone	Not tested	–	Sturrock 1977
Primary human lymphocytes	Chromosome aberrations	–	–	Kirkland et al. 1981
Chinese hamster ovary cells	Chromosome aberration	(+)	–	NTP 1988b
Primary human lymphocytes	Sister chromatid exchange	Not tested	+	Morimoto and Koizumi 1983
Primary human lymphocytes	Sister chromatid exchange	–	–	Kirkland et al. 1981
Chinese hamster ovary cells	Sister chromatid exchange	–	Not tested	White et al. 1979
Chinese hamster ovary cells	Sister chromatid exchange	–	–	NTP 1988c

Table 2-20. Genotoxicity of Chloroform *In Vitro*

Species (test system)	Endpoint	Results		Reference
		Activation		
		With	Without	
Human lymphoblastic leukemia cells	DNA damage	Not tested	–	Geter et al. 2004a
Primary human airway epithelial cells	DNA damage	Not tested	(+)	Landi et al. 2003
Human-derived hepatoma line (HepG2 cells)	DNA damage	Not tested	(+)	Zhang et al. 2012
Rat hepatocytes	DNA damage	Not tested	–	Geter et al. 2004a
Primary human lymphocytes	Unscheduled DNA synthesis	–	–	Perocco and Prodi 1981
Primary rat hepatocytes	Unscheduled DNA synthesis	Not tested	–	Larson et al. 1994a

[a]Cells transfected with rat theta-class glutathione S-transferase T1 (GST1).
[b]Tested with GSH supplemented S9 mix.

+ = positive results; (+) = weakly positive results; – = negative results; DNA = deoxyribonucleic acid; GSH = glutathione; GST = glutathione S-transferase

Table 2-21. Genotoxicity of Chloroform *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Mammals			
Mouse (inhalation)	Gene mutation (hepatocytes)	–	Butterworth et al. 1998
Mouse (gavage)	Sister chromatid exchange in bone marrow	+	Morimoto and Koizumi 1983
Rat (gavage)	Micronuclei in renal cells	+	Robbiano et al. 1998
Rat (GW, W)	DNA damage (duodenum, liver, kidney)	–	Geter et al. 2004a
Rat (GO)	DNA damage (glandular stomach, liver)	–	Wada et al. 2015
Rat (GO)	Unscheduled DNA synthesis (kidney)	+	Lipsky et al. 1993
Rat (GW)	Unscheduled DNA synthesis (kidney)	–	Lipsky et al. 1993
Rat (GO)	Unscheduled DNA synthesis (hepatocytes)	–	Mirsalis et al. 1982
Mouse (GO)	Unscheduled DNA synthesis (hepatocytes)	–	Larson et al. 1994a
Mouse (IP)	Micronuclei in bone marrow	+	NTP 2018a
Mouse (IP)	Chromosome aberrations in bone marrow	–	NTP 1987a

Table 2-21. Genotoxicity of Chloroform *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Mouse (IP)	Sister chromatid exchange in bone marrow	–	NTP 1987b
Human (multi-route; blood levels measured)	Oxidative DNA damage (urinary 8-OHdG)	+	Liu et al. 2020
Rat (W)	Oxidative DNA damage (renal 8-OxoG levels)	–	McDorman et al. 2005
Nonmammalian eukaryotic organisms			
Drosophila melanogaster	Sex-linked recessive lethals	–	Gocke et al. 1981
Grasshopper embryo	Mitotic arrest	+	Liang et al. 1983
Pleurodeles waltl (newt) larvae	Chromosomal aberrations in erythrocytes	–	Le Curieux et al. 1995

– = negative result; + = positive result; 8-oxoG = 8-oxoguanine; 8-OHdG = 8-hydroxy-2-deoxyguanosine; DNA = deoxyribonucleic acid; GO = gavage in oil; GW = gavage in water; IP = intraperitoneal injection; W = drinking water

Chloroform was nonmutagenic in the majority of *Salmonella typhimurium* assays, with or without metabolic activation (Araki et al. 2004; Gocke et al. 1981; Kargalioglu et al. 2002; Kundu et al. 2004; Le Curieux et al. 1995; NTP 2018b; Simmon et al. 1977; Uehleke et al. 1977; Van Abbé et al. 1982). However, mutagenicity was reported in one or more *S. typhimurium* strains in a few studies. One study reported a concentration-related mutagenic effect with or without metabolic activation in *S. typhimurium* strains TA98 and TA100 (Khallef et al. 2018). Varma et al. (1988) reported weak mutagenicity in several strains without metabolic activation and in TA100 with metabolic activation. Zhang et al. (2021) identified mutagenicity in TA97 and TA102 with metabolic activation, TA98 without metabolic activation, and TA100 with and without metabolic activation. A third study reported weak mutagenicity in TA1535, but only when cells were transfected with rat theta-class glutathione S-transferase T1 (Pegram et al. 1997).

Chloroform was not mutagenic in *Escherichia coli* without metabolic activation or standard S9 metabolic activation (Araki et al. 2004; Kirkland et al. 1981). However, addition of GSH to the standard S9 mix resulted in increased mutations in *E. coli* WP2/pKM1010, but not WP2uvrA/pKM101 (Araki et al. 2004). Weak mutagenicity was observed in *Saccharomyces cerevisiae* without metabolic activation (De Serres et al. 1981). No exposure-related sex-linked recessive lethal mutations were observed in *Drosophila melanogaster* following exposure to chloroform (Gocke et al. 1981).

In mammalian cells, mutations were not observed without metabolic activation in mouse lymphoma cells or Chinese hamster lung fibroblasts (Mitchell et al. 1988; Myhr and Caspary 1988; Sturrock 1977). However, when metabolic activation was added to mouse lymphoma cells, forward mutations were observed (Mitchell et al. 1988; Myhr and Caspary 1988). *In vivo*, gene mutations were not induced in mouse hepatocytes following inhalation exposure to concentrations up to 90 ppm for 10, 30, 90, or 180 days (Butterworth et al. 1998).

There is mixed evidence regarding chromosomal effects in mammalian cells following exposure to chloroform *in vitro*; examinations of cell types, metabolic activation, and exposure concentrations do not clearly explain differential findings in these studies. One study reported induction of sister chromatid exchanges in human primary lymphocyte cells in the absence of metabolic activation at concentrations ≥2,000 µg/mL (Morimoto and Koizumi 1983). At lower concentrations (maximum of 400 µg/mL), neither sister chromatid exchanges nor chromosomal aberrations were induced in human primary lymphocyte cells with or without metabolic activation (Kirkland et al. 1981). Sister chromatid exchanges were also not induced in Chinese hamster ovary (CHO) cells following exposure to concentrations up to 5,000 µg/mL with or without metabolic activation (NTP 1988c) or vapor levels of 0.71% v/v for 1 hour in the presence of metabolic activation (White et al. 1979). Chromosome aberrations were not induced in CHO cells without metabolic activation at concentrations up to 1,600 µg/mL but equivocal results were observed with metabolic activation at 5,000 µg/mL (NTP 1988b).

In *S. cerevisiae* yeast cells, chromosomal recombination was induced in *S. cerevisiae* without metabolic activation at concentrations ≥2,980 µg/mL (Brennan and Schiestl 1998). In contrast, weak evidence of recombination was observed in *Schizosaccharomyces pombe* yeast cells with metabolic activation only at the highest concentration of 6,400 µg/mL; recombination was not observed without metabolic activation (Callen et al. 1980).

A limited number of *in vivo* mammalian cells indicate chloroform is clastogenic; however, as observed with *in vitro* studies, findings are somewhat inconsistent. Increased frequency of sister chromatid exchange in bone marrow cells was seen in mice gavaged with a 50 mg/kg/day of chloroform for 4 days (Morimoto and Koizumi 1983). However, sister chromatid exchanges were not induced in bone marrow erythrocytes of male mice after a single intraperitoneal injection to doses up to 800 mg/kg (NTP 1987b). In other studies, the frequency of micronucleated kidney cells was increased approximately 3-fold in rats following a single gavage of 478 mg/kg (Robbiano et al. 1998) and the frequency of micronucleated bone marrow erythrocytes was increased 1.9-fold in male mice following intraperitoneal injections of

400 mg/kg/day for 3 days (NTP 2018a). In contrast, chromosome aberrations were not induced in male mice following a single intraperitoneal injection at doses up to 1,000 mg/kg (NTP 1987a).

In non-mammalian species, mitotic arrest was induced in grasshopper embryos exposed to chloroform (Liang et al. 1983); however, chromosomal aberrations were not observed in erythrocytes of newt larvae exposed to up to 50 µg/mL of chloroform in their swimming water for 12 days (Le Curieux et al. 1995).

There is limited evidence that chloroform may be a weak DNA damaging agent. No DNA damage was observed in *E. coli* with or without metabolic activation (Le Curieux et al. 1995). In mammalian cells, there was weak evidence of DNA damage at high exposure levels in primary human airway epithelial cells and human-derived hepatoma HepG2 cells in the absence of metabolic activation (Landi et al. 2003; Zhang et al. 2012). Chloroform did not induce DNA damage or unscheduled DNA synthesis in human lymphoblastic leukemia or primary lymphocyte cells, primary human lymphocytes, or rat hepatocytes exposed *in vitro* (Geter et al. 2004a; Larson et al. 1994a; Perocco and Prodi 1981).

In an epidemiological cohort study, blood chloroform levels were associated with increased urinary 8-hydroxy-2-deoxyguanosine (8-OHdG) levels, a marker of oxidative DNA damage (Liu et al. 2020). Evidence of oxidative DNA damage was not observed in the rat kidney following exposure to 1.8 g/L in drinking water (~250 mg/kg/day) for 3 weeks (McDorman et al. 2005). In gavage studies, unscheduled DNA synthesis (UDS) was observed in the kidney when rats were exposed once to ≥90 mg/kg via gavage in oil, but not at doses up to 180 mg/kg via gavage in water (Lipsky et al. 1993). No evidence of UDS was observed in rat or mouse hepatocytes after single gavage doses up to 400 or 477 mg/kg in oil, respectively (Larson et al. 1994a; Mirsalis et al. 1982). No DNA damage was observed in the rat gastrointestinal tract, liver, or kidney at doses up to ~2,000 mg/kg via gavage in water (single exposure) or 300 mg/kg/day via drinking water for 2 weeks (Geter et al. 2004a). Similarly, no DNA damage was observed in the glandular stomach or liver of rats exposed to doses up to 500 mg/kg/day for 3 days via gavage in oil (Wada et al. 2015).

Epigenetic changes have also been associated with chloroform exposure. Global decreases in DNA methylation have been observed in mouse liver cells following oral exposure to chloroform for 11 days (Coffin et al. 2000) or 54 days (Mostafa et al. 2009). Similarly, global DNA methylation was decreased in mouse kidney cells following oral exposure to chloroform for 7 days (Tao et al. 2005). Targeted analyses following oral exposure in mice have observed hypomethylation of the promotor region of the

c-*myc* protooncogene, which was associated with increased messenger ribonucleic acid (mRNA) expression of c-*myc,* in the liver (Coffin et al. 2000; Pereira et al. 2001) and kidney (Tao et al. 2005).