Toxicological Profile for Chloroform

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U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

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DISCLAIMER

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FOREWORD

This toxicological profile is prepared in accordance with guidelines developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance due to associated acute-, intermediate-, and chronic-duration exposures;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, intermediate, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staffs of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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Agency for Toxic Substances and Disease Registry
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VERSION HISTORY

Date	Description
October 2024	Final toxicological profile released
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September 1997	Final toxicological profile released
April 1993	Final toxicological profile released
January 1989	Final toxicological profile released

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ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

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CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

1.1 OVERVIEW AND U.S. EXPOSURES

Chloroform (also known as trichloromethane or methyl trichloride) is a volatile colorless liquid with a pleasant non-irritating odor and a slightly sweet taste. Chloroform is produced naturally via biological and physical processes. Most of the chloroform produced by industry in the United States is used as a chemical intermediate, specifically for producing refrigerants and polymers used for non-corrosive, waterproof, or nonstick liners. Additionally, chloroform may be used as a solvent in various industrial applications. Historically, chloroform was also used as an anesthetic during surgery, but it is no longer used for this purpose due to availability of safer alternatives.

Chloroform produced by industry can enter the environment from chemical companies' waste sites. Chloroform can also enter the environment as an unwanted disinfection byproduct that originates from the chlorination of drinking water. Chloroform is readily volatile and enters the air directly from factories that make or use it and by evaporating from water and soil that contain it. Chloroform enters water and soil when any wastewater containing chlorine is released into the environment and may migrate from soil to groundwater due to its low sorption. Since chloroform is produced naturally and is formed as a byproduct of chlorine in water, small amounts are likely to be found almost everywhere. Chloroform's half-life in the atmosphere is on the order of months and is persistent in aerobic environments; anaerobic biodegradation occurs more readily, especially at low chloroform concentrations.

Chloroform levels have been fairly well characterized in ambient and indoor air, food, and drinking water supplies. Detections are generally in the ppb range. Limited monitoring studies of surface water, groundwater, soil, and sediment were located. The general population is most likely to be exposed to chloroform through inhalation of indoor and outdoor air, ingestion of food or disinfected water, or dermal contact with disinfected water. Chloroform contamination of these media most likely results as a byproduct of disinfection of water by chlorine. Chloroform will readily volatize from treated water to indoor and outdoor air. Thus, low levels of chloroform vapor may be breathed in while using treated water during bathing or cleaning or during food preparation.

Populations with increased exposure to chloroform are expected to be people who work in industries that use or manufacture chloroform, or who work or reside near sources where chloroform may form as a disinfection byproduct. Limited occupational exposure data were available. Individuals who both work

in facilities that manufacture/use chloroform and live nearby (e.g., fence line communities) may have increased risk of higher cumulative exposure due to both occupational plus residential exposure. Other populations with increased risk of exposure include people who are around chlorinated water for extended periods of time, such as when swimming or cleaning, or are living near hazardous waste sites with chloroform contamination.

1.2 SUMMARY OF HEALTH EFFECTS

Information on the noncancer toxicity of chloroform in humans primarily comes from numerous case series and case reports following medical use as an anesthetic, intentional exposure (e.g., recreational, suicidal, or homicidal purposes), or accidental exposure. There are a limited number of occupational exposure studies informing noncancer toxicity of chloroform. Additionally, many epidemiological studies examine potential toxic effects following exposure to chloroform as a water disinfection byproduct. Further information on the noncancer toxicity of chloroform comes from numerous inhalation and oral studies in animals. Data following dermal exposure are very limited in humans and animals.

As illustrated in Figures 1-1 and 1-2, sensitive targets in laboratory animals following inhalation and/or oral exposure include the respiratory, hepatic, renal, and neurological systems, along with the developing organism.

A systematic review of these endpoints resulted in the following hazard identification conclusions:

- Respiratory effects are a presumed health effect for humans following inhalation exposure.
- Hepatic effects are a known health effect for humans.
- Renal effects are a presumed health effect for humans.
- Neurological effects are a known health effect for humans.
- Developmental effects are a suspected health effect for humans.

Figure 1-1. Health Effects Found in Animals Following Inhalation Exposure to Chloroform

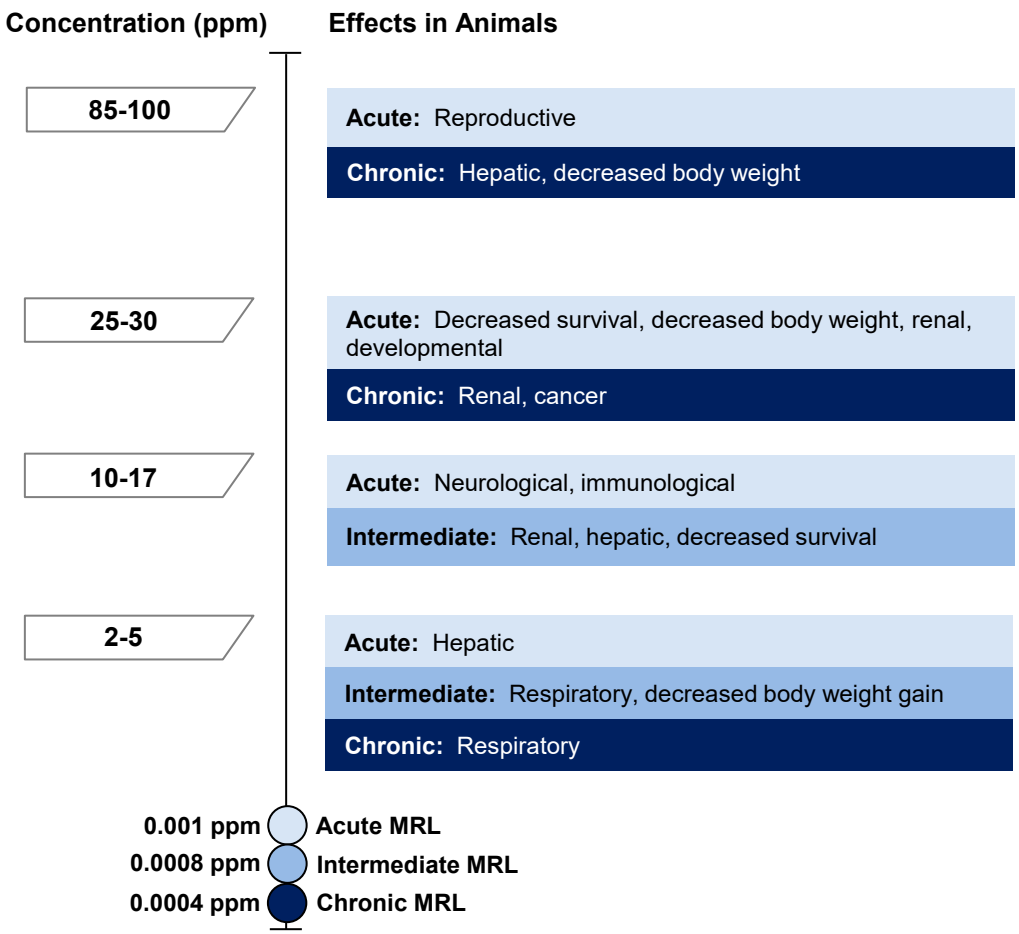
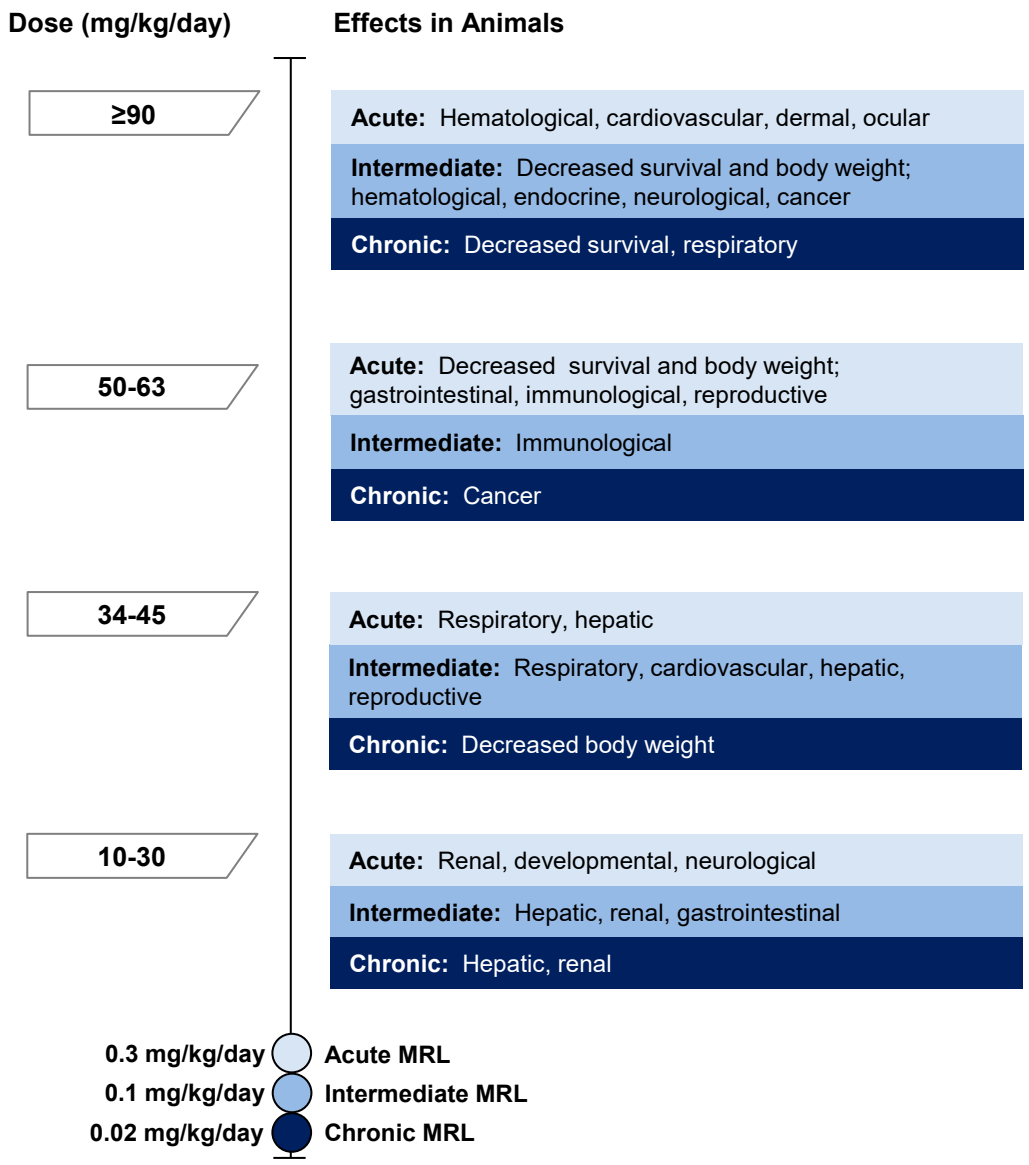


Figure 1-2. Health Effects Found in Animals Following Oral Exposure to Chloroform



Respiratory Effects. In humans, depression of respiratory rates and/or respiratory arrest have been reported in case reports of high-level exposure via inhalation (Jayaweera et al. 2017; Storms 1973; Whitaker and Jones 1965); these effects are likely secondary to central nervous system (CNS) depression. Lung damage has been reported in several fatal cases of inhalation or oral exposure (Section 2.4). In animals, the nasal epithelium and the underlying nasal bones are consistent targets of toxicity in rodents following acute-, intermediate-, and chronic-duration inhalation exposure (Constan et al. 1999; Kasai et al. 2002; Larson et al. 1994c, 1996; Mery et al. 1994; Templin et al. 1996b; Yamamoto et al. 2002) and acute- and intermediate-duration gavage exposure (Dorman et al. 1997; Larson et al. 1995b; Templin et al. 1996a).

Damage to the lower respiratory tract in animals was generally only observed at lethal exposure levels (Bowman et al. 1978; Kasai et al. 2002; NCI 1976). However, there is limited evidence of inflammatory responses in the lung at low inhalation exposure levels in mice (de Oliveira et al. 2015).

Hepatic Effects. There is some evidence of adverse hepatic effects with occupational exposure to chloroform, with effects reported in some studies (Bomski et al. 1967; Kang et al. 2014; Lin et al. 2005; Phoon et al. 1983) but not others (Callen et al. 1958; Li et al. 1993). However, the results of occupational studies should be interpreted with caution due to study limitations, including poor exposure characterization, small subject numbers, and lack of control for confounding factors (e.g., co-exposures). Despite limitations, findings reported in some workers are supported by numerous case series and case reports, which indicate that the liver is a clear target of toxicity in humans following inhalation exposure to high levels of chloroform (Giusti and Chiarotti 1981; Hwang and Kim 2022; Lionte 2010; Royston 1924; Townsend 1939). In fatal ingestion cases, acute liver failure and/or severe liver damage have also been found at autopsy (Dettling et al. 2016; Piersol et al. 1933). In numerous nonfatal cases of inhalation or ingestion, reversible clinical signs of hepatotoxicity manifest within 1–7 days of exposure (Section 2.9).

The liver is also a clear target of toxicity in animals. Hepatic lesions have been observed following acute-, intermediate-, and chronic-duration inhalation and oral exposure in rodents; intermediate- and chronic-duration oral exposure in dogs; and in an acute-duration oral exposure in rabbits (Section 2.9). Typical lesion progression begins with mild histopathological damage after low and/or brief exposures (e.g., lipid accumulation, cellular swelling and vacuolation, scattered necrosis, hepatocellular proliferation) and progresses to widespread and severe necrosis and degeneration with high level and/or

long-term exposure. In oral studies, rodents exposed via gavage were more susceptible to hepatotoxicity than those exposed via drinking water (Larson et al. 1994b, 1995a).

Renal Effects. Several case reports indicate that the kidney is a target of chloroform toxicity in humans following exposure to high levels via inhalation or oral routes. Fatal exposures have been associated with renal damage (Piersol et al. 1933; Royston 1924), while reversible changes in clinical chemistry and urinalysis have been reported in nonfatal cases (Dettling et al. 2016; Gosselink et al. 2012; Piersol et al. 1933; Schroeder 1965; Sridhar et al. 2011; Wallace 1950). The kidney is a clear target of toxicity in animals. Renal lesions have been observed following acute-, intermediate-, and chronic-duration inhalation and oral exposure in rodents; intermediate- and chronic-duration oral exposure in dogs; and acute-duration oral and dermal exposure in rabbits (Section 2.10). Typical lesion progression begins with mild histopathological damage after low and/or brief exposures (e.g., tubular dilation, single-cell necrosis, renal cell proliferation) and progresses to severe nephropathy characterized by widespread necrosis and degeneration with higher level and/or longer-term exposure. In oral studies, rodents exposed via gavage are more susceptible to renal toxicity than those exposed via drinking water.

Neurological Effects. Chloroform was previously utilized as a common general anesthetic, so it is a known CNS depressant at high exposure levels in both humans and animals (Section 2.15). 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 (2.76–6.04 ppm), including impaired hand-eye coordination, slowed reaction time, and memory impairments (Li et al. 1993). Chloroform-exposed workers also had increased subjective complaints, including dizziness, fatigue, somnolence, insomnia, increased dreaming, anorexia, depression, and anger (Challen et al. 1958; Li et al. 1993). In animals, the only reported effects at concentrations below those associated with frank CNS depression included impaired operant conditioning and paired taste aversion (Balster and Borzelleca 1982; Landauer et al. 1982). The only histopathological change reported in the neurological system is olfactory nerve loss in rats following acute-duration inhalation exposure (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.

Developmental Effects. Many epidemiological studies evaluated potential associations between developmental effects and exposure to disinfection byproducts in chlorinated water (Table 2-18). Some studies reported associations between chloroform exposure from tap water and measures of impaired growth, including low birth weight (Grazuleviciene et al. 2011; Wright et al. 2004), intrauterine growth

restriction (Kramer et al. 1992), small for gestational age (Summerhayes et al. 2012; Sun et al. 2020; Wright et al. 2004), and decreased postnatal weight gain (Botton et al. 2015). However, no associations were noted in several other studies (Bonou et al. 2017; Cao et al. 2016; Hinckley et al. 2005; Liu et al. 2021; Porter et al. 2005; Villanueva et al. 2011). No clear associations were observed between chloroform exposure and birth defects (Dodds and King 2001; Grazuleviciene et al. 2013; Kaufman et al. 2018, 2020; Zaganjor et al. 2020) or neurodevelopmental outcomes (Villanueva et al. 2018).

There is also inconsistent evidence for fetal malformations or variations in animals following exposure to chloroform. There is limited evidence for missing ribs and acaudate fetuses with imperforate anus in rats (Schwetz et al. 1974) and cleft palate in mice (Murray et al. 1979) following maternal inhalation exposure during gestation. These defects were not observed in additional developmental studies in rats exposed via inhalation (Baeder and Hofmann 1988; EPA 1978) or rats or rabbits exposed orally (Ruddick et al. 1983; Thompson et al. 1974). Delayed ossification and decreased fetal growth were reported in many developmental studies after inhalation or oral exposure, generally only at levels associated with maternal toxicity (Section 2.17).

Cancer Effects. There is limited evidence of associations between chloroform exposure and cancer in humans. One occupational study reported an increased risk of pancreatic cancer in workers with "substantial" exposure to chloroform, but no association with a wide variety of other forms of cancer (Christensen et al. 2013). Additional occupational studies found no associations between chloroform exposure and several other forms of cancer (Section 2.19). Some epidemiological studies evaluating the potential risk of cancer and exposure to disinfection byproducts in chlorinated water reported associations between urinary bladder cancer, colon cancer, rectal cancer, melanoma, breast cancer, and childhood acute leukemia and chloroform exposure from tap water (Bove et al. 2007; Doyle et al. 1997; Font-Ribera et al. 2018; Gao et al. 2014; Jones et al. 2019). However, several other epidemiological studies did not observe associations with these or other forms of cancer (Section 2.19). In animals, chronic-duration inhalation exposure is associated with renal tumors in mice (Yamamoto et al. 2002) and chronic-duration oral exposure is associated with hepatic and renal tumors in rats (Jorgenson et al. 1985; NCI 1976; Tumasonis et al. 1985, 1987) and mice (Eschenbrenner and Miller 1945; NCI 1976; Roe et al. 1979).

The U.S. Environmental Protection Agency (EPA) determined that chloroform is likely to be carcinogenic to human by all routes of exposure under dose conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues; it is not likely to be carcinogenic by any route at dose levels that do not cause those effects (IRIS 2001). The International Agency for Research on Cancer

(IARC) determined that chloroform is possibly carcinogenic to humans based on inadequate evidence in humans and sufficient evidence in experimental animals (IARC 1999). The Department of Health and Human Services (HHS) determined that chloroform is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals (NTP 2021).

1.3 MINIMAL RISK LEVELS (MRLs)

The inhalation database was considered adequate for derivation of acute-, intermediate-, and chronic-duration inhalation MRLs for chloroform. As illustrated in Figure 1-3, the respiratory, hepatic, and neurological systems appear to be the most sensitive targets of chloroform toxicity following inhalation exposure. Immunological and body weight effects also have relatively low lowest-observed-adverse-effect level (LOAEL) values. The MRL values are summarized in Table 1-1 and discussed in greater detail in Appendix A.

The oral database was considered adequate for derivation of acute-, intermediate-, and chronic-duration oral MRLs for chloroform. As illustrated in Figure 1-4, the hepatic, renal, and developmental systems appear to be the most sensitive targets of chloroform toxicity following oral exposure. The MRL values are summarized in Table 1-1 and discussed in greater detail in Appendix A.

Figure 1-3. Summary of Sensitive Targets of Chloroform – Inhalation

Available data indicate that the respiratory, hepatic, and neurological systems are the most sensitive targets of chloroform inhalation exposure.

Numbers in triangles and circles are the lowest LOAELs (ppm) among health effects in humans and animals, respectively.

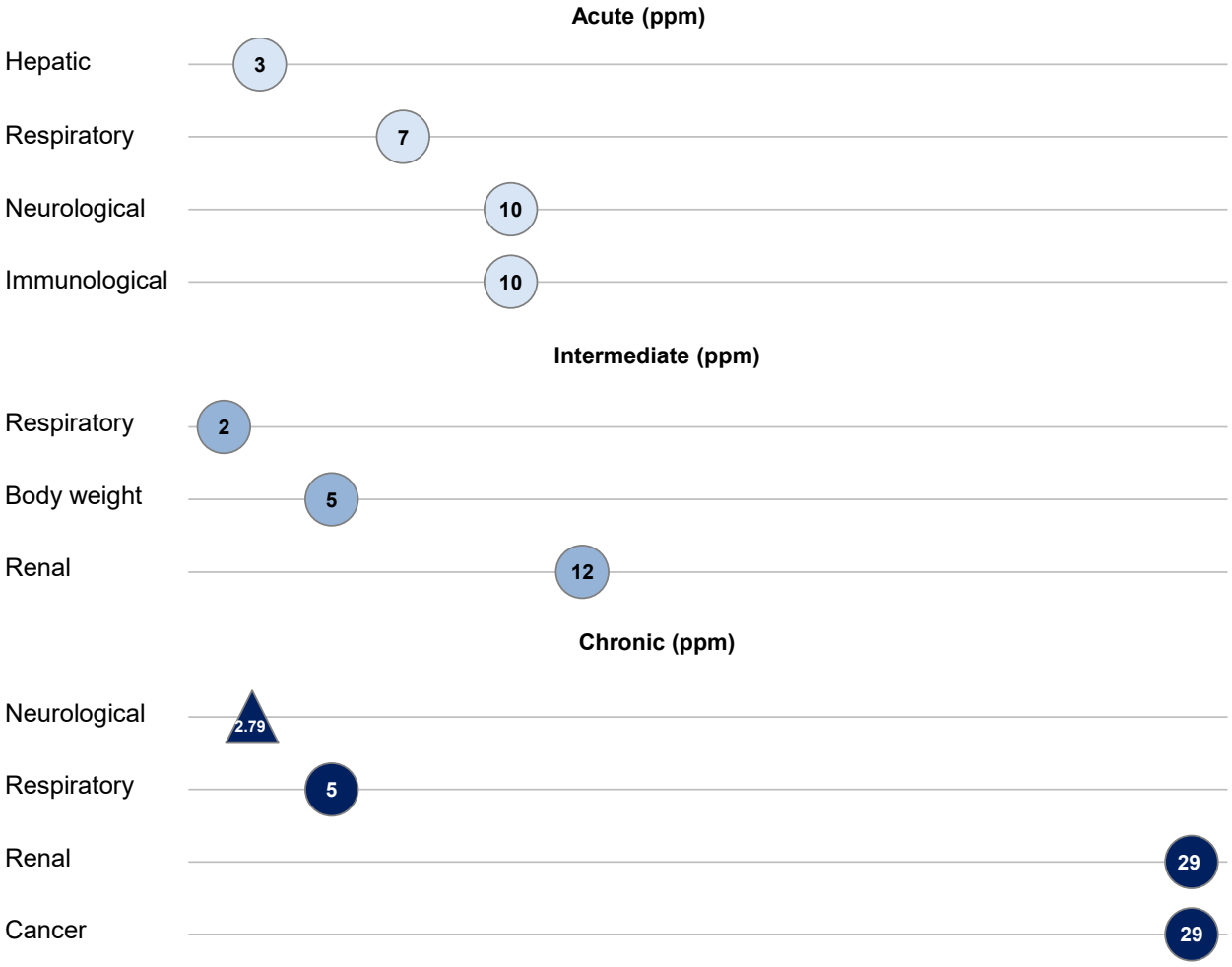


Figure 1-4. Summary of Sensitive Targets of Chloroform – Oral

Available data indicate that the hepatic, renal, and developmental systems are the most sensitive targets of chloroform oral exposure.

Numbers in circles are the lowest LOAELs for all health effects in animals; no reliable dose response data were available for humans.

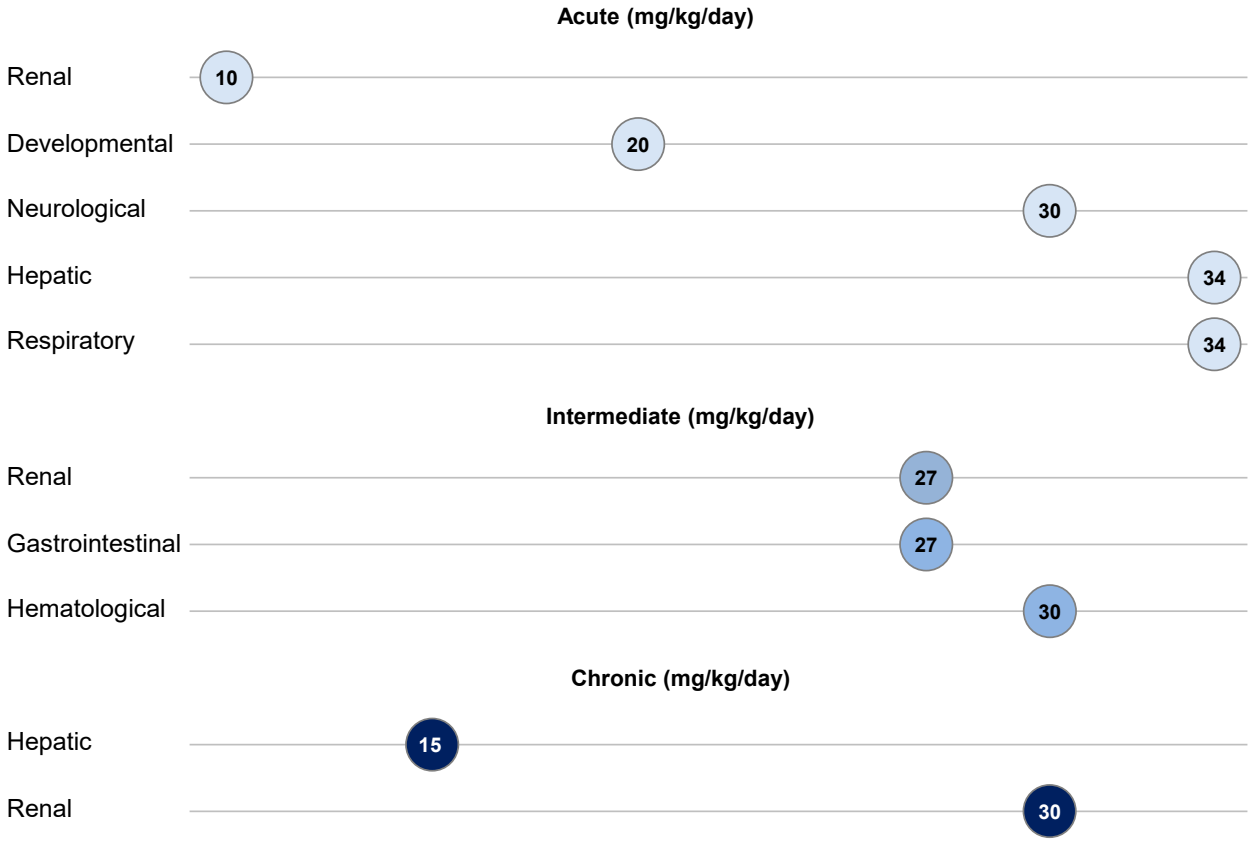


Table 1-1. Minimal Risk Levels (MRLs) for Chloroform[a]

Exposure route	Exposure duration	MRL	Critical effect	POD type	POD value	Uncertainty/ modifying factor	Reference
Inhalation	**Acute**	**0.001 ppm** (0.005 mg/m^3)	Nasal lesions	$NOAEL_{HEC}$	0.04 ppm	30	Larson et al. 1996; Templin et al. 1996b
	Intermediate	**0.0008 ppm** (0.004 mg/m^3)	Nasal lesions	$LOAEL_{HEC}$	0.07 ppm	90	Templin et al. 1996b
	Chronic	**0.0004 ppm** (0.002 mg/m^3)	Nasal lesions	$LOAEL_{HEC}$	0.11 ppm	300	Yamamoto et al. 2002
Oral	**Acute**	**0.3 mg/kg/day**	Hepatic lesions	NOAEL	26 mg/kg/day	100	Larson et al. 1994b
	Intermediate	**0.1 mg/kg/day**	Increased serum ALT (~2-fold)	$NOAEL_{ADJ}$	13 mg/kg/day	100	Heywood et al. 1979
	Chronic	**0.02 mg/kg/day**	Hepatic lesions	$BMDL_{ADJ}$	1.84 mg/kg/day	100	Heywood et al. 1979

[a]See Appendix A for additional information.

ADJ = adjusted for continuous/daily exposure; ALT = alanine aminotransferase; BMDL = benchmark dose lower confidence limit; HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

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

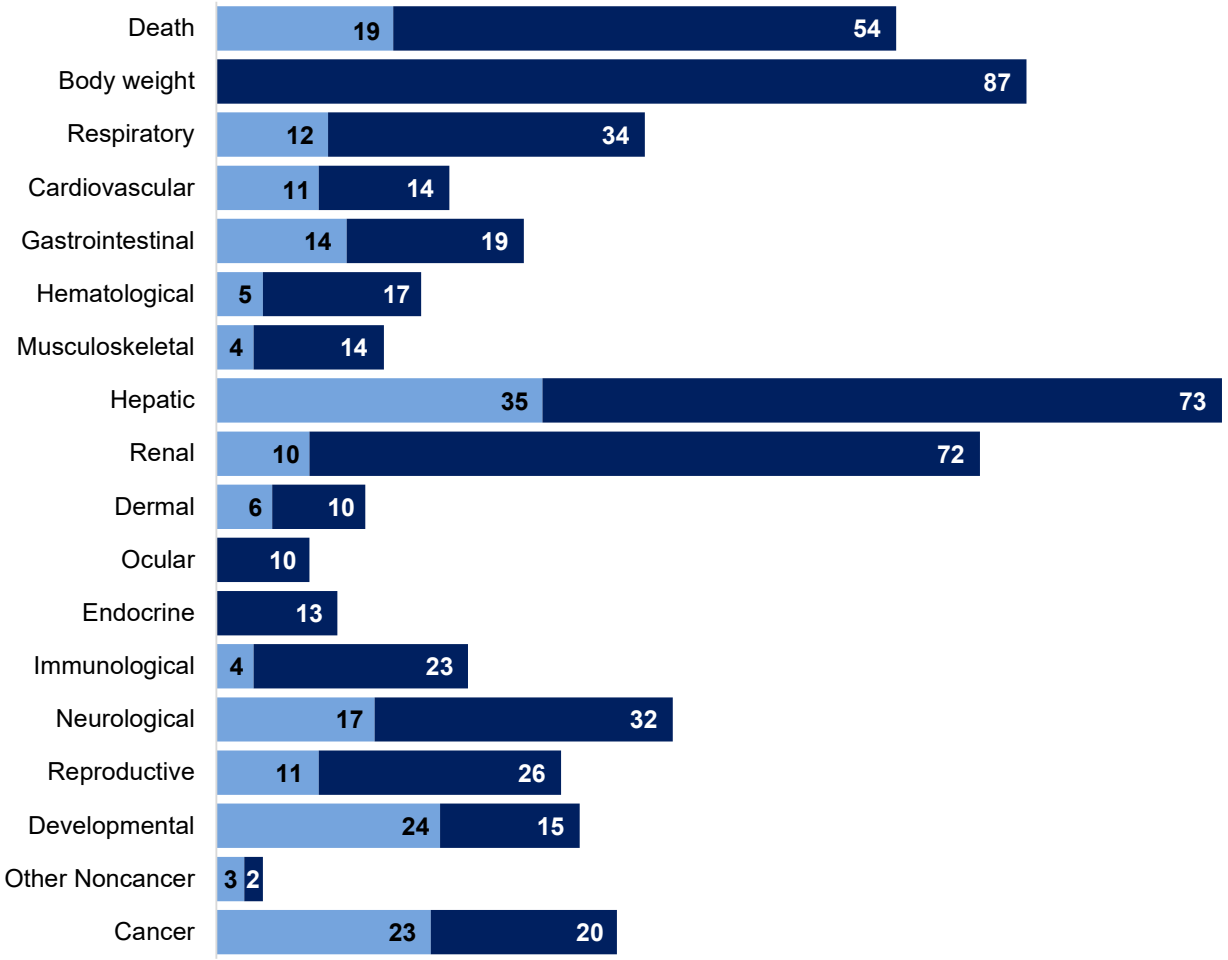
humans and animals. There is limited evidence for neurological effects at exposure levels below those associated with frank CNS depression. One epidemiological study in humans reported neurobehavioral impairments at low occupational exposure levels, and a limited number of animal studies reported alterations 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.

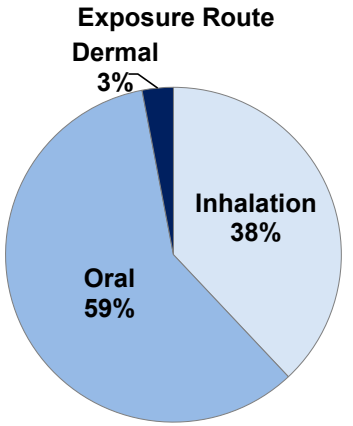
- **Developmental Endpoints.** Developmental effects are a suspected health effect for humans based on inadequate evidence in human epidemiology studies and a moderate level of evidence in animal studies. Epidemiological studies evaluating developmental effects associated with exposure to disinfection 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).

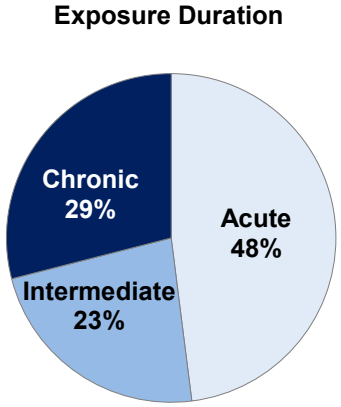
Figure 2-1. Overview of the Number of Studies Examining Chloroform Health Effects*

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

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
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)

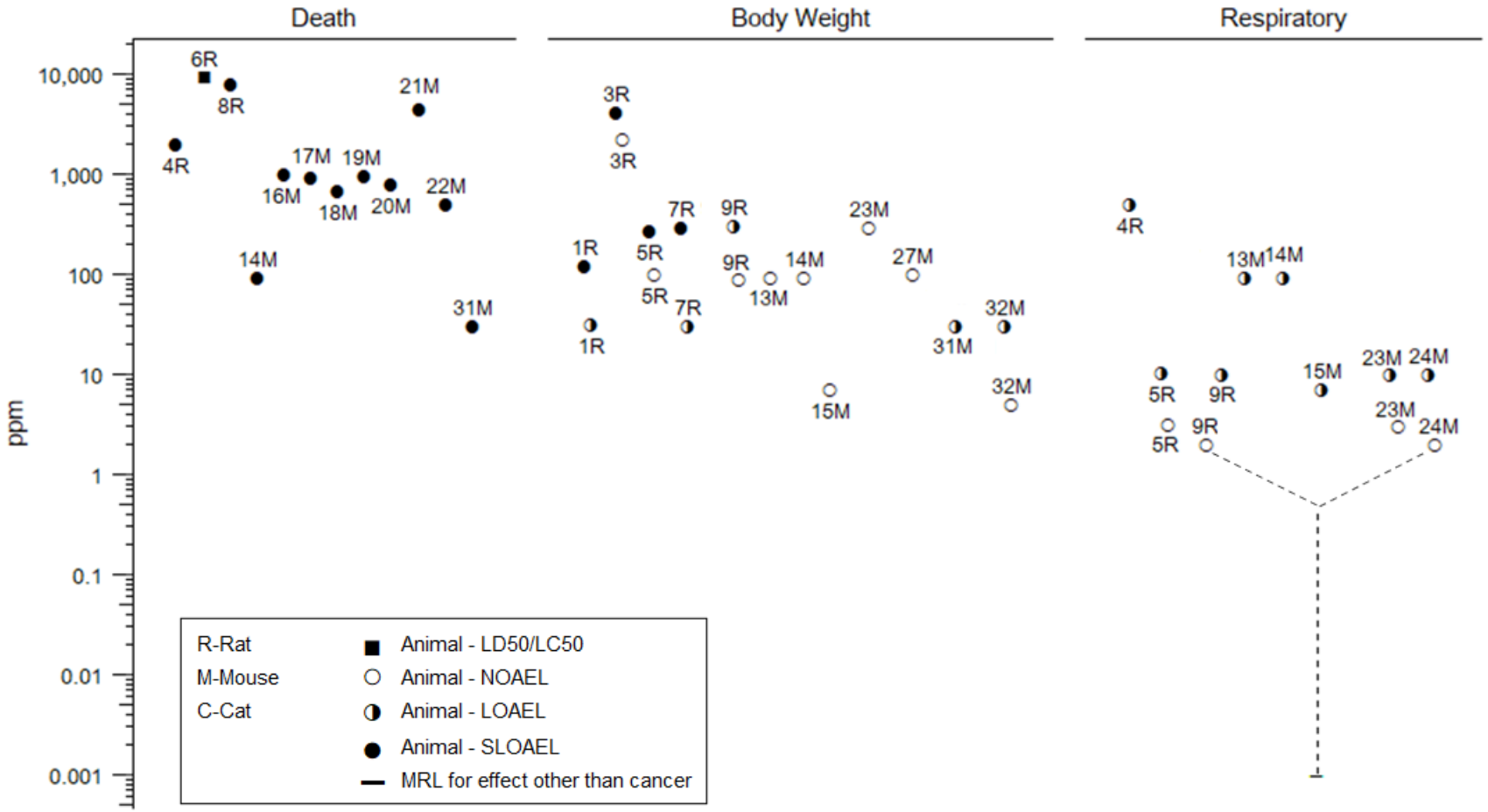


Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Acute (≤14 days)

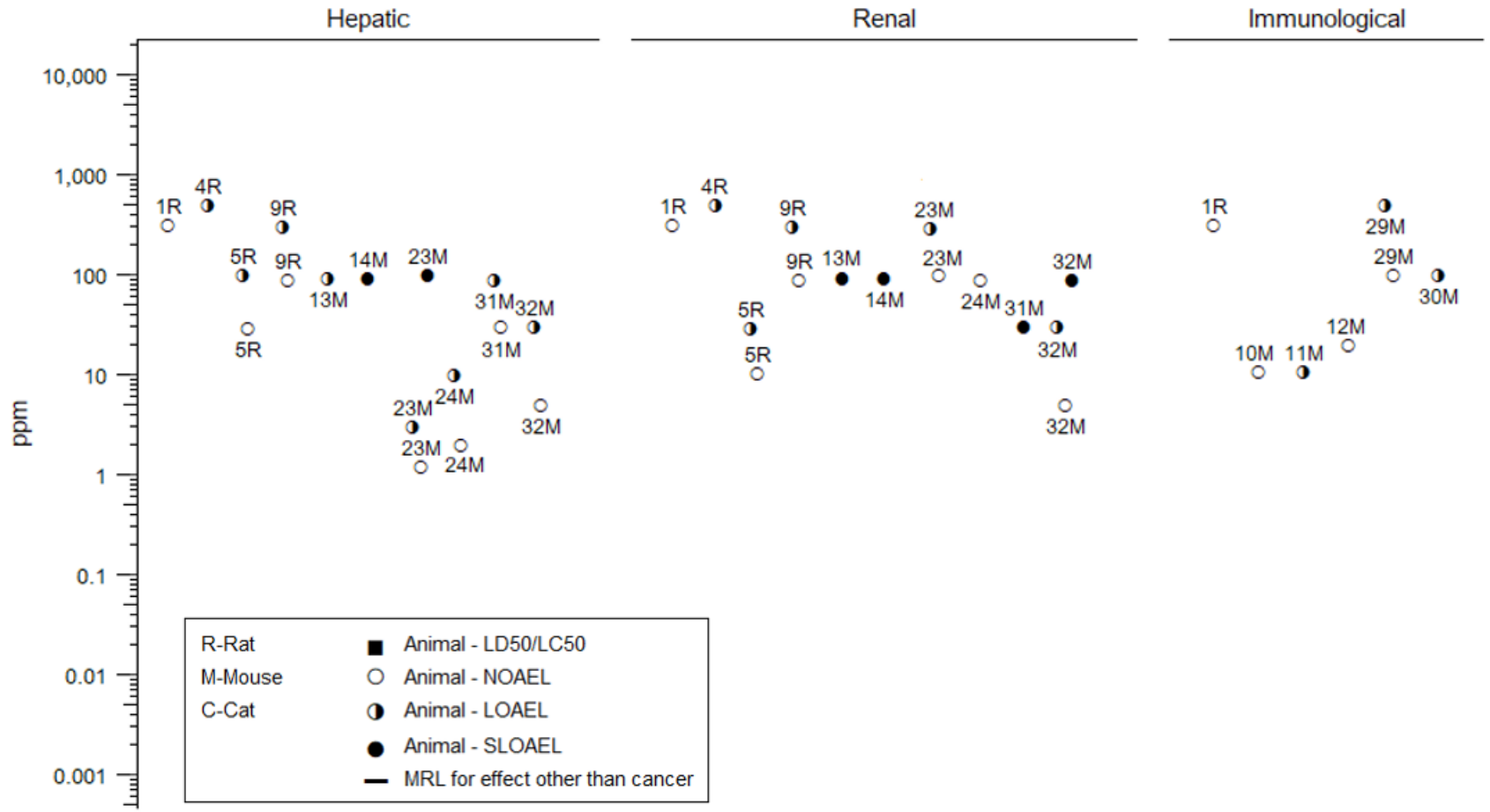


Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Acute (≤14 days)

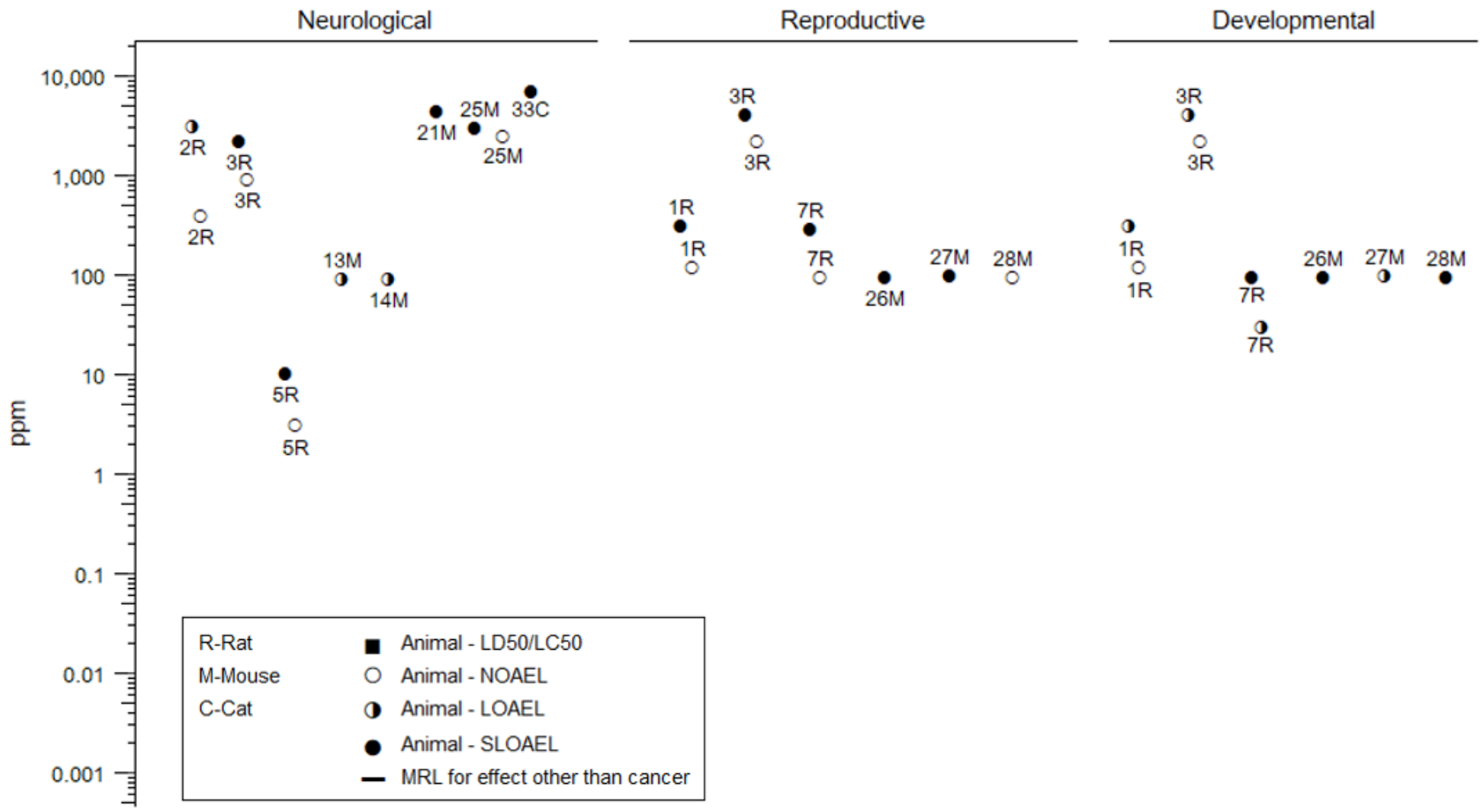


Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Intermediate (15–364 days)

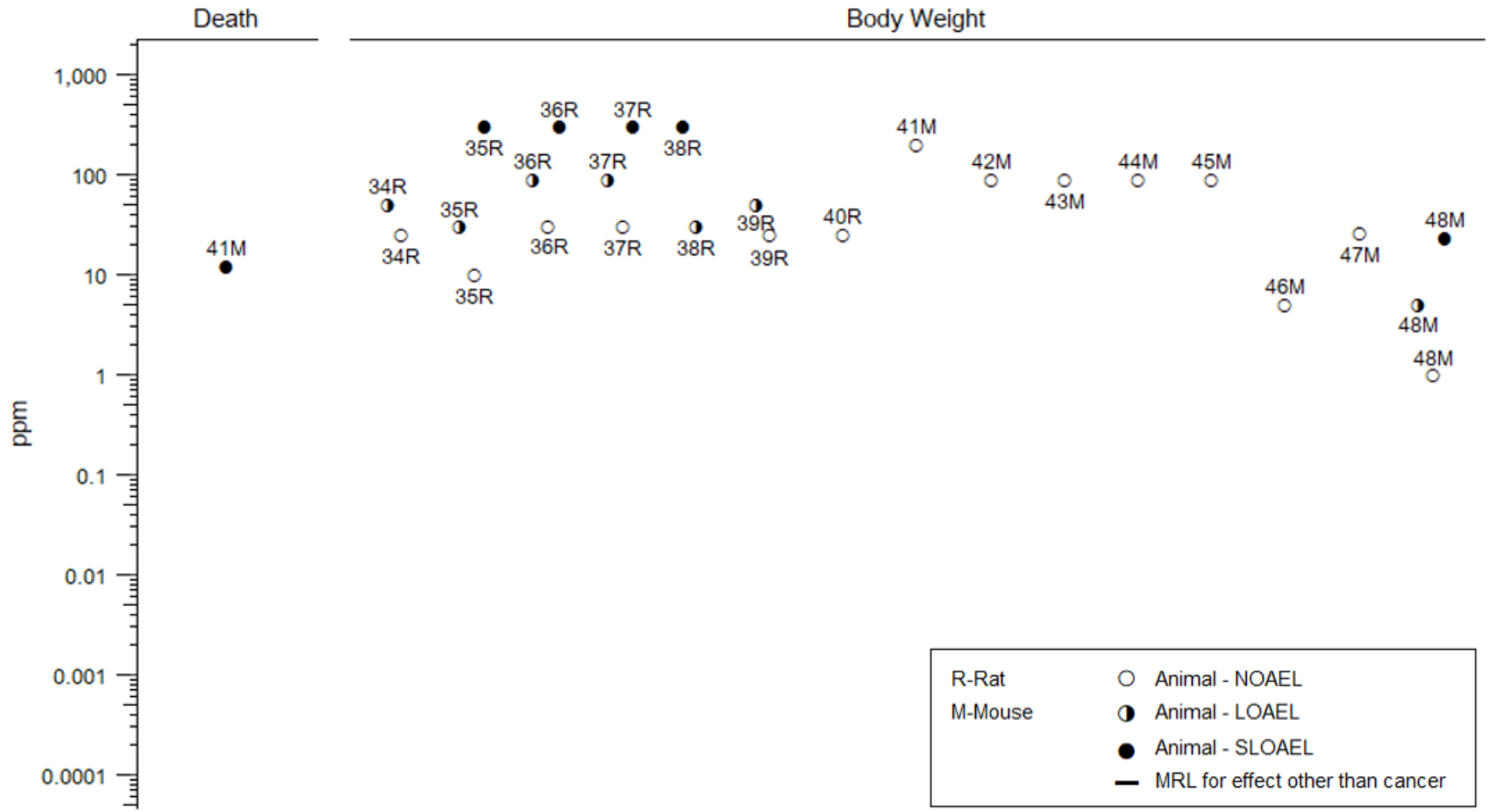


Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Intermediate (15–364 days)

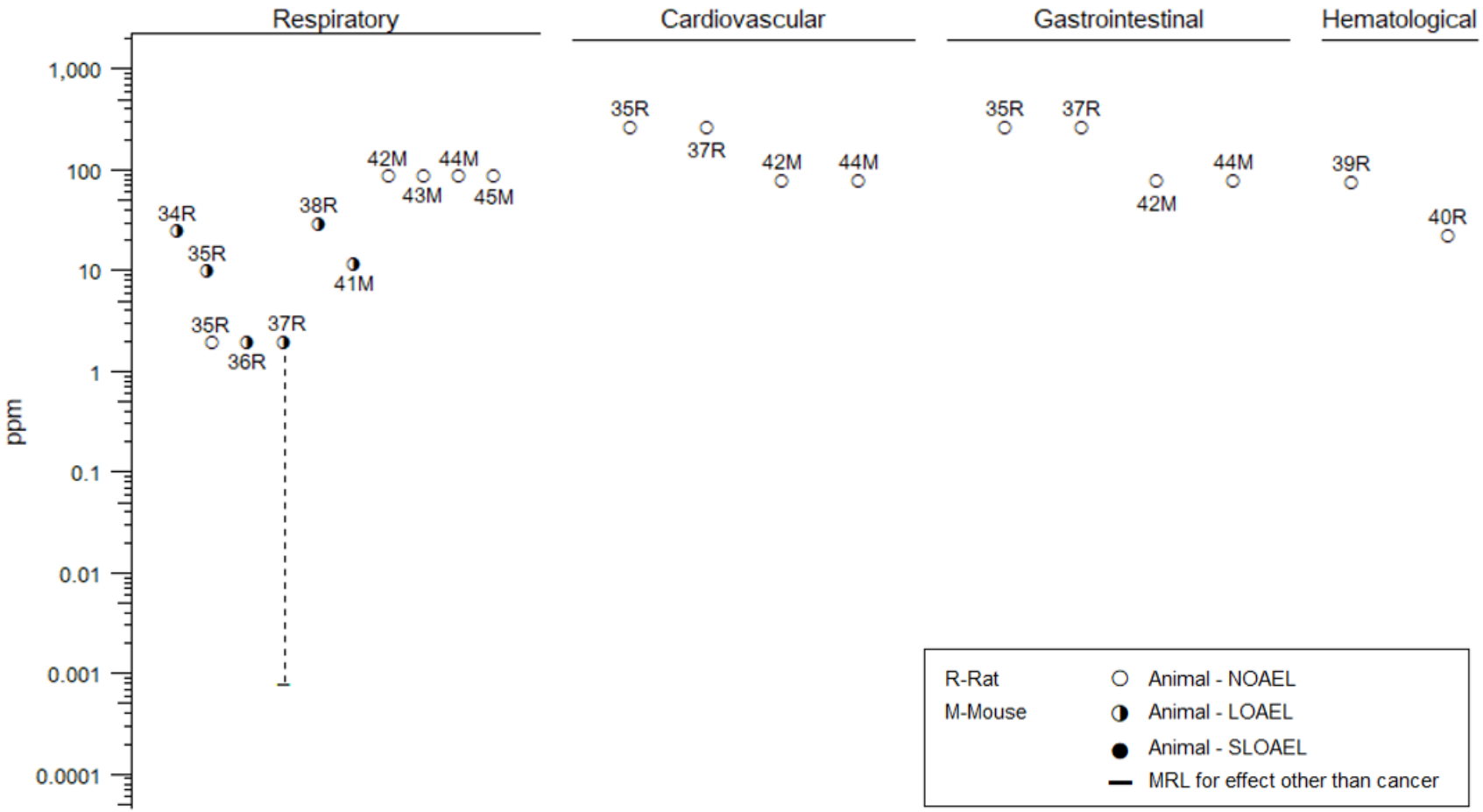


Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Intermediate (15–364 days)

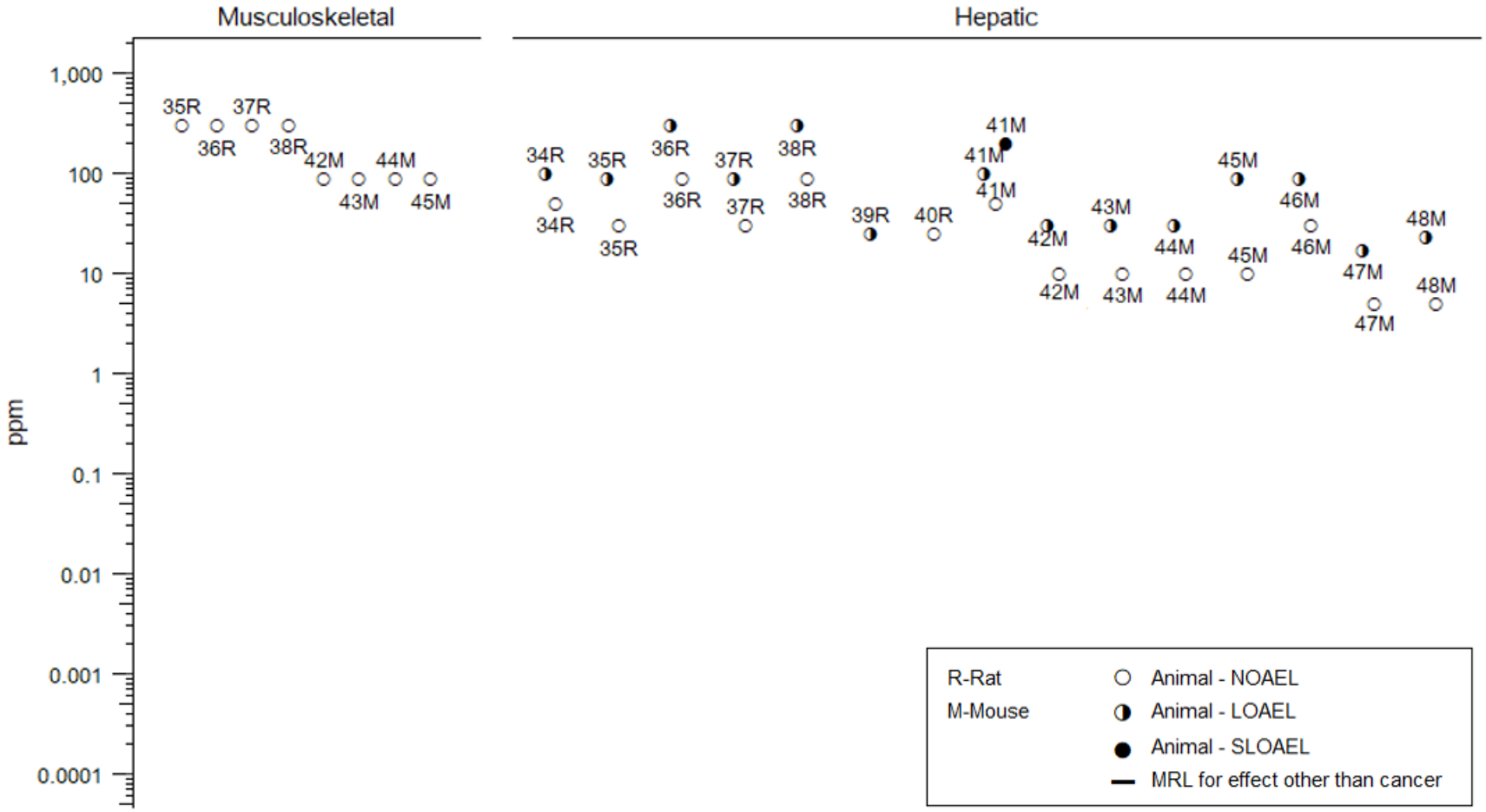


Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Intermediate (15–364 days)

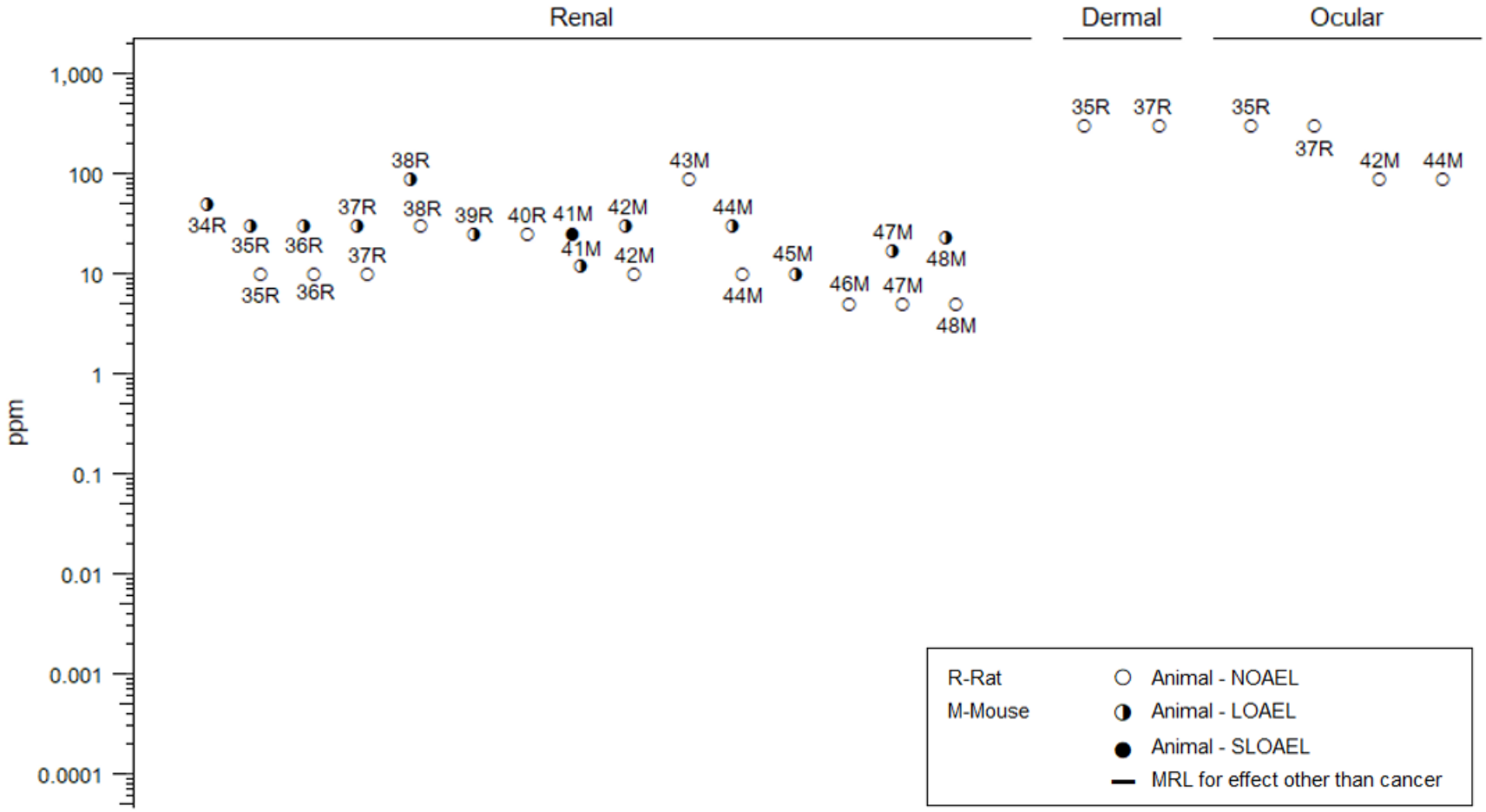


Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Intermediate (15–364 days)

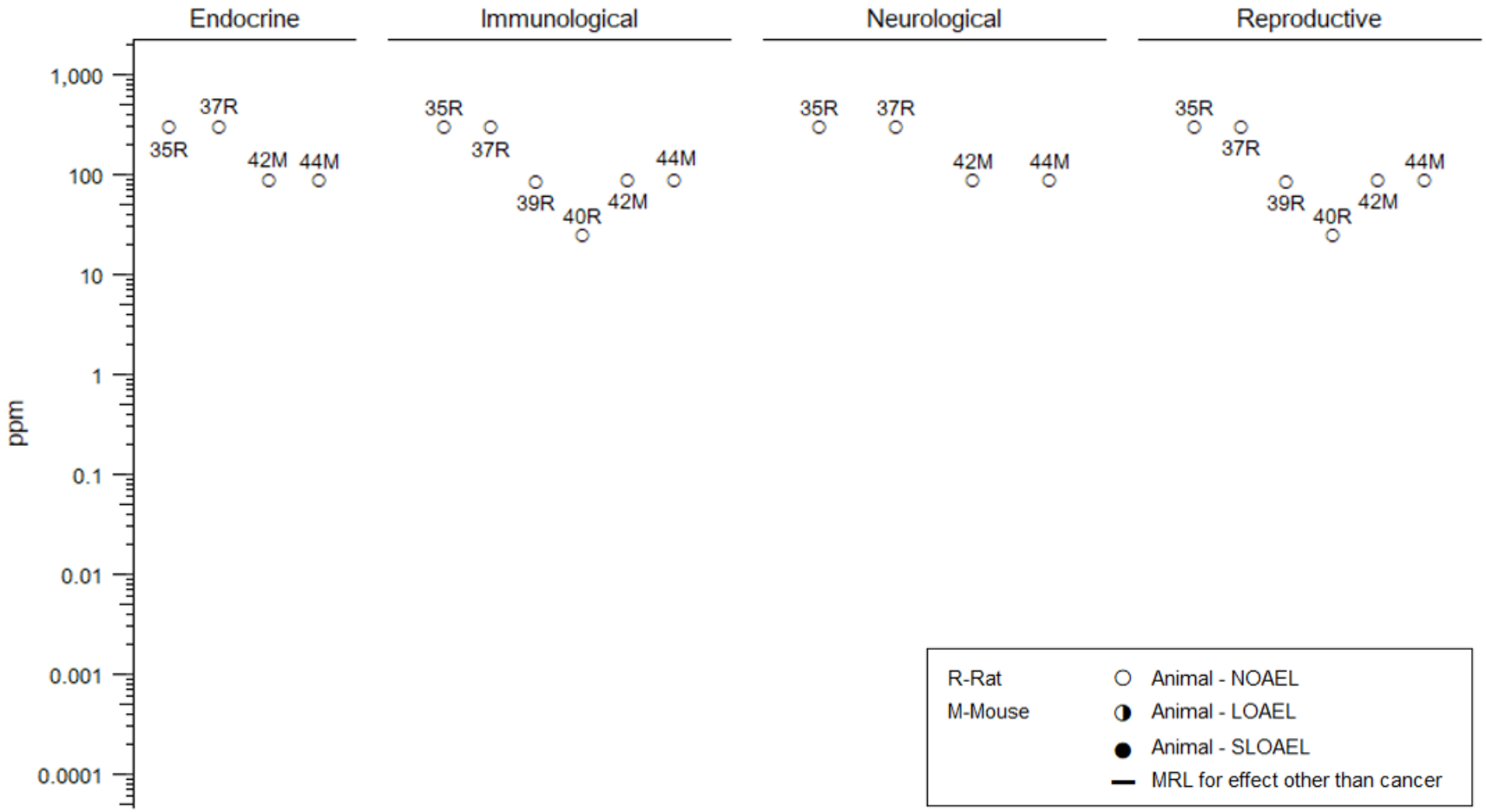


Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Chronic (≥365 days)

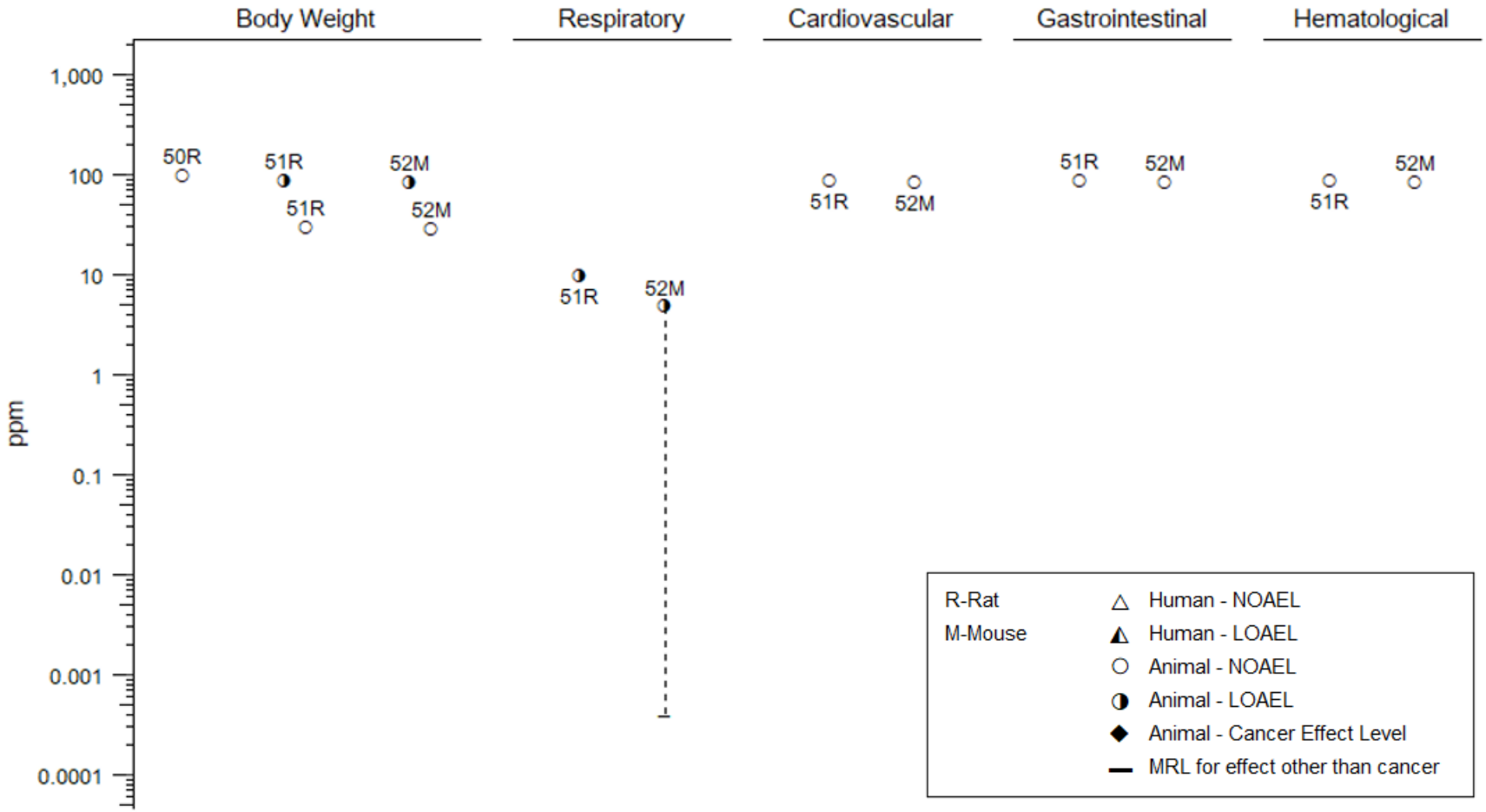


Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Chronic (≥365 days)

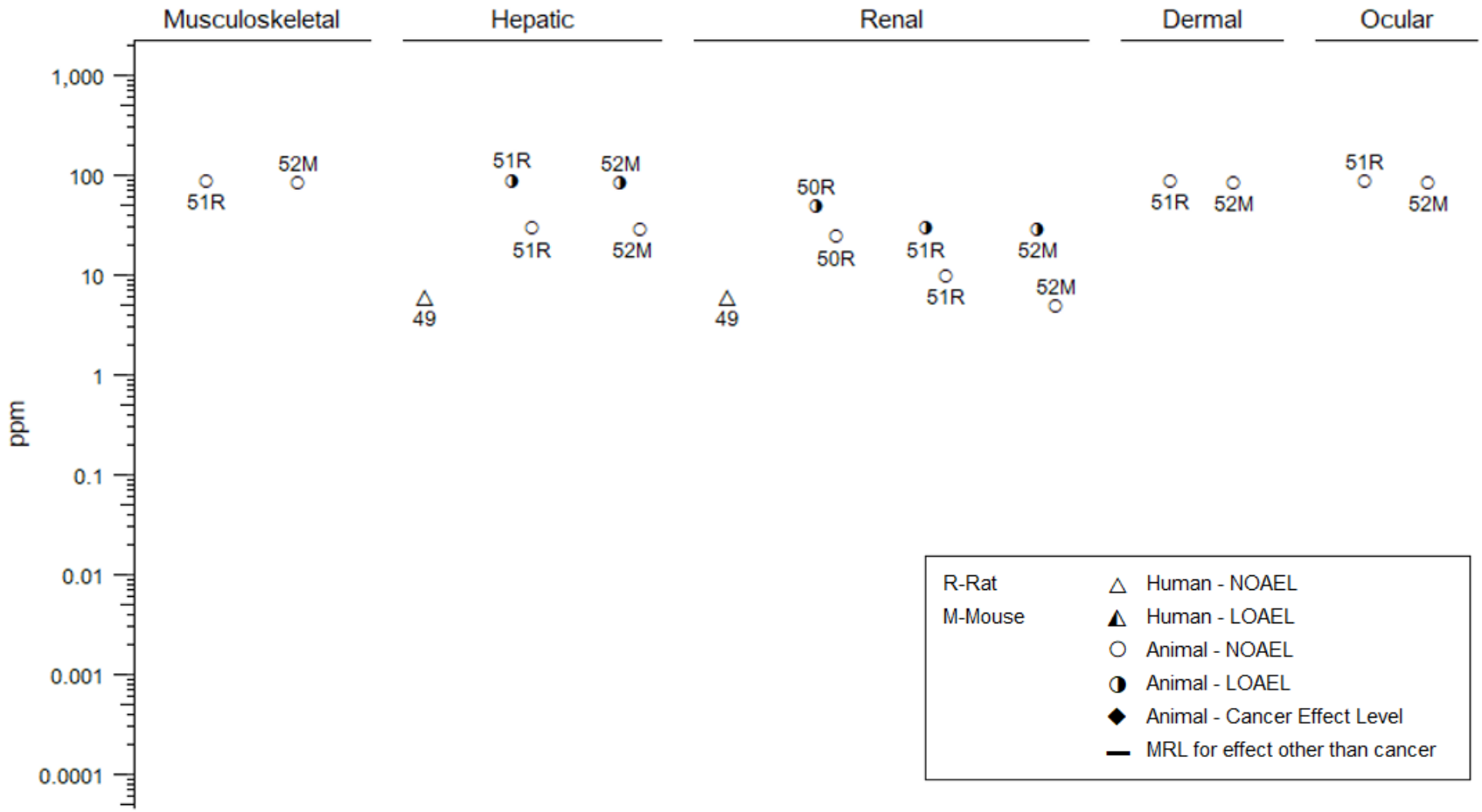


Figure 2-2. Levels of Significant Exposure to Chloroform – Inhalation
Chronic (≥365 days)

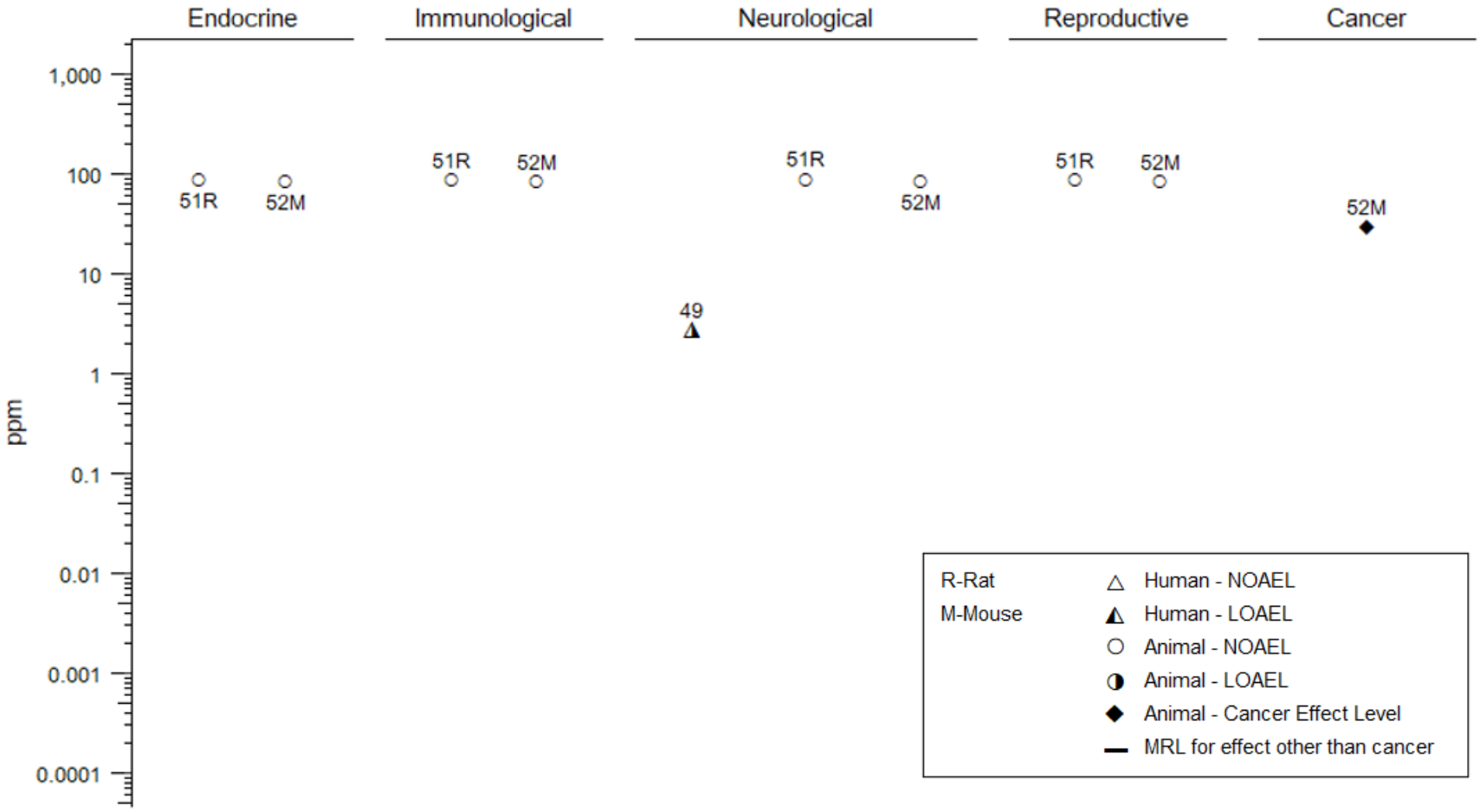


Table 2-2. Levels of Significant Exposure to Chloroform – Oral (mg/kg/day)

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)

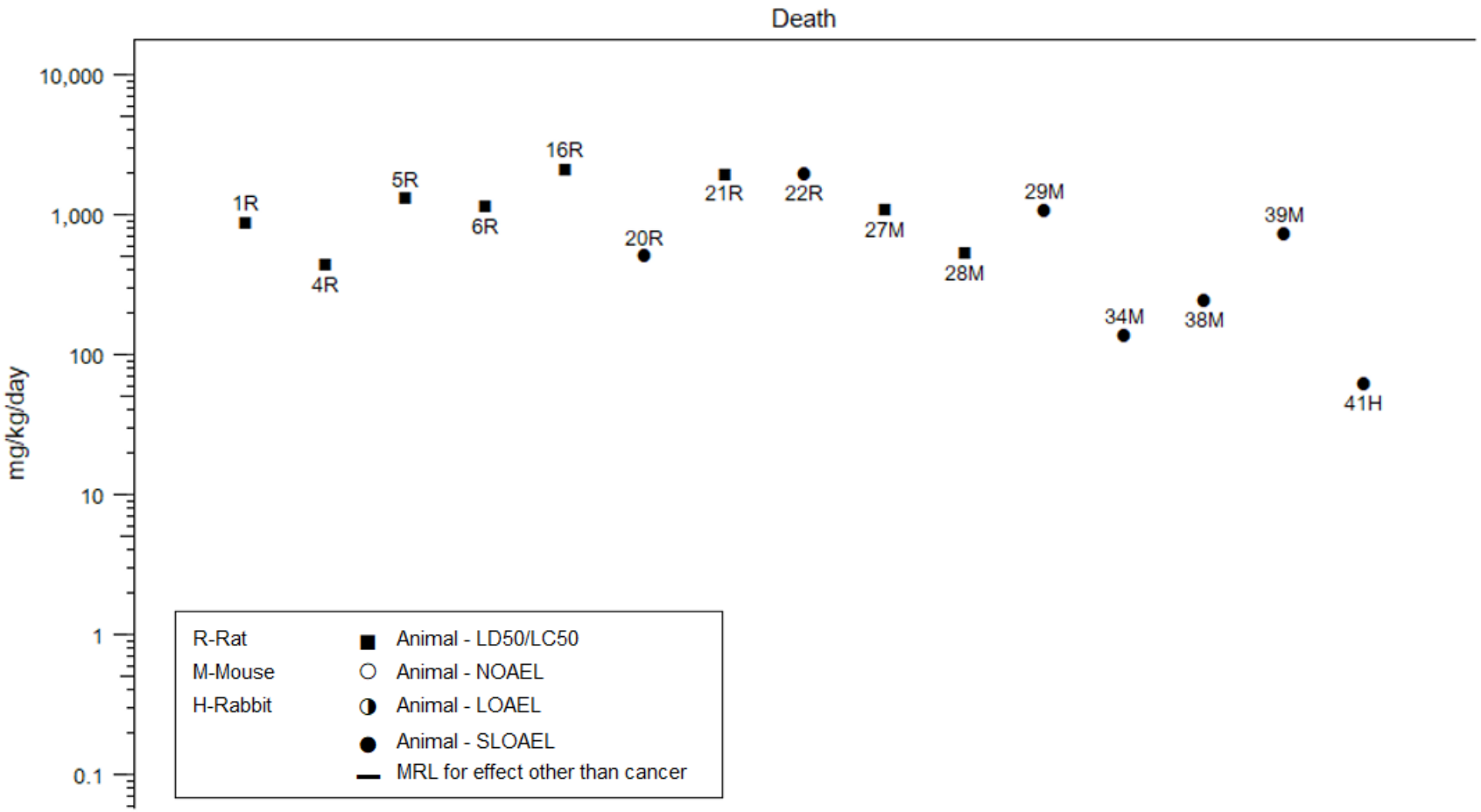


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

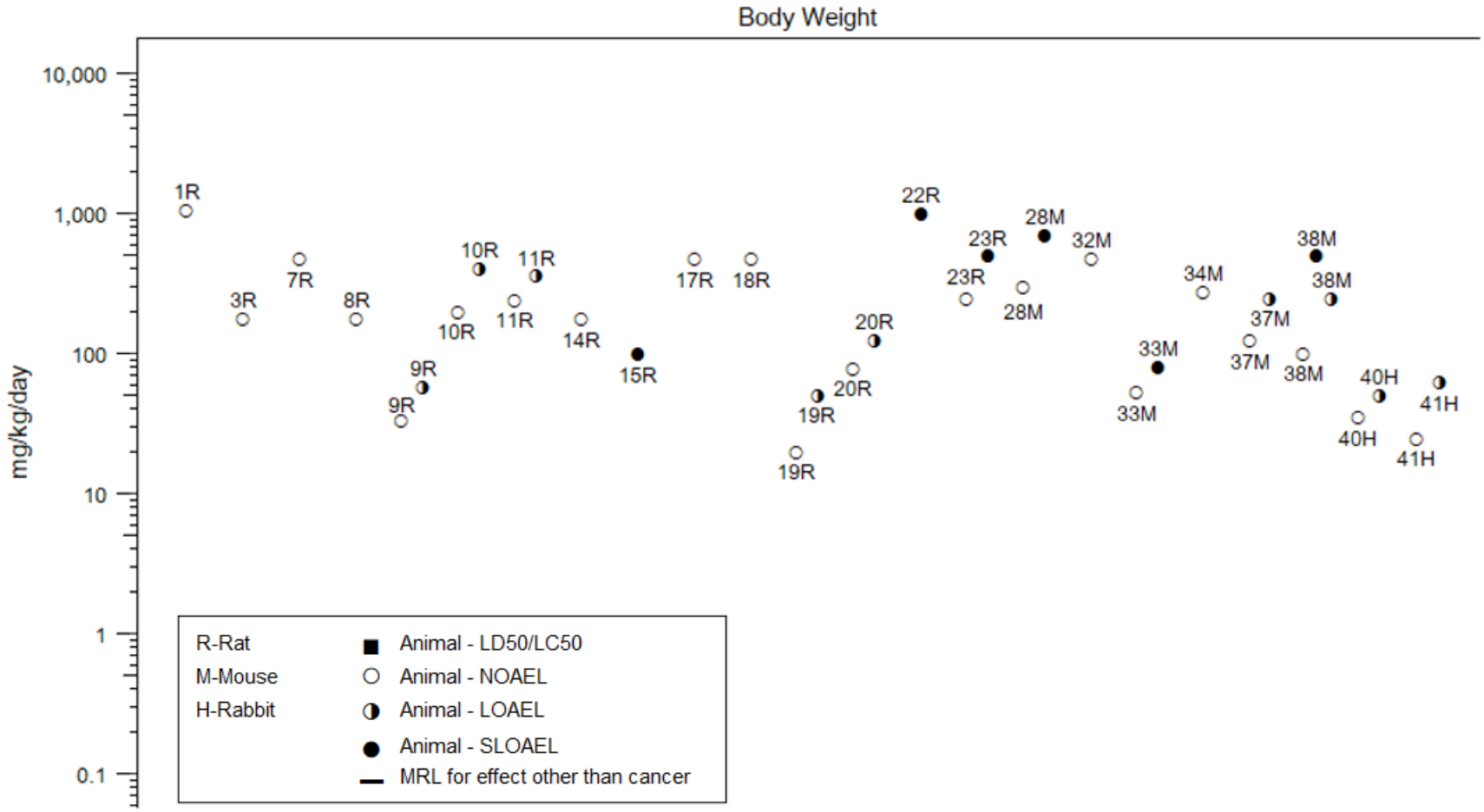


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

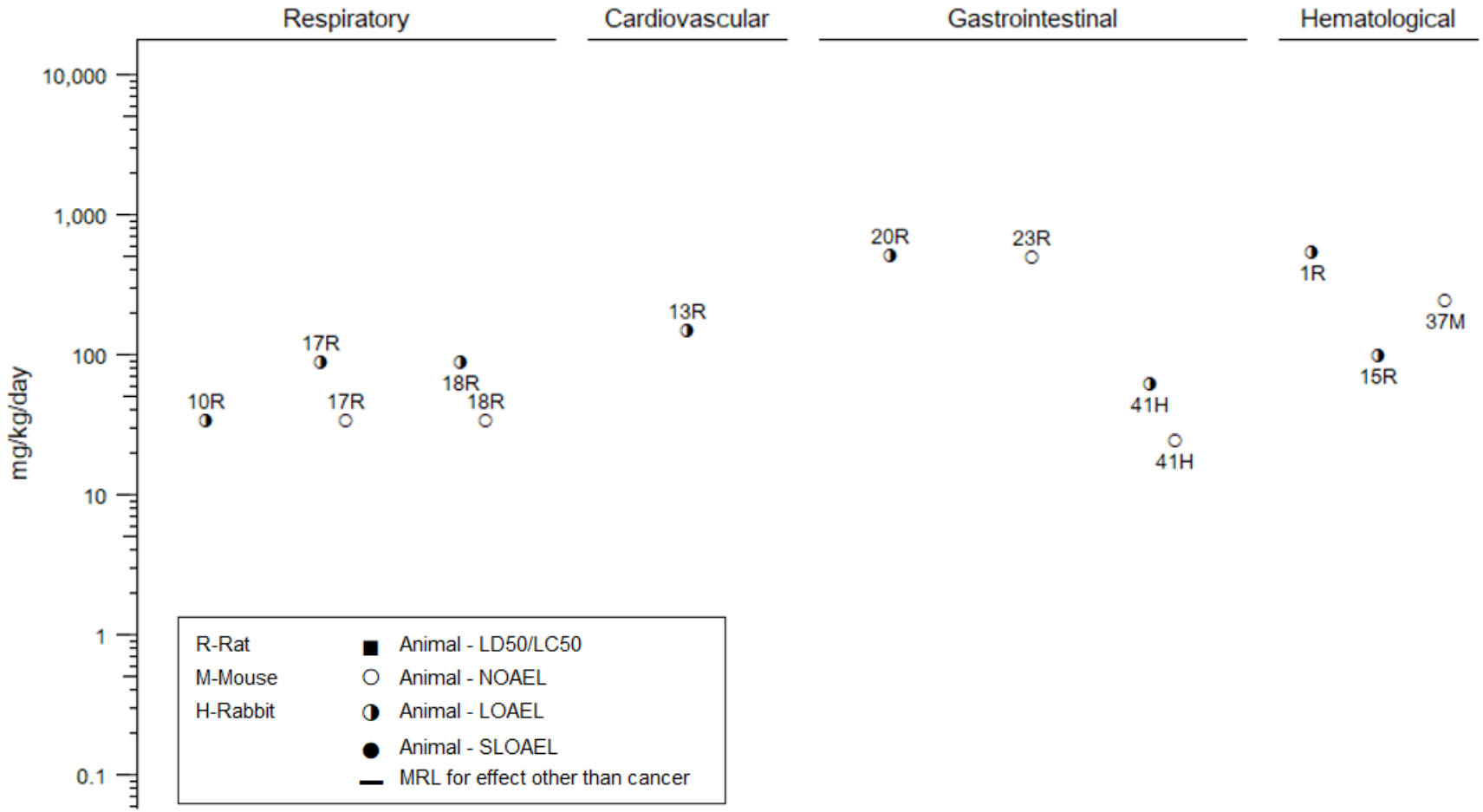


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

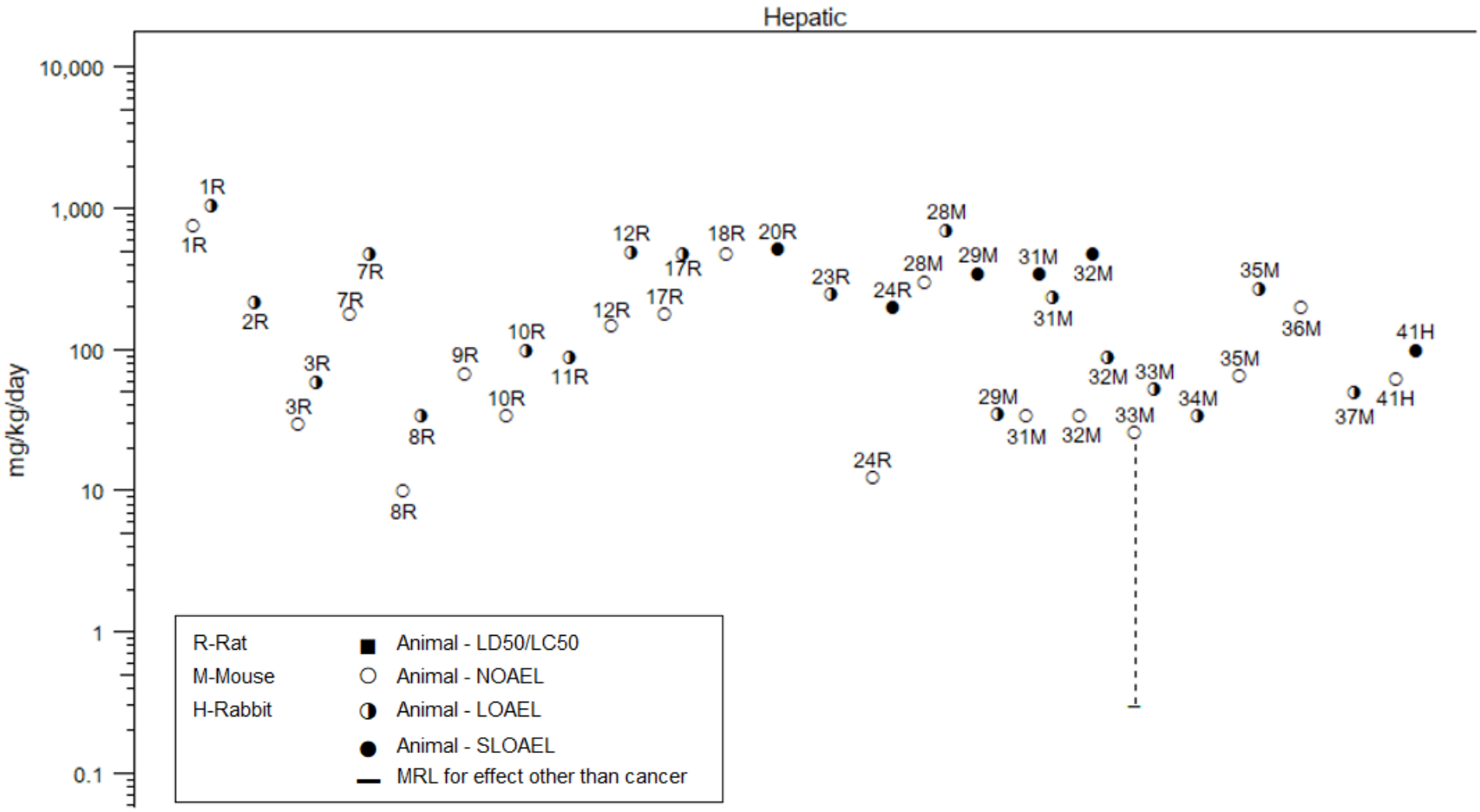


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

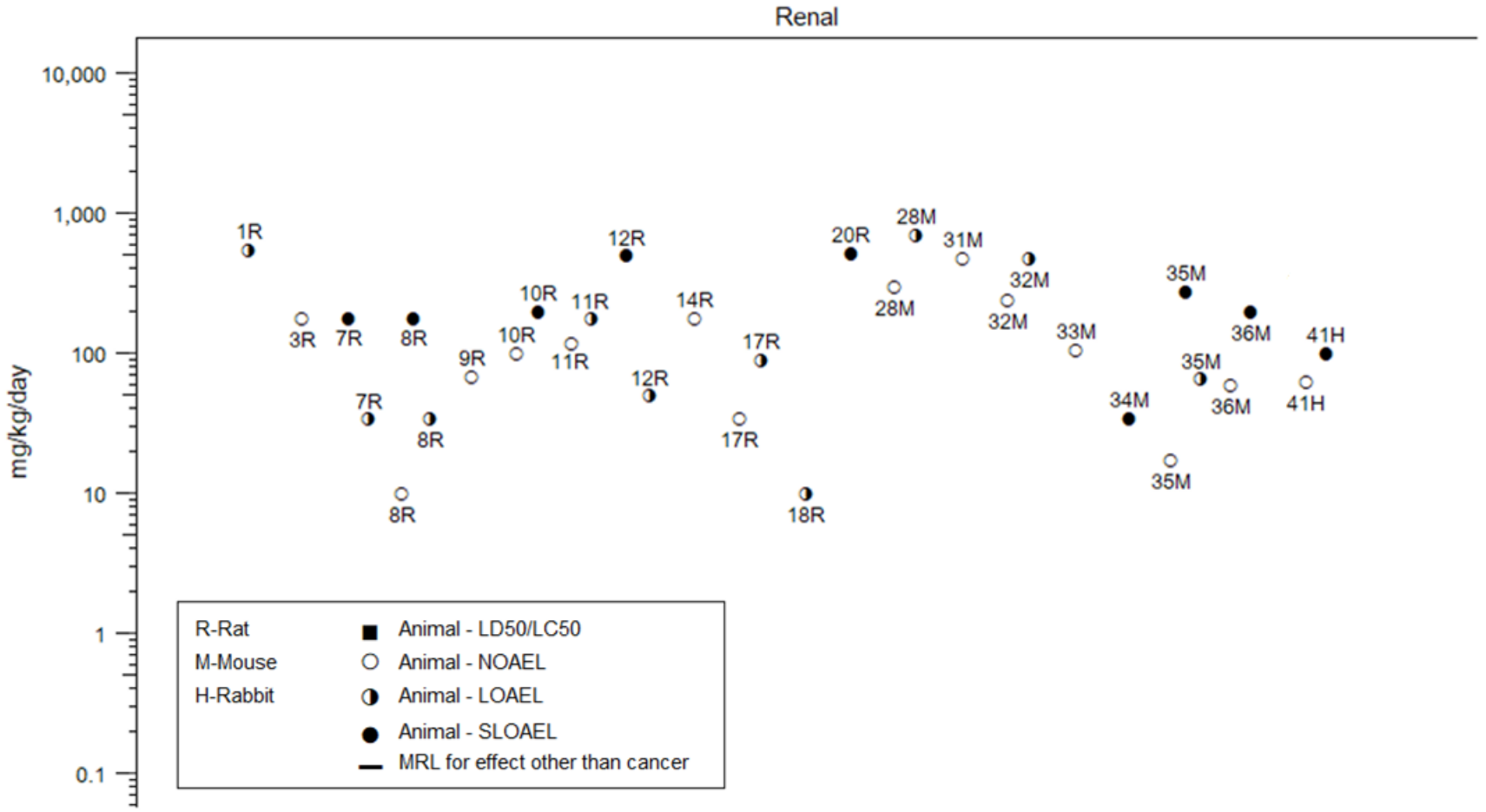


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

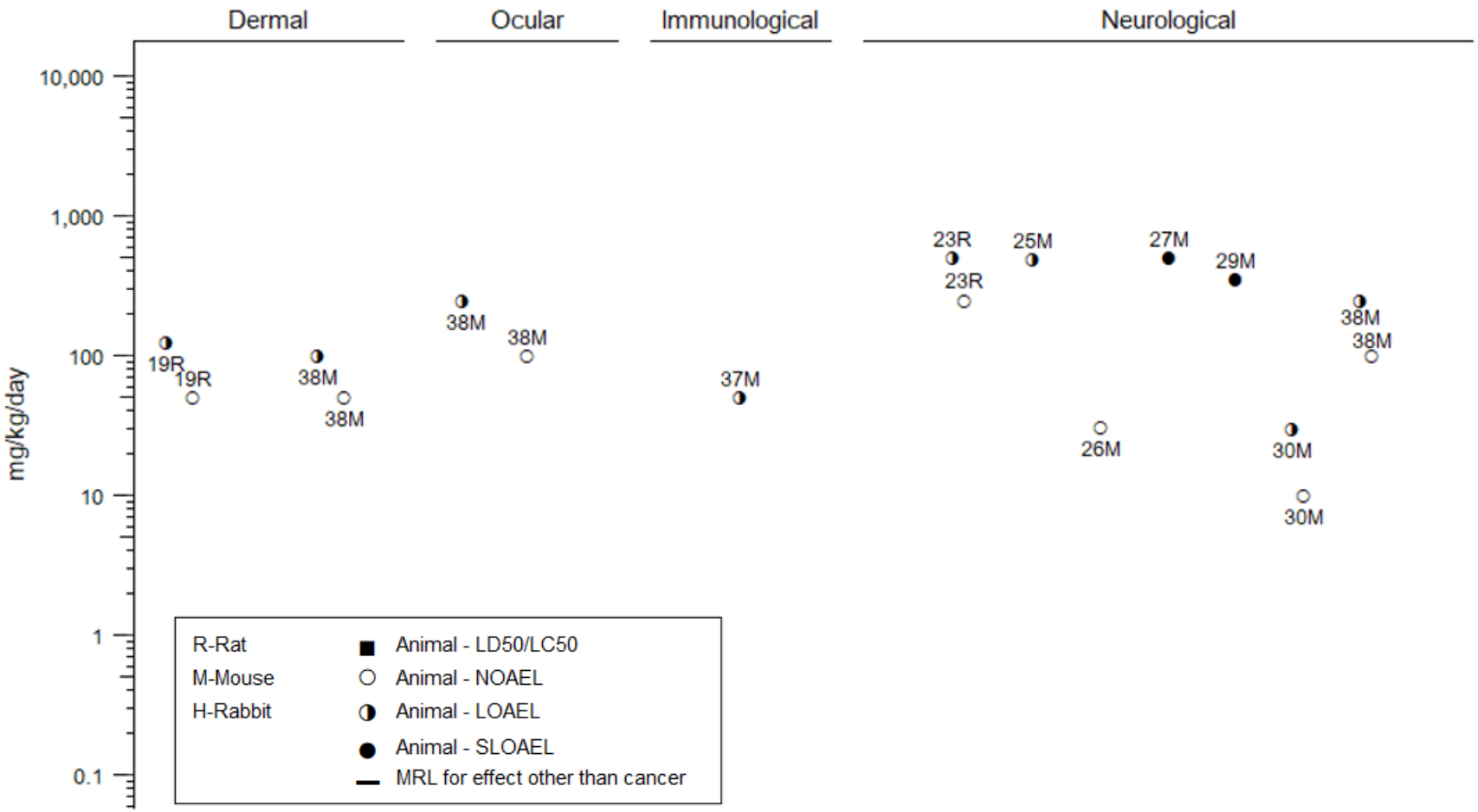


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Acute (≤14 days)

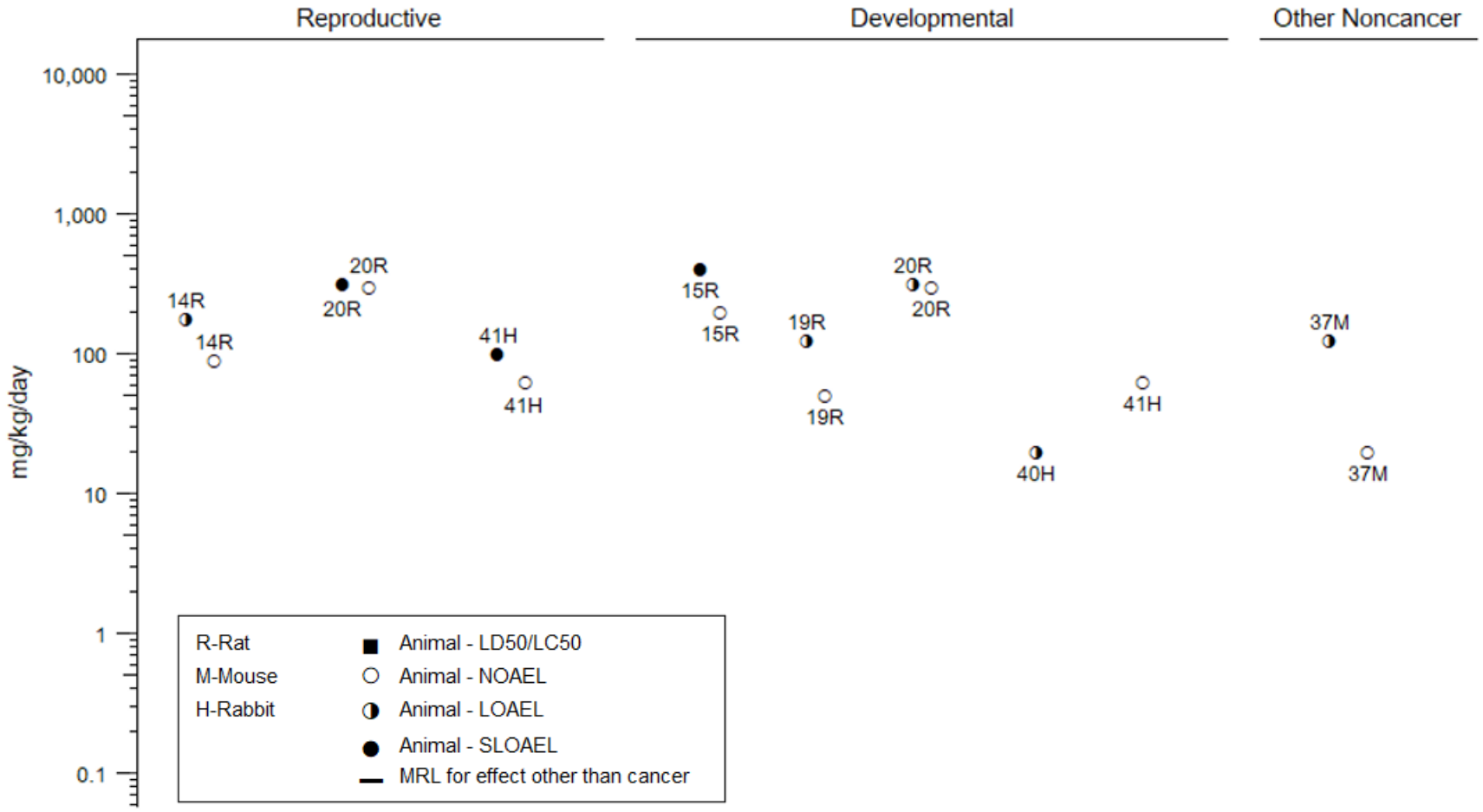


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

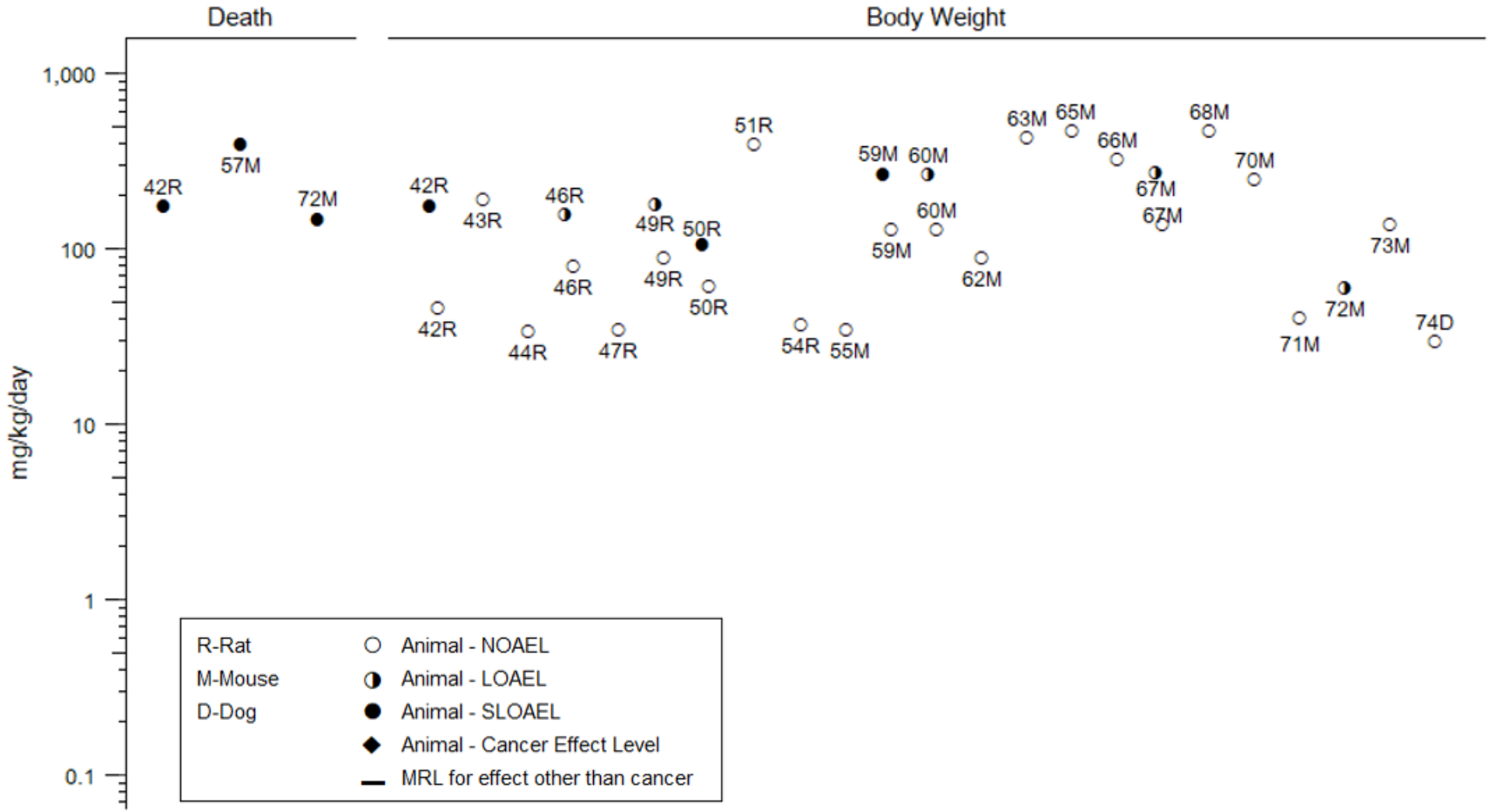


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

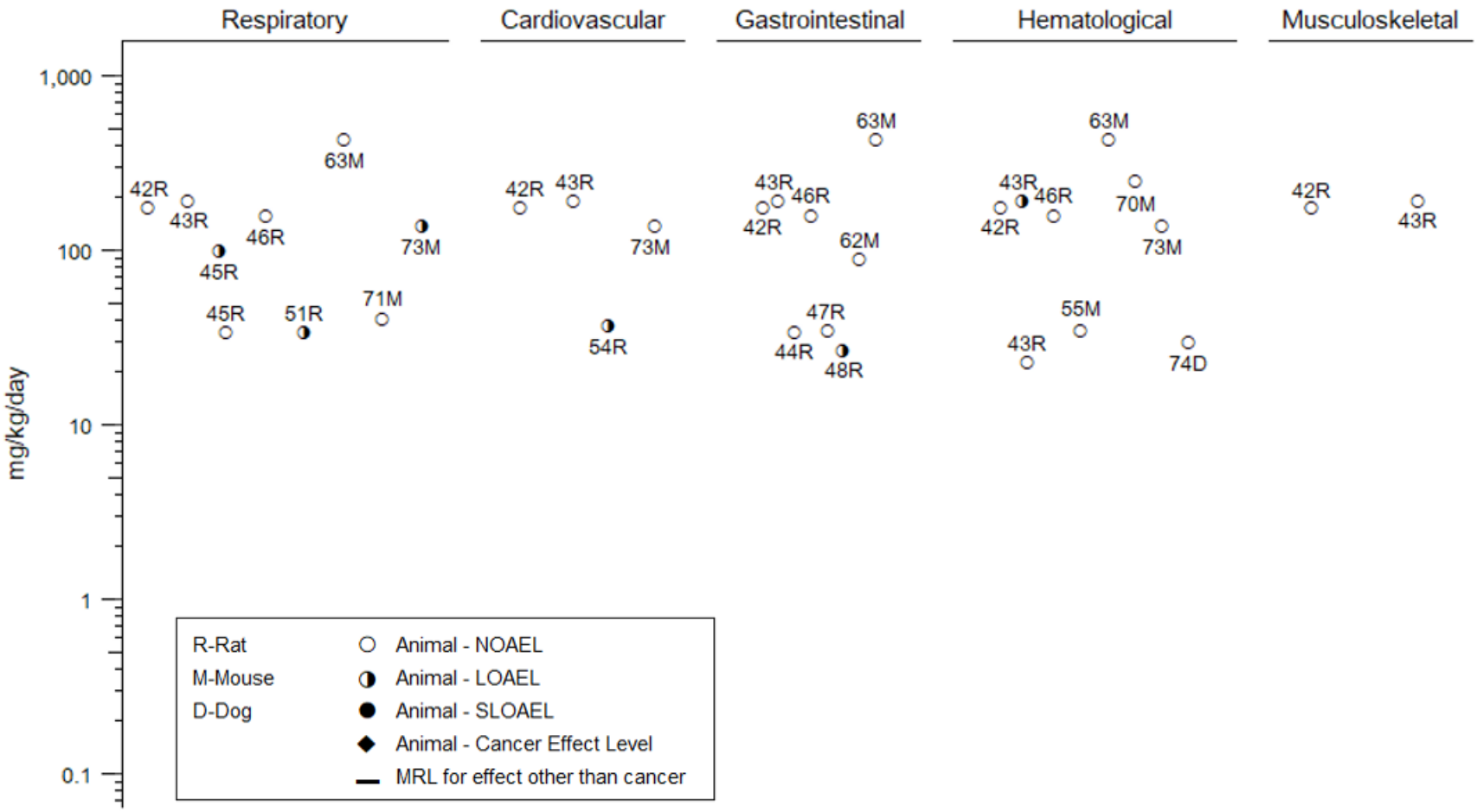


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

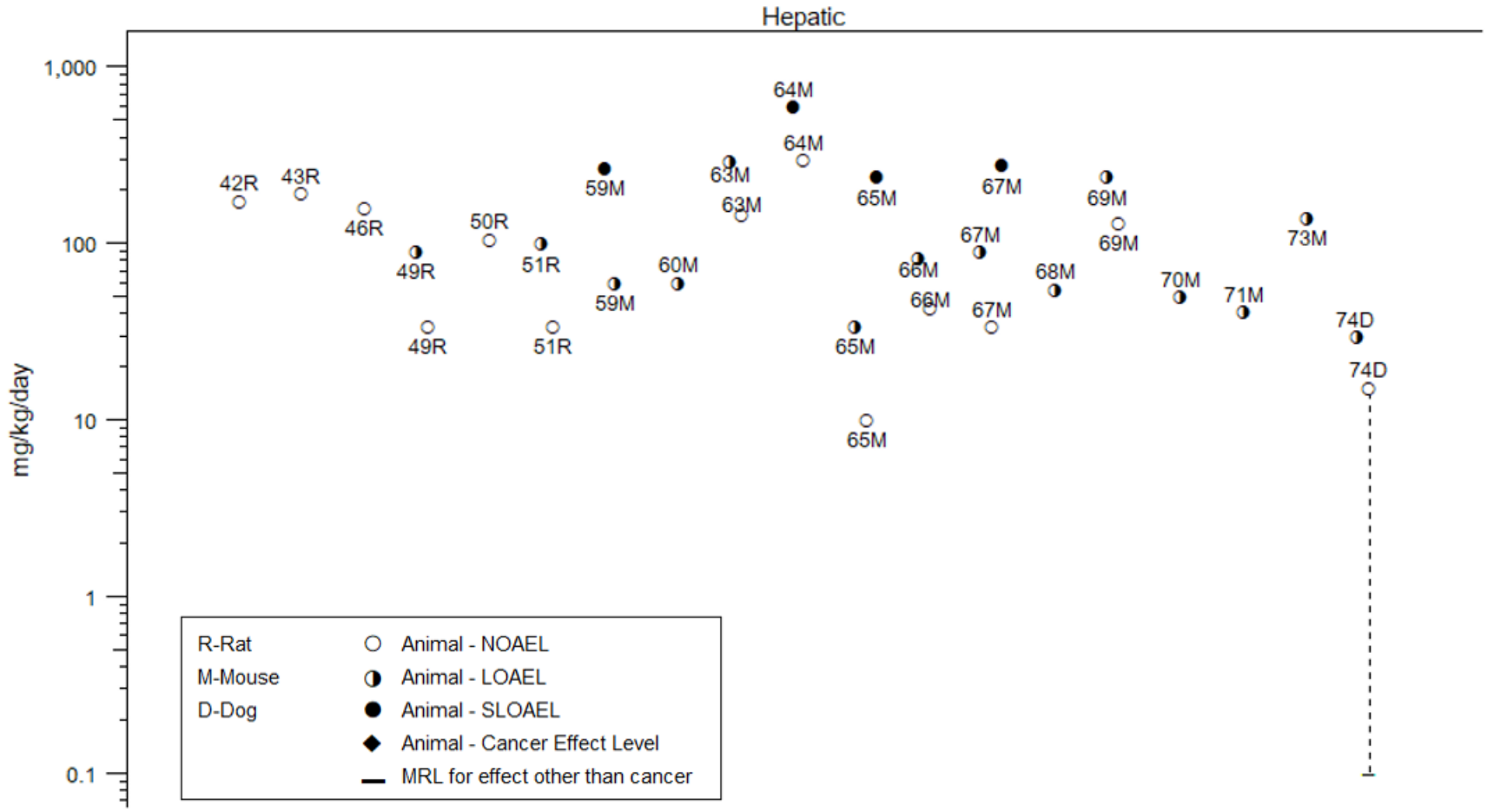


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

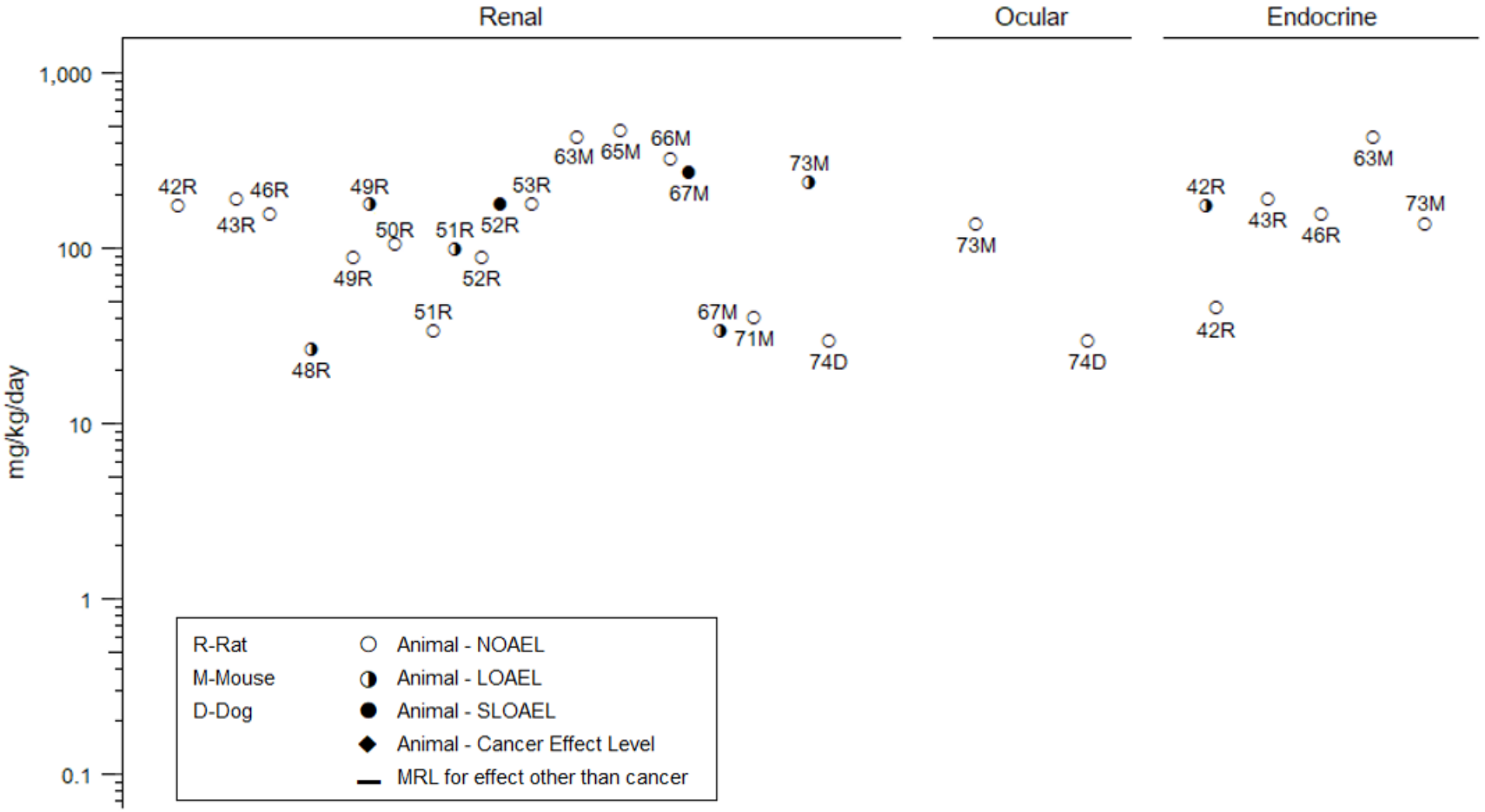


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

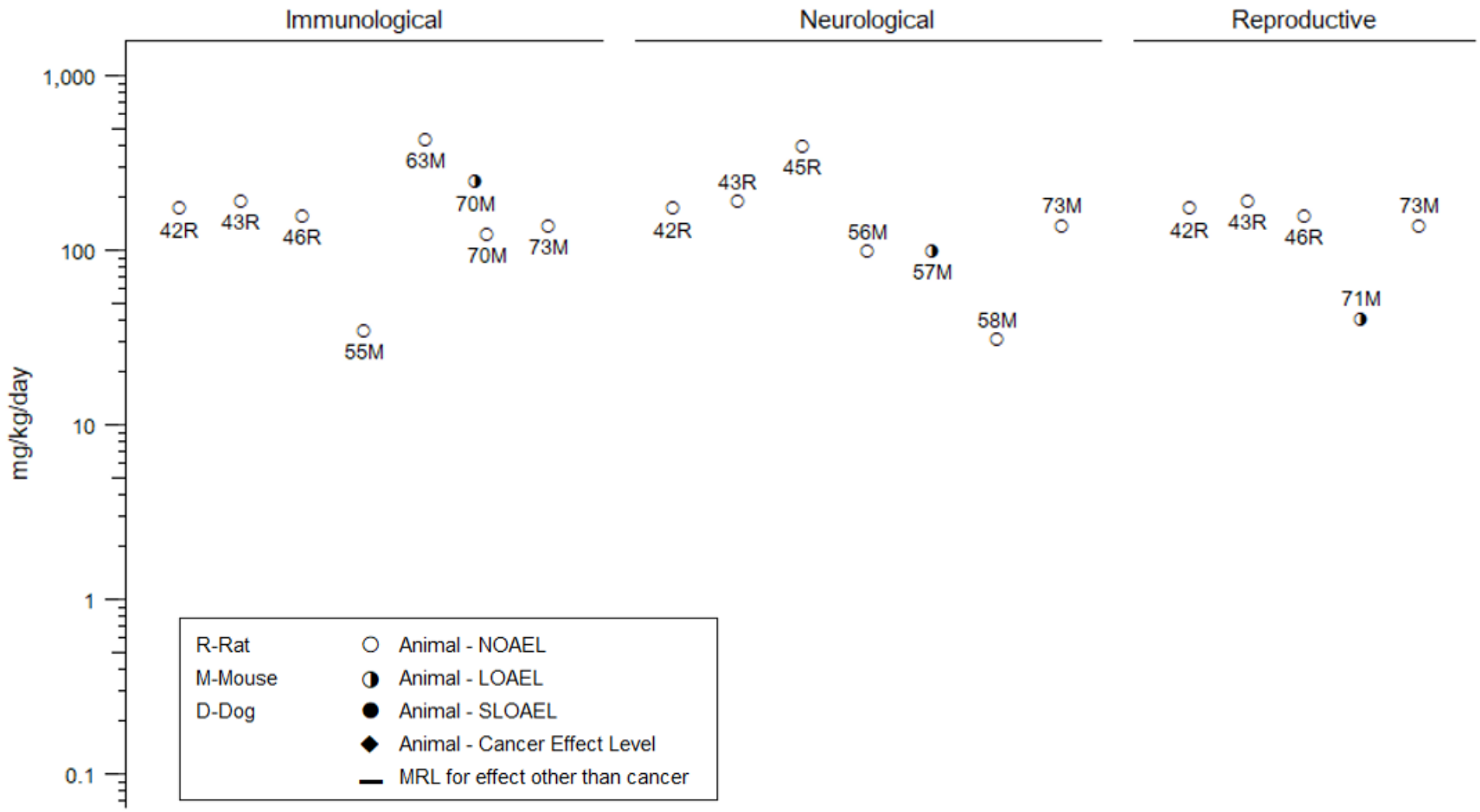


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Intermediate (15–364 days)

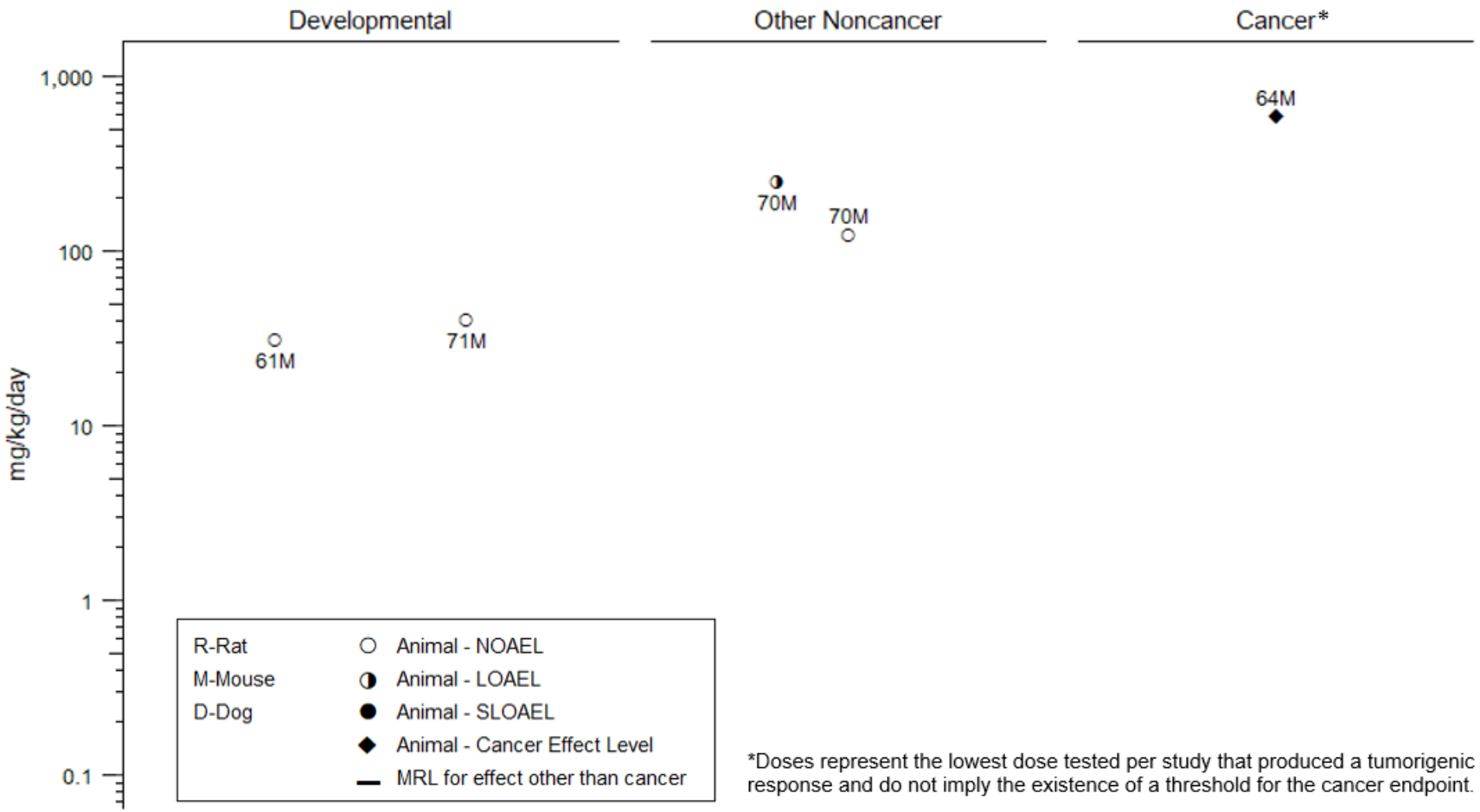


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Chronic (≥365 days)

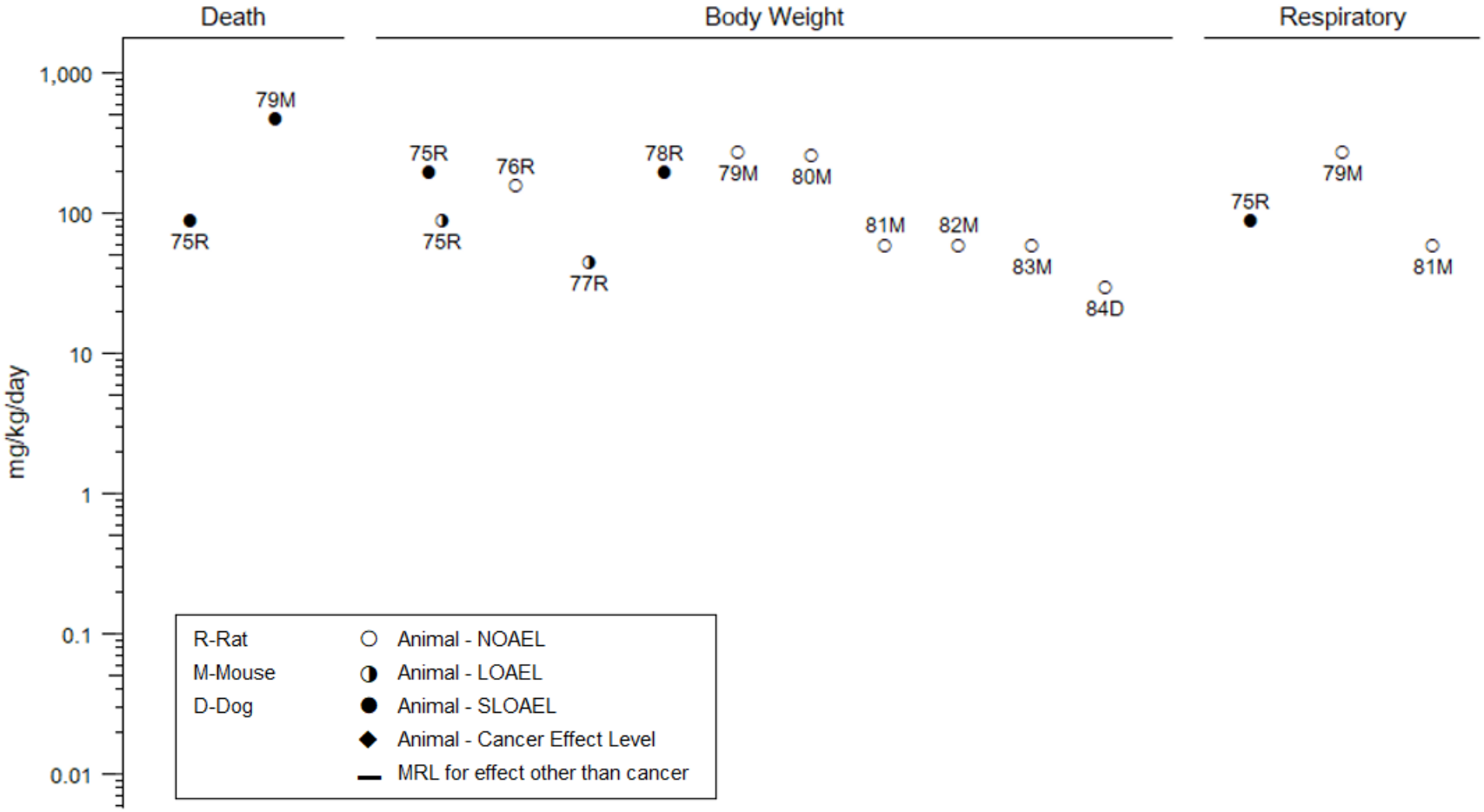


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Chronic (≥365 days)

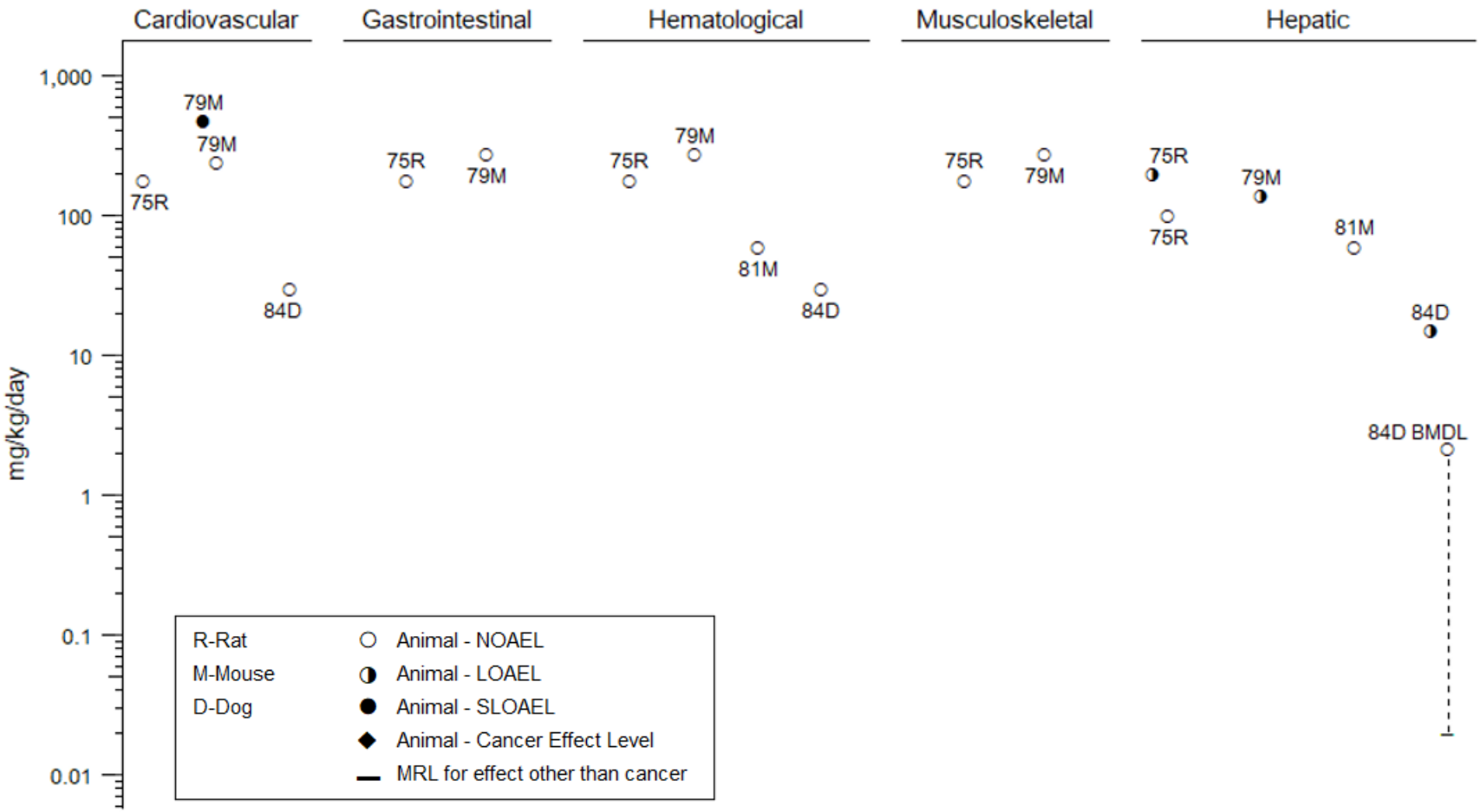


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Chronic (≥365 days)

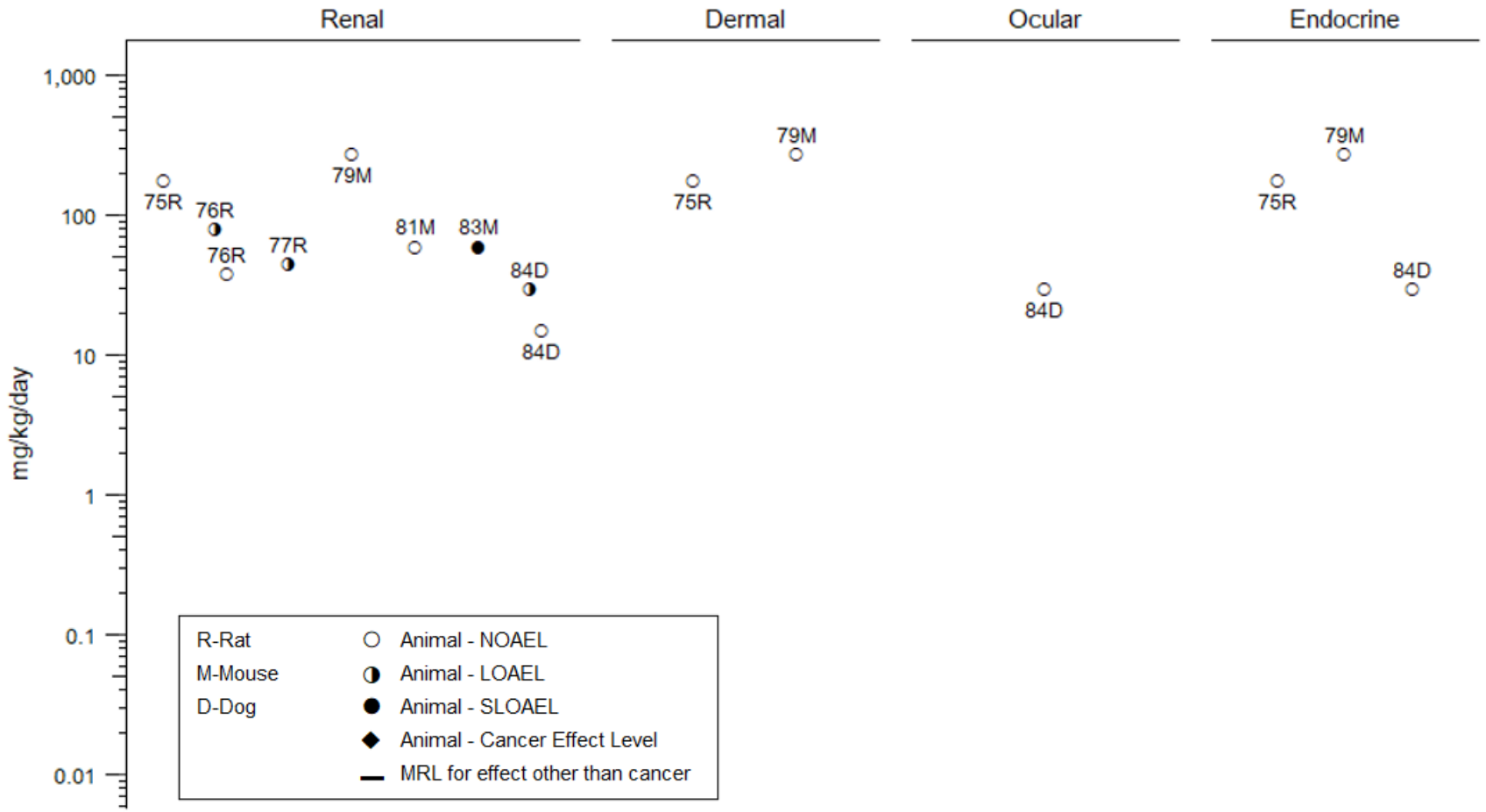


Figure 2-3. Levels of Significant Exposure to Chloroform – Oral
Chronic (≥365 days)

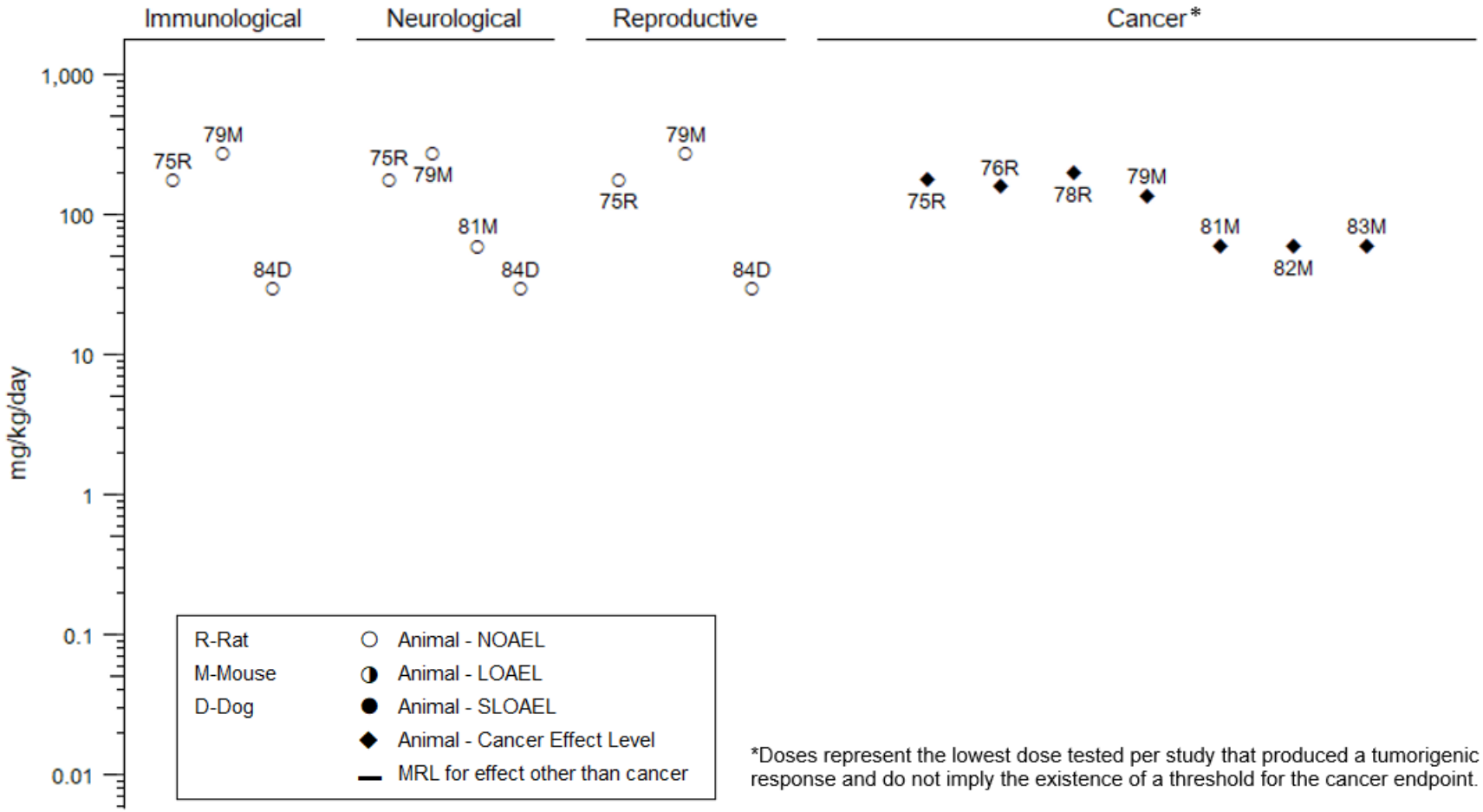


Table 2-3. Levels of Significant Exposure to Chloroform – Dermal

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).

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

3.1 TOXICOKINETICS

- Absorption of chloroform can occur through the lungs, gastrointestinal tract, and skin.
- Absorbed chloroform is distributed throughout the body. Based on blood-tissue partition coefficients, the equilibrium distribution would be in the following order: fat>>liver>kidney≥ other tissues.
- Chloroform is metabolized by mixed function oxidases (CYP2E1) in the liver, kidney, and other tissues to form reactive intermediates such as phosgene.
- Absorbed chloroform is excreted primarily through the lungs as chloroform. Metabolites are excreted primarily through the lungs as carbon dioxide and in urine to a lesser extent.
- Numerous physiologically based pharmacokinetic (PBPK) models of chloroform have been developed and applied to interspecies and route-to-route dosimetry extrapolation.

3.1.1 Absorption

Inhalation. Absorption of inhaled chloroform depends on many factors, including air concentration, exposure duration, solubility in blood and tissues, and physical activity level, which influences the ventilation rate and cardiac output (Silva et al. 2013). Pulmonary absorption of chloroform is also influenced by total body weight and total fat content, with uptake and storage in adipose tissue increasing with increasing body weight and fat.

Absorption of inhaled chloroform is governed, in part, by its solubility in blood. The blood/air partition coefficient has been estimated in a variety of ways, including experimental measurements made under equilibrium conditions (Batterman et al. 2002; Béliveau and Krishnan 2000a; Gargas et al. 1989; Kaneko et al. 2000) and predictions from physical and/or chemical properties (Abraham et al. 2005; Basak et al. 2004; Poulin and Krishnan 1996). Values from experimental determinations are 7–11 in human blood and 15–21 in rodent blood.

The blood/air partition coefficient has shown concentration dependency when evaluated in rat blood, with values of 16–21 at concentrations <10 µmol (injected into a sealed vial) and 6–9 at concentrations of 37–187 µmol (Béliveau and Krishnan 2000b; Béliveau et al. 2001). Jia et al. (2012) derived a population-based estimate of the blood/air distribution coefficient for chloroform based on data collected on blood

chloroform levels and personal air volatile organic compound (VOC) monitoring in a subset of the 1999–2000 NHANES sample. The blood/air distribution coefficient is a partition coefficient measured under "real-world" scenarios, rather than a controlled laboratory setting that cannot account for human variability and often does not evaluate various air concentrations and/or mixed exposure scenarios. Based on NHANES data, the mean distribution coefficient was 51.3 (standard error [SE]=7.0; N=195 adults). This study also found a significant inverse association between the distribution coefficient and the air chloroform concentration, consistent with the experimental data in rat blood.

In inhalation exposures, the arterial blood concentration of chloroform is directly proportional to the concentration in inhaled air. At anesthetic concentrations (8,000–10,000 ppm), steady-state arterial blood concentrations of chloroform were 7–16.2 mg/mL (Smith et al. 1973). Total body equilibrium with inspired chloroform concentration required at least 2 hours at resting ventilation and cardiac output (Smith et al. 1973).

Xu and Weisel (2005) measured blood chloroform kinetics in six adult subjects who inhaled chloroform released from shower water while protected from dermal contact. Observations of air and blood chloroform concentrations were fit to a one-compartment model to estimate an absorption percentage of 71% (range 40–80%).

Aggazzotti et al. (1993, 1995) measured the amount of chloroform absorbed from swimming in indoor swimming pools. Alveolar air samples were collected from both swimmers and observers who did not swim. The chloroform concentration in plasma was correlated with the concentration in air (Spearman's coefficient 0.74). No differences were found between males and females in any exposure group.

Cammann and Hübner (1995) attempted to correlate chloroform exposure with blood and urine chloroform concentrations in persons using indoor swimming pools. Water and air samples were collected from three swimming pools in Germany, with blood and urine samples collected from attendants, normal swimmers, and agonistic swimmers before and after environmental exposure. Pool water chloroform levels ranged from 3.04 to 27.8 µg/L, while air concentrations ranged from 7.77 to 191 µg/m^3. In general, blood chloroform levels increased with exposure. Blood levels were lowest in attendants (0.13–2.45 µg/L), followed by normal swimmers (0.56–1.65 µg/L), and agonistic swimmers (1.14–5.23 µg/L). Based upon the differences seen in the two swimming groups, the study authors concluded that increased physical activity leads to increased absorption and/or ingestion of chloroform.

In a similar study, Lévesque et al. (1994) attempted to quantitate the body burden of chloroform following exposure in an indoor pool. Scuba divers were exposed to chloroform-laden water and air on each of 7 days. On each exposure day, the subjects exercised for a 55-minute period; alveolar air samples were collected before exercise and after 35 or 55 minutes of exercise. Pre-exercise alveolar levels of chloroform averaged 52.6 ppb; this was attributed to air contamination in the locker room. Alveolar air concentrations of chloroform after 35 and 55 minutes of exercise increased steadily through day 5, averaging 0.1–0.95 and 0.104–1.093 ppm, respectively. On day 6, when scuba gear was worn by the subjects, alveolar air concentrations after 35 and 55 minutes of exercise were 0.196 and 0.209 ppm, respectively. The study authors concluded that the average proportions of body burden due to inhalation after 35 and 55 minutes of exercise were 76 and 78%, respectively.

Nashelsky et al. (1995) described one nonfatal assault and three deaths in which chloroform was utilized. Blood and/or tissue concentrations of chloroform were determined in the assault victim and one decedent within 24 hours, within 10 days in another decedent who was frozen for the majority of that period, and after 5 months without preservation in the last decedent. Blood concentrations in two decedents were 2 and 3 µg/mL; fat concentrations were 10 and 42 µg/mL; brain concentrations were 3 and 46 µg/mL; and the liver concentration in one decedent was 24 µg/mL. Due to the nature of the tissues analyzed, these data should be regarded as qualitative indicators of chloroform absorption only.

Yoshida et al. (1999) measured uptake of inhaled chloroform in rats exposed to chloroform in a closed chamber. The kinetics of chloroform uptake from the chamber slowed as the chamber concentration increased from 0.01 to 100 ppm. This observation is consistent with saturable metabolic elimination of absorbed chloroform.

Oral. Peak blood levels were reached 1 hour following ingestion of ^{13}C-labeled chloroform (0.5 g) in a gelatin capsule (Fry et al. 1972). Based on measurements of exhaled chloroform, approximately 100% of the dose was estimated to have been absorbed.

Experiments in mice, rats, and monkeys indicate that oral doses (60 mg/kg) of ^{14}C-labeled chloroform in olive oil were almost completely absorbed as indicated by an 80–96% recovery of radioactivity in expired air, urine, and carcass (Brown et al. 1974a; Taylor et al. 1974). Absorption in mice and monkeys was rapid with peak blood levels reached 1 hour after oral administration of 60 mg/kg chloroform in olive oil.

Studies conducted in mice and rats have found that oral absorption of chloroform is affected by the vehicle in which it is administered. In general, absorption is higher when doses were dissolved in water, compared to corn oil or aqueous 2% Emulphor (Dix et al. 1997; Pereira 1994). Intestinal absorption of chloroform in either water or corn oil administered intragastrically to rats was rapid with both vehicles, but the rate and extent of absorption varied greatly (Withey et al. 1983). The peak concentrations of chloroform in blood were 39.3 µg/mL when administered in water and 5.9 µg/mL when administered in corn oil in rats. The greater degree of absorption following administration in water can be explained by the faster partitioning of a lipophilic compound such as chloroform with mucosal lipids from an aqueous vehicle. Peak blood concentrations were reached somewhat more rapidly with the water vehicle (5.6 minutes as opposed to 6 minutes for corn oil). The uptake from a corn oil solution was more complex (pulsed) than from aqueous solution. A possible explanation for this behavior is that the chloroform in corn oil was broken up into immiscible globules, some of which did not come into contact with the gastric mucosa. Another possible explanation was that intragastric motility may have separated the doses into aliquots that were differentially absorbed from the gastrointestinal tract.

Pereira (1994) investigated the uptake and protein binding of chloroform in the liver and kidney in female B6C3F1 mice. Animals received single doses of chloroform by gavage in either water or corn oil. Uptake of chloroform from water into the liver peaked in 1.5 minutes, and hepatic uptake during the first 20 minutes exceeded that of chloroform delivered in oil. During the first 20 minutes after dosing, binding of chloroform to macromolecules in the liver was greater when water vehicle was utilized; beyond 20 minutes, the amount of binding was equivalent between the two vehicle groups. Renal uptake of chloroform from water exceeded uptake of chloroform from oil over the entire 4-hour period. The extent of binding to macromolecules in kidneys was consistently greater in the group given chloroform in water. Differences in chloroform toxicity based on the vehicle have also been reported elsewhere (Larson et al. 1994b, 1995a).

Dermal. Dermal absorption of chloroform is dependent on ambient temperature. In a study of 10 adult subjects who bathed in water containing 100 µg/L chloroform, the amount of chloroform exhaled when the bath water temperature was 40°C was approximately 40 times that observed when the temperature was 30°C (Gordon et al. 1998). Dick et al. (1995) estimated dermal absorption of chloroform in seven adult subjects. Each subject was exposed to [^{14}C]-chloroform applied to a covered 3.1 cm^2 area of the forearm. The applied doses were 50 µg in water or 250 µg in ethanol and the exposure duration was 8 hours. Absorption was estimated from the sum of ^{14}C exhaled and excreted during a 4-hour period

following the exposure. Mean absorption was 7.8% (standard deviation [SD]=1.4%) when the vehicle was water and 1.6% (SD=0.3%) when the vehicle was ethanol.

Dermal absorption of chloroform is governed, in part, by its rate of diffusion through the skin. Fan et al. (2007) estimated the dermal permeability coefficient (K_P, cm/hour) for chloroform in 11 adult subjects. Each subject was exposed by immersing their hand and forearm in 100 µg chloroform/L water contained in a closed chamber. The K_P was estimated from the rate of change in chloroform concentration in the chamber reservoir. The mean was 0.166 cm/hour (SD: 0.108 cm/hour). In a study in which adult subjects bathed in water containing 40 µg/L chloroform (38°C) while wearing a breathing mask, the K_P, estimated from the rate of chloroform exhaled, was 0.015 cm/hour (Xu and Weisel 2005).

Several studies have estimated the dermal K_P from measurements made in excised human skin preparations. Estimates ranged from 0.016 to 0.16 cm/hour (Dick et al. 1995; Nakai et al. 1999; Poulin and Krishnan 2001; Xu et al. 2002). Differences in estimates may reflect differences in methods used to estimate the K_P (Bunge et al. 1995).

Lévesque et al. (1994) attempted to quantitate the body burden of chloroform following dermal and inhalation exposure in an indoor swimming pool. Male scuba divers were exposed to chloroform-laden water and air on each of 7 days. On each exposure day the subjects exercised for a 55-minute period. On day 6 of the experiment, subjects wore scuba gear to determine the percentage body burden due to dermal exposure. On day 6, when scuba gear was worn by the subjects, alveolar air concentrations after 35 and 55 minutes of exercise were 196 and 209 ppb, respectively. From these data, it would appear that the average proportions of body burden due to dermal exposure after 35 and 55 minutes of exercise were 24 and 22%, respectively.

Cammann and Hübner (1995) attempted to correlate chloroform exposure with blood and urine chloroform concentrations in persons using indoor swimming pools. Water and air samples were collected from three pools in Germany, and blood and urine samples were collected from attendants, normal swimmers, and agonistic swimmers before and after exposure. Pool water chloroform levels ranged from 3.04 to 27.8 µg/L, while air concentrations ranged from 7.77 to 191 µg/m^3. Blood chloroform levels generally increased with higher chloroform exposure levels. Blood levels were lowest in attendants (0.13–2.45 µg/L), followed by normal swimmers (0.56–1.65 µg/L), and agonistic swimmers (1.14–5.23 µg/L). Based upon the differences seen in the two swimming groups, the study authors concluded that increased physical activity leads to increased absorption and/or ingestion. With the

exception of the inclusion of attendants, the study authors did not attempt to differentiate between inhalation and dermal absorption of chloroform. However, the increased blood concentrations seen in the swimmers seems to indicate that dermal absorption did indeed occur.

According to dermal absorption studies with solvents other than chloroform, the absorption of such solvents in guinea pigs is more rapid than metabolism or pulmonary excretion (Jakobson et al. 1982). A dermal absorption rate of 329 nmol/minute/cm^2 (±60 nmol/minute/cm^2) was calculated for the shaved abdominal skin of mice (Tsuruta 1975). This is equivalent to a human absorption rate of 29.7 mg/minute, assuming that a pair of hands are immersed in liquid chloroform (Tsuruta 1975). However, this calculation was based on the assumptions that the rate of chloroform penetration is uniform for all kinds of skin and that the total surface area of a pair of human hands is 800 cm^2; the former assumption is especially dubious.

Islam et al. (1995, 1996, 1999a, 1999b) investigated the fate of topically applied chloroform in male hairless rats. Hairless rats were exposed by immersion in an aqueous solution of chloroform (Islam et al. 1996). Inhalation was prevented by isolating the head in an enclosed chamber. Chloroform was detected in blood within 4 minutes of immersion. Systemic absorption was estimated from the blood chloroform profile (area under the curve [AUC]) observed during and following dermal exposure or an intravenous dose. A 30-minute exposure to 0.44 mg/mL resulted in absorption of approximately 10.2 mg of chloroform. Islam et al. (1999a) estimated absorption of chloroform from dermal exposures to neat chloroform applied to the back of hairless rats. Systemic absorption was estimated from the dermal and intravenous blood chloroform AUC. Rats exposed to 1557 mg chloroform over a 5.46 cm^2 area absorbed approximately 2.8 mg following a 1-minute exposure, 2.5 mg following a 3-minute exposure, and 13.3 mg following an 8-minute exposure. Following cessation of dermal exposure, chloroform was rapidly eliminated from the skin surface by evaporation with a half-time of 2–3 minutes (Islam et al. 1999b). Dermal permeability coefficients (cm/hour) in rats have been estimated from *in vivo* dermal exposure studies (Bogen and Keating 2000).

3.1.2 Distribution

Chloroform is lipid soluble and readily passes through cell membranes, causing narcosis at high concentrations. Blood chloroform concentrations during anesthesia (presumed concentrations 8,000–10,000 ppm) were 7–16.2 mg/mL in 10 patients (Smith et al. 1973). An arterial chloroform concentration of 0.24 mg/mL during anesthesia corresponded to the following partition coefficients: blood/gas, 8;

blood/vessel rich compartment, 1.9; blood/muscle compartment, 1.9; blood/fat compartment, 31; blood/vessel poor compartment, 1; and blood/liver, 2 (Feingold and Holaday 1977). Partition coefficients were calculated for humans based on results in mice and rats, and in human tissues *in vitro*: blood/air, 7.4; liver/air, 17; kidney/air, 11; and fat/air, 280 (Corley et al. 1990).

Tissue/blood partition coefficients for chloroform have been estimated in a variety of ways, including experimental measurements under equilibrium conditions (Gargas et al. 1989; Kaneko et al. 2000; Mahle et al. 2007; Paixao et al. 2013; Thrall et al. 2002) and predictions from physical and/or chemical properties (Abraham and Ibrahim 2006; Abraham et al. 2006; DeJongh et al. 1997; Derricott et al. 2015; Poulin and Krishnan 1996). In general, the highest partition coefficients have been measured in adipose tissue (20–40 times that of other tissues). The value for the tissue/blood partition coefficient depends on the composition of the tissue (Poulin and Krishnan 1996) and varies across species, age, and other factors that affect tissue composition (Mahle et al. 2007).

The chloroform levels in seven patients who died after excessive administration during anesthesia were: brain, 372–480 mg/kg; lungs, 355–485 mg/kg; and liver, 190–275 mg/kg (Gettler and Blume 1931). The chloroform levels in patients under anesthesia who died from other causes were: brain, 120–182 mg/kg; lungs, 92–145 mg/kg; and liver, 65–88 mg/kg tissue wet weight. Nashelsky et al. (1995) described one nonfatal assault and three deaths in which chloroform was utilized. Blood and/or tissue concentrations of chloroform were determined in the assault victim and one decedent within 24 hours, within 10 days in another decedent who was frozen for the majority of that period, and after 5 months without preservation in the last decedent. Blood concentrations in two decedents were 2 and 3 µg/mL; fat concentrations were 10 and 42 µg/mL; brain concentrations were 3 and 46 µg/mL; and the liver concentration in one decedent was 24 µg/mL.

After whole-body autoradiography to study the distribution of ^{14}C-labeled chloroform in mice, most of the radioactivity was found in fat immediately after exposure, while the concentration of radioactivity in the liver increased during the postanesthetic period, most likely due to covalent binding to lipid and protein in the liver (Cohen and Hood 1969). Partition coefficients (tissue/air) for mice and rats were 21.3 and 20.8 for blood; 19.1 and 21.1 for liver; 11 and 11 for kidney; and 242 and 203 for fat, respectively (Corley et al. 1990). Arterial levels of chloroform in mongrel dogs reached 0.35–0.40 mg/mL by the time animals were in deep anesthesia (Chenoweth et al. 1962). Chloroform concentrations in the inhaled stream were not measured, however. After 2.5 hours of deep anesthesia, there was 392 mg/kg chloroform in brain tissue, 1,305 mg/kg in adrenals, 2,820 mg/kg in omental fat, and 290 mg/kg in the liver.

Radioactivity from ^{14}C-labeled chloroform was detected in the placenta and fetuses of mice shortly after inhalation exposure (Danielsson et al. 1986). During early gestation, accumulation of radioactivity was observed in the embryonic neural tissues, while the respiratory epithelium was more involved in chloroform metabolism in the late fetal period.

Due to its lipophilic character, chloroform accumulates to a greater extent in tissues of high lipid content. As shown by the results presented above, the relative concentrations of chloroform in various tissues decreased as follows: adipose tissue > brain > liver > kidney > blood.

No studies were located regarding distribution in humans after oral exposure to chloroform.

Take et al. (2010) compared the distribution of chloroform in rats following separate or simultaneous oral and inhalation exposure. Rats received an oral dose of 55 mg/kg deuterated chloroform ($CDCl_3$) separately or simultaneously with an exposure to 100 ppm chloroform for 360 minutes in a closed chamber. The highest chloroform or $CDCl_3$ concentrations were observed in fat. Combined oral and inhalation exposure increased concentrations of orally administered $CDCl_3$ in blood, fat, kidney, and liver, compared to oral exposure alone. This suggests that the inhaled chloroform altered the disposition of orally administered $CDCl_3$, possibly by inhibiting $CDCl_3$ metabolism.

Take et al. (2014) found that the blood AUC/kg for chloroform following inhalation exposure of rats showed a strong linear correlation (r-0.99) with the inhalation exposure concentration. Based on a linear regression model of inhalation dose and AUC, Take et al. (2014) estimated the inhalation exposure that would be equivalent to the AUC/kg observed following oral exposure to chloroform in corn oil. Over the range of oral doses explored (12.5–100 mg/kg), the inhalation equivalent dose ranged from 19 to 187 ppm.

High concentrations of radioactivity were observed in body fat and livers of rats, mice, and squirrel monkeys given oral doses of 60 mg/kg ^{14}C-labeled chloroform (Brown et al. 1974a). The maximum levels of radioactivity in the blood appeared within 1 hour and were 3 µg equivalents chloroform/mL for mice and 10 µg equivalents chloroform/ml for monkeys, which represented ≈0.35 and 1%, respectively, of the total radioactivity. In monkeys, bile concentrations peaked within 6 hours. The distribution of radioactively labeled chloroform was studied in three strains of mice (Taylor et al. 1974). No strain-related differences were observed; however, higher levels of radioactivity were found in the renal cortex

of males and in the liver of females. The renal binding of radioactive metabolites may have been altered by variations in the testosterone levels as a result of hormonal pretreatment in females or castration in males. Sex-linked differences in chloroform distribution were not observed in rats or monkeys (Brown et al. 1974a). Chloroform accumulates in the adipose tissue of rats after oral exposure of intermediate duration (Pfaffenberger et al. 1980).

Islam et al. (1995) investigated the fate of topically applied chloroform in male hairless rats. For exposures <4 minutes, chloroform-laden water was applied to shaved back skin; for exposures of 4–30 minutes, rats were submerged in baths containing chloroform-laden water. Selected skin areas were tape-stripped a various number of times after various delay periods. The study authors found that the accumulated amount of chloroform declined rapidly with depth of stratum corneum. As the time of exposure decreased, smaller amounts of chloroform were found in the deeper layers of stratum corneum; by 5 minutes postexposure, the amount of chloroform at the first tape strip (skin surface) dropped to negligible levels. It appeared that there was an incremental build-up of chloroform in the skin over the first 4 minutes. When compared to uptake measured by bath concentration differences, approximately 88% of the chloroform dose was not accounted for in the stratum corneum and was assumed to be systemically absorbed.

3.1.3 Metabolism

The metabolism of chloroform is well understood. Approximately 50% of an oral dose of 0.5 g chloroform was metabolized to carbon dioxide in humans (Fry et al. 1972). Metabolism was dose-dependent, decreasing with higher exposure. A first-pass effect was observed after oral exposure (Chiou 1975). Approximately 38% of the dose was converted in the liver, and 17% was exhaled unchanged from the lungs before reaching the systemic circulation. *In vivo* metabolic rate constants of $V_{max}C$=15.7 mg/hour/kg and K_M=0.448 mg/L were defined for humans based on pharmacokinetic results obtained from inhalation studies in rats and mice and *in vitro* enzymatic studies in human tissues (Corley et al. 1990). The metabolic activation of chloroform to its toxic intermediate, phosgene, was slower in humans than in rodents.

Metabolic pathways of chloroform biotransformation are shown in Figure 3-1. Metabolism studies indicated that chloroform was, in part, exhaled from the lungs or was converted by oxidative dehydrochlorination of its carbon-hydrogen bond to form phosgene (Pohl et al. 1981; Stevens and Anders 1981). This reaction was mediated by cytochrome P450 and was observed in the liver and kidneys (Ade et al. 1994; Branchflower et al. 1984; Liu et al. 2013; Smith et al. 1984). The dominant isozyme mediating chloroform metabolism in rats and humans is CYP2E1 (Constan et al. 1999; Gemma et al. 2003; Lipscomb et al. 2004; Testai et al. 1996). However, other isoenzymes contribute to the low-affinity phase of oxidative metabolism, including CYP2A6 in humans and CYP2B1/2 in rats (Gemma et al. 2003; Testai et al. 1996). In renal cortex microsomes of DBA/2J mice, the majority of chloroform metabolism was oxidative under ambient oxygen conditions, while anoxic conditions resulted in reductive metabolism (Ade et al. 1994).

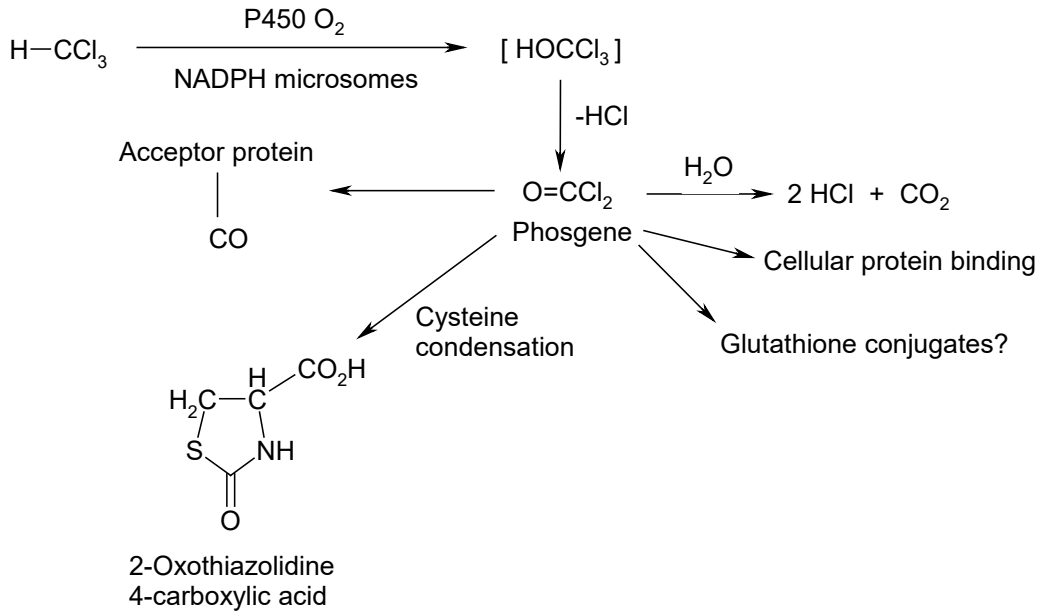
Phosgene may react with two molecules of GSH to form diglutathionyl dithiocarbonate, which is further metabolized in the kidneys, or it may react with other cellular elements and induce cytotoxicity (Pohl and Gillette 1984). *In vitro* studies indicate that phosgene and other reactive chloroform metabolites bind to lipids and proteins of the endoplasmic reticulum proximate to the cytochrome P450 (Sipes et al. 1977; Wolf et al. 1977). The metabolism of chloroform to reactive metabolites occurs not only in microsomes but also in nuclear preparations (Gomez and Castro 1980). Covalent binding of chloroform to lipids can occur under anaerobic and aerobic conditions, while binding to the protein occurs only under aerobic conditions (Testai et al. 1987).

It was further demonstrated that chloroform can induce lipid peroxidation and inactivation of cytochrome P450 in rat liver microsomes under anaerobic conditions (de Groot and Noll 1989). Covalent binding of chloroform metabolites to microsomal protein *in vitro* was intensified by microsomal enzyme inducers and prevented by GSH (Brown et al. 1974b). It was proposed that the reaction of chloroform metabolites with GSH may act as a detoxifying mechanism. When GSH is depleted, however, the metabolites react with microsomal protein, and may cause necrosis. This is supported by observations that chloroform doses that caused liver GSH depletion produced liver necrosis (Docks and Krishna 1976). In fasted animals, chloroform has been found to be more hepatotoxic (Brown et al. 1974b; Docks and Krishna 1976) even though animals were found to have lower blood chloroform concentrations (Wang et al. 1995); this phenomenon would apparently be explained by a decreased GSH content and resultant inability to bind toxic metabolites. This may explain the clinical finding of severe acute hepatotoxicity in women exposed to chloroform via anesthesia during prolonged parturition.

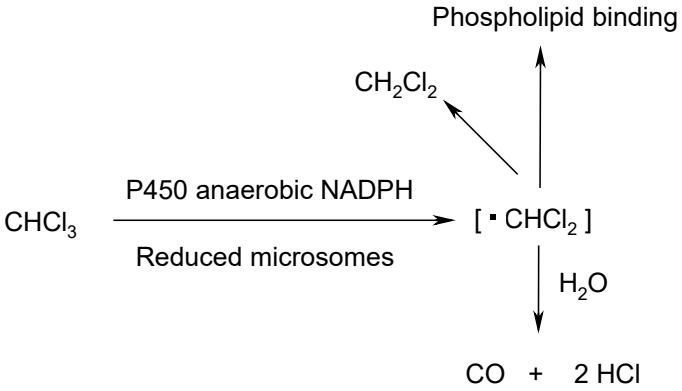
Evidence that chloroform is metabolized at its carbon-hydrogen bond is provided by experiments using the deuterated derivative of chloroform (Branchflower et al. 1984; McCarty et al. 1979; Pohl et al. 1980). Deuterated chloroform was one-half to one-third as cytotoxic as chloroform, and its conversion to phosgene was much slower. The results confirmed that the toxicity of chloroform is primarily due to its metabolites.

Figure 3-1. Metabolic Pathways of Chloroform Biotransformation

Major aerobic pathway



Minor aerobic pathway



An *in vitro* study of mice hepatic microsomes indicated that a reductive pathway may also play an important role in chloroform hepatotoxicity (Testai et al. 1990, 1995). It was demonstrated that radical chloroform metabolites bind to macromolecules (proteins, lipids) and the process can be inhibited by reduced GSH.

The final product of the aerobic metabolic pathway of chloroform is carbon dioxide (Brown et al. 1974a; Fry et al. 1972). This carbon dioxide is mostly eliminated through the lungs, but some is incorporated into endogenous metabolites and excreted as bicarbonate, urea, methionine, and other amino acids (Brown et al. 1974a). Chloride ions are an end product of chloroform metabolism found in the urine (Van Dyke et al. 1964). Carbon monoxide was a minor product of the anaerobic metabolism of chloroform *in vitro* (Ahmed et al. 1977) and *in vivo* in rats (Anders et al. 1978; Pankow and Damme 1999).

A sex-related difference in chloroform metabolism was observed in mice (Taylor et al. 1974). Chloroform accumulated and metabolized in the renal cortex of males to a greater extent than in females, while liver chloroform concentrations were greater in females than in males; the results may have been influenced by testosterone levels. This effect was not observed in any other species and may explain why male mice were more susceptible to the lethal and renal effects of chloroform than were females (Deringer et al. 1953).

Wang et al. (1994) found that, in male Wistar rats, pretreatment with ethanol increased chloroform metabolism about 2-fold but did not affect hepatic microsomal protein of cytochrome P450 content. In addition, intraperitoneal administration of chloroform resulted in greater blood concentrations, peak values, and AUCs, as compared to oral administration. AUCs in rats administered chloroform orally were 0.34–6.45 versus 0.58–8.78 in rats administered chloroform intraperitoneally. The study authors concluded that differences between route groups in hepatotoxicity were due to differences in the proportion of dose exposed to first-pass metabolism. Since oral dosing results in the greatest first-pass exposure, this route resulted in the greatest hepatotoxicity. The degree of hepatic exposure also influenced the enhancing effect of ethanol; the group receiving chloroform orally was affected the most by ethanol pretreatment. The study authors also concluded that intraperitoneal exposure produced data most like that of inhalation exposure, presumably due to the smaller proportion of dose going through first-pass metabolism.

Interspecies differences in the rate of chloroform conversion were observed in mice, rats, and squirrel monkeys, with species differences in metabolism being highly dependent on dose. The conversion of

chloroform to carbon dioxide was highest in mice (80%) and lowest in squirrel monkeys (18%) (Brown et al. 1974a). Similarly, chloroform metabolism was calculated to be slower in humans than in rodents. Therefore, it was estimated that the exposure to equivalent concentrations of chloroform would lead to a much lower delivered dose in humans (Corley et al. 1990). Inter-strain differences in kinetics of metabolism of chloroform in rodents has also been observed (Vittozzi et al. 2000, 2001).

A study by Gearhart et al. (1993) was conducted to determine the interactions of chloroform exposure with body temperature, gas uptake, and tissue solubility in mice as possible explanations for the difficulty in fitting a physiologically based pharmacokinetic/pharmacodynamic (PBPK/D) model to chloroform gas-uptake data to derive *in vivo* metabolic constants. Male mice were exposed to air concentrations of 100, 800, 2,000, or 5,500 ppm chloroform for 6 hours and their core body temperatures were monitored frequently over the exposure period. After exposure, blood, liver, thigh muscle, and fat tissues were removed for tissue/air and tissue/blood partition coefficient analysis at three temperatures (25, 31, and 37°C). For all tissues, tissue/air partition coefficients exhibited temperature-dependent decreases with increasing temperature. The rate of decrease was greatest for the blood/air partition coefficient. Average body temperatures for each exposure group decreased as the exposure concentrations increased. Temperature-dependent decreases in core body temperature were hypothesized to decrease overall metabolism of chloroform in mice. The data collected were also used to develop a PBPK model for chloroform disposition.

3.1.4 Excretion

Xu and Weisel (2005) measured blood chloroform kinetics in six adult subjects who inhaled chloroform released from shower water while protected from dermal contact with the water. Observations of air and blood chloroform concentrations were fit to a one-compartment model to estimate a residence time (τ) of 13.1 min (±1.62 SD) which corresponds to a first-order half-time of 9.1 minutes ($\tau \cdot \ln[2]$). A two-compartment model fit to the same data yielded half-times of 4.7 and 39 minutes.

Chloroform was detected in the exhaled air of volunteers exposed to a normal environment, heavy automobile traffic, or air in a dry-cleaning establishment (Gordon et al. 1988). Higher chloroform levels in the breath corresponded to higher exposure levels. The calculated biological half-time for chloroform was 7.9 hours.

Following a single, oral exposure, most of the 0.5 g of radioactively labeled chloroform administered to volunteers was exhaled during the first 8 hours after exposure (Fry et al. 1972). A slower rate of pulmonary excretion was observed during the first 8 hours in volunteers who had more adipose tissue than the other volunteers. Up to 68.3% of the dose was excreted unchanged, and up to 50.6% was excreted as carbon dioxide. A positive correlation was made between pulmonary excretion and blood concentration. Less than 1% of the radioactivity was detected in the urine.

Dick et al. (1995) examined the excretion of chloroform in seven adult subjects following dermal exposure to chloroform. Each subject was exposed to [^{14}C]-labeled chloroform applied to a covered 3.1-cm^2 area of the forearm. The applied doses were 50 µg in water or 250 µg in ethanol and the exposure duration was 8 hours. Urinary excretion of ^{14}C was measured for a period of 3 days following the start of dermal exposure and exhaled ^{14}C was measured for the first 48 hours following the start of exposure. When administered in water, mean urinary excretion was 0.42% of the applied dose and excretion from the lungs was 7.8%. When chloroform was administered in ethanol, the mean urinary excretion was 0.07% of the applied dose and excretion from the lungs was 0.83%.

Excretion of radioactivity in mice and rats was monitored for 48 hours following exposure to ^{14}C-labeled chloroform (Corley et al. 1990). In general, 92–99% of the total radioactivity was recovered in mice and 58–98% was recovered in rats; the percentage of recovery decreased with increasing exposure. With increasing concentration, mice exhaled 80–85% of the total radioactivity recovered as ^{14}C-labeled carbon dioxide, 0.4–8% as ^{14}C-labeled chloroform, and 8–11 and 0.6–1.4% as urinary and fecal metabolites, respectively. Rats exhaled 48–85% of the total radioactivity as ^{14}C-labeled carbon dioxide, 2–42% as ^{14}C-labeled chloroform, and 8–11 and 0.1–0.6% in the urine and feces, respectively. A 4-fold increase in exposure concentration was followed by a 50- and 20-fold increase in the amount of exhaled, unmetabolized chloroform in mice and rats, respectively.

Approximately 80% of a single dose of 60 mg/kg ^{14}C-labeled chloroform was converted within 24 hours to ^{14}C-labeled carbon dioxide in mice (Brown et al. 1974a; Taylor et al. 1974), while only ≈66% of the dose was converted to ^{14}C-labeled carbon dioxide in rats (Brown et al. 1974a). Eight hours after administration of 100–150 mg/kg of ^{14}C-labeled chloroform, 49.6 and 6.5% of radioactivity was converted to carbon dioxide, 26.1 and 64.8% was expired as unmetabolized parent compound, and 4.9 and 3.6% was detected in the urine in mice and rats, respectively (Mink et al. 1986). These results indicate that mice metabolize high doses of chloroform to a greater degree than rats. Only 18% of a chloroform dose was metabolized to ^{14}C-labeled carbon dioxide in monkeys, and ≈79% was detected as

unchanged parent compound or toluene soluble metabolites (Brown et al. 1974a). Within 48 hours after exposure, ≈2, 8, and 3% of the administered radioactivity was detected in the urine and feces of monkeys, rats, and mice, respectively.

Islam et al. (1996, 1999a) measured the systemic clearance of chloroform in rats following dermal exposures. Elimination of chloroform from blood was biphasic with estimated rate constants (k) of 0.030 and 0.007 $minute^{-1}$ (Islam et al. 1996). The equivalent first-order half-times (ln[2]/k) were 23 and 99 minutes.

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

Models are simplified representations of a system with the intent of reproducing or simulating its structure, function, and behavior. PBPK models are more firmly grounded in principles of biology and biochemistry. They use mathematical descriptions of the processes determining uptake and disposition of chemical substances as a function of their physicochemical, biochemical, and physiological characteristics (Andersen and Krishnan 1994; Clewell 1995; Mumtaz et al. 2012a; Sweeney and Gearhart 2020). PBPK models have been developed for both organic and inorganic pollutants (Ruiz et al. 2011) and are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Mumtaz et al. 2012b; Ruiz et al. 2011; Sweeney and Gearhart 2020; Tan et al. 2020). PBPK models can also be used to more accurately extrapolate from animal to human, high dose to low dose, route to route, and various exposure scenarios and to study pollutant mixtures (El-Masri et al. 2004). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints (Clewell 1995).

3.1.5.1 Summary of PBPK/PD Models

Several rodent and human PBPK models have been used to predict the absorption (oral, inhalation, and dermal) from water and air, distribution, metabolism, and excretion of chloroform (Chinery and Gleason 1993; Corley et al. 1990, 2000; Evans et al. 2020; Gearhart et al. 1993; Haddad et al. 2006; Norman et al. 2008; Reitz et al. 1990; Roy et al. 1996a, 1996b; Sarangapani et al. 2002). Steady-state solutions to chloroform PBPK models for predicting steady-state blood levels have also been reported (Aylward et al. 2010). Some of the above models have been used to support interspecies extrapolation of biologically

based dose response models (Conolly and Butterworth 1995; Luke et al. 2010; Pelekis et al. 2001; Sasso et al. 2013; Smith et al. 1995; Tan et al. 2003) or to evaluate the relative contributions of dermal, inhalation, and oral exposure pathways to internal doses of chloroform from environmental exposures such as showering (Haddad et al. 2006; Lyons et al. 2008; Tan et al. 2006, 2007). Population-based models have been developed that account for parameter variability and uncertainty (Delic et al. 2000; Yang et al. 2010).

In a PBPK model that used simulations with mice, rats, and humans (Corley et al. 1990), the tissue delivered dose from equivalent concentrations of chloroform was highest in the mouse, followed by rats, and then humans. The study authors suggested that this behavior is predicted by the model because of the lower relative rates of metabolism, ventilation, and cardiac output (per kg of body weight) in the larger species. Assuming equivalent target doses produce equivalent toxicities in target tissues, the relative sensitivities of the three species used in the study (mouse > rat > human) predicted by the model under identical exposure conditions are quite different from the relative sensitivity to chloroform assumed by the "uncertainty factor."

In a PBPK/PD model based closely on the Corley model, Reitz et al. (1990) described a pharmacodynamic endpoint (cytotoxicity) in the livers of chloroform-exposed animals produced by phosgene, the reactive metabolite of chloroform.

In gas-uptake experiments, Gearhart et al. (1993) demonstrated a dose-dependent decrease in core body temperature with increased inhaled concentrations of chloroform. The decrease in body temperature could account for decreased *in vivo* chloroform metabolism, partition coefficients, pulmonary ventilation, and cardiac output rates in mice.

Chinery and Gleason (1993) used a shower model for chloroform-contaminated water to predict breath concentration (as a quantifiable function of tissue dose) and actual absorbed dose from a measured water supply concentration following exposure while showering. The model's predictions demonstrated that dose information based only on dermal absorption (without considering an inhalation component) may underestimate actual dose to target organs in dosimetric assessment for chloroform in water supplies during showering. The model also predicted a steady-state stratum corneum permeability of chloroform in human skin in the range of 0.16–0.36 cm/hour, with the most likely value being 0.2 cm/hour. The study authors suggested that the results predicted by this model could be used to estimate household exposures to chloroform or other exposures which include dermal absorption.

McKone (1993) demonstrated that chloroform in shower water had an average effective dermal permeability between 0.16 and 0.42 cm/hour for a 10-minute shower. The model predicted that the ratio of chloroform dermally absorbed in the shower (relative to chloroform-contaminated water concentration) ranged between 0.25 and 0.66 mg per mg/L. In addition, the McKone model demonstrated that chloroform metabolism by the liver was not linear across all dermal/inhalation exposure concentrations and became nonlinear at higher (60–100 mg/L) dose concentrations.

3.1.5.2 Chloroform PBPK Model Comparison

Several chloroform PBPK models that describe the disposition of chloroform in animals and humans have been identified from the open literature (from the early 1980s to 1994). Based on the information presented in these models, there is evidence to suggest that PBPK models for chloroform are fairly refined and have the potential for use in human health risk assessments when key conditions are met (e.g., exposure route and duration, evaluated species, target tissue).

The PBPK model developed by Corley et al. (1990) has provided a basic model for the fate of chloroform in humans and laboratory animals. The Corley et al. (1990) model has been modified in various ways for use in dosimetry extrapolation and exposure pathway apportionment studies (Corley et al. 2000; Delic et al. 2000; Liao et al. 2007; Norman et al. 2008; Roy et al. 1996a, 1996b; Sarangapani et al. 2002; Sasso et al. 2013; Yang et al. 2010). The models of Corley et al. (1990) and Reitz et al. (1990) have described several aspects of chloroform metabolism and disposition in laboratory animals and humans; however, they do not address the dermal routes of exposure.

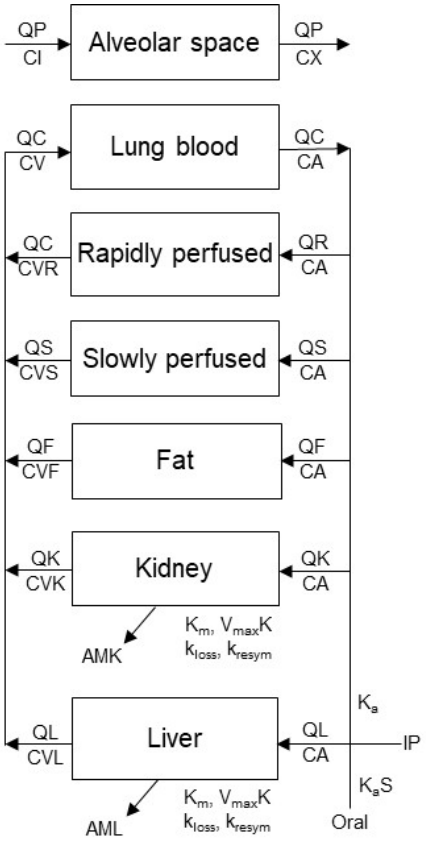
The models of McKone (1993), Chinery and Gleason (1993), and Corley et al. (2000) address both the inhalation and dermal exposure routes in humans. Several different approaches to modeling the skin have been reported, including single-compartment, well-mixed models (Corley et al. 2000; McKone 1993), multicompartment skin models (Chinery and Gleason 1993; Norman et al. 2008; Roy et al. 1996a, 1996b), and membrane diffusion models (Norman et al. 2008). Further discussion of each model and its application in human risk assessments is presented below.

3.1.5.3 Discussion of Chloroform Models

The Corley et al. (1990, 2000) Model

The Corley model (Corley et al. 1990) was the first chloroform PBPK model to describe and ultimately predict the fate of chloroform in several species (including humans) under a variety of exposure conditions. Many subsequent PBPK models for chloroform are based on the Corley model. Therefore, the Corley model is shown schematically in Figure 3-2 and discussed in-depth below, with subsequent models discussed more briefly. The Corley model has been used for cancer risk assessment (Reitz et al. 1990).

Figure 3-2. Parameters Used in the Corley et al. (1990) Physiologically Based Pharmacokinetic Model



Physiological model used to describe the pharmacokinetics in rats, mice, and humans during inhalation, oral, and intraperitoneal (IP) exposures.

AMK = amount metabolized in kidney; AML = amount metabolized in liver

Risk Assessment. This model successfully described the disposition of chloroform in rats, mice, and humans following various exposure scenarios and developed dose surrogates more closely related to toxicity response. With regard to target tissue dosimetry, the Corley model predicts the relative order of susceptibility to chloroform toxicity to be mouse > rat > human based on macromolecular binding (MMB).

Description of the Model. The Corley chloroform PBPK model was based on an earlier PBPK model developed by Ramsey and Andersen (1984) to describe the disposition of styrene exposure in rats, mice, and humans. A schematic representation of the Corley model (taken from Corley et al. 1990) is shown in Figure 3-2 with oral, inhalation, and intraperitoneal routes represented. Liver and kidney are represented as separate compartments since both are target organs for chloroform. The Corley et al. (1990) model has been modified to include two kidney compartments representing cortex and medulla regions of the kidney with metabolism assigned to renal cortex and liver (Liao et al. 2007; Sasso et al. 2013). This modification enabled simulation of dosimetry cell death in renal cortex resulting from exposures to chloroform.

The physiologic, biochemical constants and partition coefficients required for the model are shown in Table 3-1. Physiologic constants (organ weight, blood flows, etc.) were similar to those used by Andersen et al. (1987) or were taken from other literature sources. Tissue and blood partition coefficients were determined in tissues by vial equilibration techniques in the rat and human, with extrapolated values used for the mouse. All metabolism of chloroform was assumed to occur only in the liver and kidneys through a single metabolic pathway (mixed function oxidase) that followed simple Michaelis-Menten kinetic parameters. Metabolic rate constants were obtained from the gas uptake experiments. Human metabolic rate constants were obtained from *in vitro* human microsomal fractions of liver and kidney samples using ^{14}C-$CHCl_3$ as the substrate. MMB of chloroform metabolites (phosgene) was assumed to occur in bioactivating tissues (liver and kidney) in a non-enzymatic, nonspecific, and dose-independent fashion. MMB constants for the liver and kidney were estimated from *in vivo* MMB data obtained from rats and mice exposed to ^{14}C-$CHCl_3$ via inhalation.

Table 3-1. Parameters Used in the Corley et al. (1990) Physiologically Based Pharmacokinetic Model

Parameters	Mouse	Rat	Human
		Weights (kg)	
Body	0.02858	0.230	70.0

Table 3-1. Parameters Used in the Corley et al. (1990) Physiologically Based Pharmacokinetic Model

Parameters	Mouse	Rat	Human
	Percentage of body weight (%)		
Liver	5.86	2.53	3.14
Kidney	1.70	0.71	0.44
Fat	6.00	6.30	23.10
Rapidly perfused tissues	3.30	4.39	3.27
Slowly perfused tissues	74.14	77.07	61.05
	Flow (L/hour/kg)		
Alveolar ventilation	2.01	5.06	347.9
Cardiac output	2.01	5.06	347.9
	Percentage of cardiac output (%)		
Liver	25.0	25.0	25.0
Kidney	25.0	25.0	25.0
Fat	2.0	5.0	5.0
Rapidly perfused tissues	26.0	26.0	26.0
Slowly perfused tissues	19.0	19.0	19.0
	Partition coefficients		
Blood/air	21.3	20.8	7.43
Liver/air	19.1	21.1	17.00
Kidney/air	11.0	11.0	11.00
Fat/air	242.0	203.0	280.00
Rapidly perfused/air	19.1	21.2	17.0
Slowly perfused/air	13.0	13.9	12.0
	Metabolic and macromolecular binding constants		
$V_{max}C$ (mg/hour/kg)	22.8	6.8	15.7
K_M (mg/L)	0.352	0.543	0.448
K_{loss} (L/mg)	0.000572	0	0
K_{resyn} ($hour^{-1}$)	0.125	0	0
A (kidney/liver)	0.153	0.052	0.033
fMMB ($hour^{-1}$), liver	0.003	0.00104	0.00202
fMMB ($hour^{-1}$), kidney	0.010	0.0086	0.00931
	Gavage absorption rate constants		
k_{aS} ($hour^{-1}$), corn oil	0.6	0.6	0.6
k_{aS} ($hour^{-1}$), water	5.0	5.0	5.0
	Intraperitoneal injection absorption rate constant		
K_a ($hour^{-1}$)	1.0	1.0	1.0

The gas-uptake data for rats were well described using a single Michaelis-Menten equation to describe metabolism. For the mouse inhalation studies, a simple Michaelis-Menten equation failed to adequately describe the chloroform-metabolizing capacity based on the data collected and model constants. The

study authors suspected that, following the administration of chloroform (particularly at higher concentrations), destruction of microsomal enzymes and subsequent resynthesis of microsomal enzymes was important in the mouse. This phenomenon has been documented in phenobarbital-induced rats but not naive rats. To account for this phenomenon, a first-order rate constant for the loss and subsequent regeneration of metabolic capacity was incorporated into the model for mice only.

The model also provided a good description of the *in vivo* levels of MMB in both rats and mice, with good agreement between observed and predicted values.

Corley et al. (2000) expanded the Corley et al. (1990) model to include a skin compartment for simulating dermal exposures. Dermal absorption of chloroform to blood is simulated as a diffusion process governed by a dermal permeability coefficient (cm/hour), the concentration gradient between the exposure vehicle and skin, a skin/vehicle partition coefficient, and blood flow to the skin. The value for the permeability coefficient was calibrated to data from human studies. In these studies, adult subjects bathed for 30 minutes in an aqueous solution of chloroform while breathing into a face mask to minimize inhalation exposure and to allow measurements of chloroform in exhaled air. The estimated values for the permeability coefficient varied with temperature of the bath. In male subjects, the coefficient decreased from 0.059 cm/hour at 40°C to 0.010 at 30°C. The temperature-related decrease was greater in females compared to males. In the Corley et al. (2000) model the skin is represented as a single well-mixed compartment. Alternatives to this structure have been evaluated. This includes multi-compartment skin models with and without time lags, which attempt to more acutely represent concentration gradients and diffusion rates in various layers of the skin (Norman et al. 2008; Roy et al. 1996a, 1996b). Norman et al. (2008) compared single-compartment and membrane models of dermal absorption of chloroform and concluded that single-compartment, well-mixed models tend to predict faster uptake and lower cumulative uptake than membrane diffusion models or time-lag models.

Sarangapani et al. (2002) developed a modification of Corley et al. (1990) model that included a multicompartment representation of the respiratory tract. The respiratory tract model includes compartments representing the nasal cavity, conducting airways, and pulmonary airways. The nasal cavity and conducting airways have subcompartments representing the lumen, epithelial, and submucosal layers (Morris et al. 1993). Chloroform in the lumen is transferred to the epithelial layer where it can be metabolized or transferred to the submucosa. Absorption to blood occurs from the submucosa. Exchanges between chloroform in air and the epithelial layer are assumed to occur by diffusion, governed by the air-mucus concentration gradient, the mucus surface area, a mass transfer coefficient (cm/hour) and

a tissue/air partition coefficient. Absorption of chloroform from the submucosa is assumed to be flow-limited and governed by the concentration gradient between the blood and submucosa and blood flow rate to the respiratory tract region. Metabolism in the epithelial layer is simulated as Michaelis-Menten processes (K_M, V_{max}). Values for tissue/blood partition coefficients and metabolism parameters were derived from various sources (see Table 4 of Sarangapani et al. 2002).

Validation of the Model. The Corley model was validated using chloroform data sets from oral (Brown et al. 1974a) and intraperitoneal (Ilett et al. 1973) routes of administration and from human pharmacokinetic studies (Fry et al. 1972). Metabolic rate constants obtained from the gas-uptake experiments were validated by modeling the disposition of radiolabeled chloroform in mice and rats following inhalation of chloroform at much lower doses. For the oral data set, the model accurately predicted the total amounts of chloroform metabolized for both rats and mice. After adjustment of the model parameter that describes skin blood flow for the different temperatures and after calibration of the permeability coefficient, the dermal model predicted levels and the temporal pattern of exhaled chloroform in adult subjects who bathed in aqueous solutions of chloroform (90–97 ppb) at temperatures ranging from 30 to 40°C (Corley et al. 2000).

Target Tissues. The model provided excellent predictions of MMB in both the target tissues of chloroform (liver and kidney) after intraperitoneal administration in mice (rat data were not generated). The model adequately predicted the amount of unchanged material exhaled at infinite time and the total amount metabolized by groups of male and female humans of widely varying age and weight.

Species Extrapolation. The Corley model used species-specific information to outline the model parameters; little extrapolation of information among mice, rats, and humans was required. Certain parameters previously reported in the scientific literature were assumed, however, such as body weight, percentage of body weight, and percentage of blood from the heart (i.e., percentage of cardiac output of body organs). The Corley et al. (1990) model has been combined with biologically based dose-response models to predict exposure response relationships in humans (Conolly and Butterworth 1995; Luke et al. 2010; Sasso et al. 2013; Smith et al. 1995; Tan et al. 2003). A sensitivity analysis of the Corley et al. (1990) model examined effects of parameter variability and uncertainty on interspecies extrapolation of internal dose metrics for chloroform (Delic et al. 2000). Yang et al. (2010) derived probability distributions for parameters in the Corley et al. (2000) model that account for population variability and uncertainty.

High-Low Dose Extrapolation. The Corley model was designed to facilitate extrapolations from high doses (similar to those used for chronic rodent studies) to low doses that humans may potentially be exposed to at home or in the workplace.

Inter-route Extrapolation. The Corley model used three routes of administration (intraperitoneal, oral, and inhalation) in rats and mice to describe the disposition of chloroform. These data were validated for humans by comparing the model output using the animal data with actual human data from human oral chloroform pharmacokinetic studies. Using the human pharmacokinetic constants from the *in vitro* studies conducted by Corley, the model made adequate predictions of the amount of chloroform metabolized and exhaled in both males and females. The Corley et al. (2000) model has been applied to investigate relative contributions of dermal, inhalation, and oral pathways to blood chloroform levels resulting from showering activities (Lyons et al. 2008; Tan et al. 2006, 2007).

The Reitz et al. (1990) Model

Risk Assessment. The Reitz model (Reitz et al. 1990) assumes that cytotoxicity and reparative hyperplasia are responsible for liver neoplasia. Dose surrogates, a more sophisticated and more accurate measure of target tissue dose derived from measuring a pharmacodynamic effect, were used.

Description of the Model. The Reitz et al. (1990) PBPK model was largely based on the Corley et al. (1990) model but differed in the use of a pharmacodynamic end point, cytotoxicity in the livers of chloroform-exposed animals (mice) produced by phosgene (the reactive metabolite of chloroform). The Reitz model focused on the liver as the target organ for chloroform; thus, the kidney compartment toxicity was not addressed. The kidney compartment was combined with the rapidly perfused tissue group. The Reitz et al. (1990) model used two types of dose measurement, referred to as dose surrogates. One type of dose surrogate used was covalent binding to macromolecules, which provided a rate-independent parameter of average daily macromolecular binding (AVEMMB). The second type of dose surrogate was cytotoxicity (PTDEAD), a rate-dependent parameter that measured cell death (by histopathological analysis and ^{3H}thymidine uptake) due to the formation of reactive chloroform metabolites (i.e., phosgene). Model calculations of PTDEAD were based on several assumptions: liver cells have a finite capability for repairing damage caused by chloroform metabolites; liver cells differ from cell to cell in their capabilities to repair this damage; and induction of cytotoxicity in liver cells does not occur instantaneously. A sensitivity analysis of the Reitz et al. (1990) model examined effects of parameter variability and uncertainty on AVEMMB and PTDEAD (Allen et al. 1996). Pelekis et al. (2001) applied the Reitz et al.

(1990) model to quantify the uncertainty factor needed to account for differences in internal dosimetry between human adults and children.

Validation of the Model. The model simulations of PTDEAD were compared with two experimental measures of cytotoxicity: the percentage of nonviable cells observed microscopically in mice gavaged with solutions of chloroform in corn oil, and the rate of incorporation of 3H-thymidine into normal DNA during compensatory cell replication (CCR). CCR was measured following exposure of mice to chloroform vapor for 5–6 hours. Model predictions were in good agreement (within 10%) with observed percentages of dead liver cells evaluated microscopically. Agreement between predicted and observed values of cell killing based on CCR was less satisfactory.

Target Tissues. The Reitz model only applies to the metabolism of chloroform and the induction of cytotoxicity in liver tissue following exposure by inhalation, drinking water, and gavage routes using rat and mouse data.

Species Extrapolation. The Reitz model used the same species and physiologic parameters that the Corley model utilized (average body weights, organ percentage of body weight, blood flow, etc.) for model predictions. However, the model assumed equivalent intrinsic sensitivity of mouse and human hepatocytes.

High-Low Dose Extrapolation. The Reitz model was designed to facilitate extrapolations from high doses (similar to those used for chronic rodent studies) to low doses that humans may potentially be exposed to at home or in the workplace.

Inter-route Extrapolation. Inhalation and oral routes of administration were examined in the Reitz model; however, inter-route extrapolations were not specifically addressed in the Reitz model.

The Gearhart et al. (1993) Model

Risk Assessment. The Gearhart et al. (1993) model provided strong evidence that temperature changes play an important role in predicting chloroform metabolism in mice and also provided a testable hypothesis for the lack of fit of the Corley model prediction with respect to the mouse data. These data strengthen the Corley model and its implications for human risk assessment (see the Corley model description above).

Description of the Model. Gearhart et al. (1993) developed a PBPK model that described the effects of decreased core body temperature on the analysis of chloroform metabolic data. Experimental data showed that when male B6C3F1 mice were exposed for 6 hours to chloroform vapor concentrations of 100–5,500 ppm, a dose-dependent drop in core body temperature occurred, with the least amount of temperature drop occurring at the 100-ppm concentration and the most dramatic drop in temperature occurring at the 5,500-ppm level. The Gearhart model incorporated a model previously used by Ramsey and Andersen (1984) (the same model and parameters that the Corley et al. [1990] model was based on) in conjunction with a separate model reflecting changes in body core temperature to drive equations accounting for changes in partition coefficients, cardiac output, minute ventilation volumes, and rate of chloroform metabolism.

The model predicted that the V_{max} for chloroform metabolism without correcting for core temperature effects was 14.2 mg/hour/kg (2/3 of that reported in the Corley model) and the K_M was 0.25 mg/L. Without body temperature corrections, the model underpredicted the rate of metabolism at the 5,500-ppm vapor concentration. Addition of a first-order kinetic rate constant (kf=1.86 $hour^{-1}$) to account for liver metabolism of chloroform at high doses of chloroform did provide a small improvement in model predictions at 5,500 ppm but was still considered inadequate for predicting metabolism at high concentrations.

Validation of the Model. The Gearhart et al. (1993) model was not validated against a comparable data set. Corrections for the temperature effects (V_{max} increased to 15.1 mg/hour/kg) and inclusion of a first-order metabolism correction equation provided an accurate prediction of chloroform metabolism across all concentrations tested.

Target Tissues. The liver was the target tissue for this model.

Species Extrapolation. No species extrapolation was specifically addressed by the Gearhart et al. (1993) model.

High-Low Dose Extrapolation. No high-low dose extrapolation was specifically addressed by the Gearhart et al. (1993) model.

Inter-route Extrapolation. No inter-route extrapolation was specifically addressed by the Gearhart et al. (1993) model.

The Chinery and Gleason (1993) Model

Risk Assessment. The Chinery and Gleason (1993) model has been applied to estimating exposures to chloroform in a household environment as well as for occupational exposures that result from dermal exposure.

Description of the Model. The Chinery and Gleason (1993) model is a combination of the Corley et al. (1990) model and other existing models that includes a multicompartment skin component similar to that of Shatkin and Szejnwald-Brown (1991). This compartment is used to simulate penetration of chloroform into the skin while showering for 10 minutes with water containing chloroform. The skin module for this new model assumed a physiologic skin compartment consisting of three linear compartments: the dilute aqueous solution compartment; the stratum corneum (the primary barrier to the absorption of most chemicals, including chloroform); and the viable epidermis.

Validation of the Model. The model was validated using published data on experimentally derived exhaled breath concentrations of chloroform following exposure in a shower stall (Jo et al. 1990).

Target Tissues. Based on the data set of Jo et al. (1990), the Chinery and Gleason (1993) model predicted the stratum corneum permeability coefficient for chloroform to be 0.2 cm/hour (range 0.6–2.2) and the estimated ratio of the dermally and inhaled absorbed doses to be 0.75 (range 0.6–2.2) cm/hour. This new model showed that a simple steady-state model can be used to predict the degree of dermal absorption for chloroform. It was also shown that the model would be useful in predicting the concentrations of chloroform in shower air and in the exhaled breath of individuals exposed both dermally and by inhalation routes while showering with water containing low amounts (20 µg/L) of chloroform. At this concentration, the model predicted a dermal absorption dose of 0.0047 mg and inhalation absorption dose of 0.0062 mg. In addition, the model also demonstrated that as the concentration of chloroform rises due to increases in chloroform vapor, the absorbed inhalation dose increases faster and becomes larger than the absorbed dermal dose.

Species Extrapolation. No species extrapolation was specifically addressed by this model.

High-Low Dose Extrapolation. No high-low dose extrapolation was specifically addressed by this model.

Inter-route Extrapolation. The Chinery and Gleason (1993) model examined two routes of exposure: inhalation-only exposure and inhalation/dermal exposure. The model was useful in predicting the concentration of chloroform in shower air and in the exhaled breath of individuals exposed by the dermal and inhalation routes.

The McKone (1993) Model

Risk Assessment. The McKone (1993) model has had some use in human chloroform risk assessments, in that the model defined the relationship between the dermal and inhalation exposure to measures of dose and the amounts that can be metabolized by the liver by each route. The model also provided information about the inhalation and dermal exposure concentrations at which chloroform metabolism becomes nonlinear in humans.

Description of the Model. The McKone (1993) model addressed potential exposure to chloroform by the inhalation and dermal routes. McKone (1993) revised existing shower-compartment, dermal uptake, and PBPK models to produce a revised PBPK model for simulating chloroform breath levels in persons exposed in showers by the inhalation route only and by the inhalation and dermal routes combined. Parameters used by this model were taken primarily from two main sources: Jo et al. (1990) and Corley et al. (1990).

The model was also used to assess the relationship of dermal and inhalation exposure to metabolized dose in the liver, as well as to determine the tap-water concentrations at which hepatic metabolism of dermal and inhalation doses of chloroform become nonlinear. This information is especially useful for risk assessment on persons exposed to a wide range of chloroform concentrations. Experimentally measured ratios of chloroform concentrations in air and breath to tap water concentration (Jo et al. 1990) were compared with the model predictions.

Validation of the Model. The McKone (1993) model used one data set to evaluate the model results (Jo et al. 1990). The McKone (1993) model results were also compared to other existing chloroform models, with an in-depth discussion of similarities and differences between those models.

Target Tissues. The skin and lung were the target tissues studied in this model. Based on the information presented, the McKone (1993) model is appropriate for simulating chloroform breath levels in persons exposed in showers by both exposure routes. A major difference between the McKone (1993) model and the Chinery and Gleason (1993) model is that the McKone (1993) model assumes the skin to be a one compartment organ, whereas the Chinery and Gleason (1993) model assumed three compartments within the skin. The McKone (1993) model indicated that the ratio of chloroform dermally absorbed in the shower to the concentration in tap water ranges from 0.25 to 0.66 mg/L, and that chloroform can effectively permeate through the skin at a rate of 0.16–0.42 cm/hour during a 10-minute shower.

Species Extrapolation. The human was the only species addressed by the McKone (1993) model. No extrapolation between species was addressed in this model.

High-Low Dose Extrapolation. For tap-water concentrations <100 mg/L, the model predicted a linear relationship between potential dose (i.e., amounts present in the drinking water, inhaled in a shower, or skin surface contact) and the cumulative metabolized dose. At tap-water concentrations >100 mg/dL for inhalation-only showers and >60 mg/L for normal showers, however, the relationship was no longer linear and modifications to this model may be required.

Inter-route Extrapolation. The dermal and inhalation routes were addressed in this model. The McKone (1993) model did not specifically address inter-route extrapolations for chloroform.

The Haddad et al. (2006) Model

Description of the Model. The structure of Haddad et al. (2006) PBPK model is similar to that of the Corley et al. (2000) model. The model simulates 6 compartments (lung, liver, fat, skin, richly perfused tissues, poorly perfused tissues) and enables simulations of dermal, inhalation, and oral exposures. All exchanges between blood and tissues are assumed to be flow limited. Metabolism of chloroform is assigned to the liver compartment (K_M, V_{max}). Dermal absorption is simulated with a single well-mixed compartment in which absorption is governed by a dermal permeability coefficient (cm/hour), a skin/vehicle partition coefficient, the concentration difference between the vehicle and skin, and skin blood flow. The dermal permeability coefficient was assigned the value estimated by Xu et al. (2002), which was measured at 25°C. Oral absorption is governed by a bioavailability coefficient (fraction of ingested). Absorption from the lung is assumed to be flow limited and governed by the air concentration,

a blood/air partition coefficient, and blood flow to the lung. Values for tissue/blood partition coefficients and metabolism were from various sources (see Table 4 of Haddad et al. 2006). Physiological parameters for adults were from Tardif et al. (1997). These were scaled to age-dependent anthropomorphic data derived from NAHNES III (Price et al. 2003).

Validation of the Model. The model was evaluated against data from human studies in which concentrations of chloroform in exhaled air were measured during dermal exposures from swimming in an aqueous solution of chloroform (Lévesque et al. 2000). When the permeability coefficient was set to 0.00267 cm/minute (Xu et al. 2002), the predicted blood concentrations were within 1–3 SDs of observed means.

Target Tissues. The Haddad model simulates time-dependent concentrations of chloroform in blood and rates of metabolism of chloroform in the liver. Rates of metabolism could be applied to internal dosimetry of the induction of cytotoxicity in liver tissue following dermal, inhalation, or oral exposures.

Species Extrapolation. Interspecies extrapolations were not investigated in Haddad et al. (2006).

High-Low Dose Extrapolation. High-low dose extrapolations were not investigated in Haddad et al. (2006). Model performance was evaluated at near steady-state concentrations of 10–100 ppb in exhaled air.

Inter-route Extrapolation. The Haddad et al. (2006) model simulates internal doses of chloroform resulting from dermal, inhalation, and oral exposures. The model was used to predict the relative contributions dermal, inhalation, and oral pathways had to blood chloroform levels resulting from showering (Haddad et al. 2006).

The Evans et al. (2020) Model

Description of the Model. Evans et al. (2020) developed a PBPK model of the F344 rat. The structure is similar to that of the Corley et al. (1990) model with the addition of compartments representing the brain and exposure chamber (for simulating closed chamber studies). All exchanges between blood and tissues are assumed to be flow limited. Metabolism of chloroform is assigned to the liver and kidney compartments (K_M, V_{max}). Absorption from the lung is assumed to be flow limited and governed by the air concentration difference, a blood/air partition coefficient, and blood flow to the lung. Values for

tissue/blood partition coefficients were measured (see Table 7 of Evans et al. 2020). The values for the metabolism parameters were calibrated to chamber clearance rates measured in closed chamber studies of rats (Evans et al. 2020).

Validation of the Model. The model was evaluated against data from closed chamber studies of rats conducted at starting concentrations ranging from 100 to 3,000 ppm. After calibration of the K_M and V_{max} parameters, the models precited the time course for the decline in chamber chloroform concentrations (Evans et al. 2020).

Target Tissues. The Evans et al. (2020) model simulates time-dependent concentrations of chloroform in blood and rates of metabolism of chloroform in the liver and kidney. Rates of metabolism could be applied to dosimetry of the induction of cytotoxicity in rat kidney tissues following dermal, inhalation, or oral exposures.

Species Extrapolation. Interspecies extrapolations were not investigated in Evans et al. (2020).

High-Low Dose Extrapolation. Model performance was evaluated at near steady-state initial chamber concentrations ranging from 100 to 3,000 ppm. The model predicted the concentration dependency of chloroform clearance resulting from saturable metabolism of chloroform.

Inter-route Extrapolation. The Evans et al. (2020) model simulates internal doses of chloroform in the F344 rat resulting from inhalation exposures.

3.1.6 Animal-to-Human Extrapolations

Many laboratory animal models have been used to describe the toxicity of chloroform, including rats, mice, rabbits, dogs, and cats (see Tables 2-1, 2-2, and 2-3). By far, rats and mice are the most well-studied laboratory animal species. As discussed in preceding sections of Chapter 3, toxicokinetic data are available from a limited number of human studies, several studies in rats and mice, and a limited number of studies in other laboratory animals (monkeys, guinea pigs). Generally, the pharmacokinetic and toxicokinetic data gathered from rats and mice compare favorably with the limited information available from human studies, with no indication of clear species-related differences that would drastically impact default extrapolation assumptions. PBPK models, such as Corley et al. (2000), have been developed using pharmacokinetic and toxicokinetic data, and some of these have used species-specific information

to define model parameters to reduce the amount of extrapolation needed between rodents and humans under some exposure conditions and target tissues (Section 3.1.5). PBPK model conditions, species, and target tissues need to be evaluated for suitability for the selected critical effect prior to use in dose extrapolation for risk assessment in humans.

As mentioned previously, male rodents, particularly mice, have a sex-related tendency to develop severe renal disease when exposed to chloroform, particularly by the inhalation and oral exposure routes. This effect appears to be species-related, since experiments in rabbits and guinea pigs found no sex-related differences in renal toxicity. However, there is no mechanistic data to suggest that the renal disease observed in mice and rats is not relevant to humans.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to chloroform are discussed in Section 5.7, Populations with Potentially High Exposures.

Age and Sex. Numerous animal studies indicate that some male rodents may be more susceptible to the lethal and renal effects of chloroform than female rodents, particularly in mice (Kasai et al. 2002; Larson et al. 1996, 1994b, 1994d; Templin et al. 1996c; Torkelson et al. 1976; Yamamoto et al. 2002). The greater susceptibility of male mice is attributable to increased levels of CYP2E1 activity due to influence of testosterone on CYP2E1 gene transcription (Deringer et al. 1953; Eschenbrenner and Miller 1945; Trevisan et al. 2012). When female mice are co-exposed to androgens and chloroform, renal toxicity was

comparable to that observed in exposed males (Culliford and Hewitt 1957; Weir et al. 2005). Conversely, when male mice were castrated, renal toxicity was comparable to that observed in exposed females (Culliford and Hewitt 1957).

Acute lethality studies suggest evidence for age-related susceptibility to chloroform. In acute-duration inhalation lethality studies, young male mice (2 months of age) were slightly susceptible to toxic effects compared to adult mice (Deringer et al. 1953). In rats, acute oral toxicity was similar in young adult and aged animals; however, the LD_{50} value was significantly lower in 14-day-old rats (Kimura et al. 1971).

Pre-existing Conditions, Diseases, and Exposure to Other Substances. Since the liver and kidney are the two main organs responsible for chloroform metabolism, individuals who have hepatic or renal impairment may be more susceptible to chloroform toxicity; one such population would be those who misuse alcohol (Kutob and Plaa 1962; Wang et al. 1994). Also, exhaustion and starvation may potentiate chloroform hepatotoxicity, as indicated in some human clinical reports of women exposed to chloroform as an anesthetic during labor (Royston 1924; Townsend 1939). Chloroform is also more hepatotoxic in fasted animals (Brown et al. 1974b; Docks and Krishna 1976; Ekstrom et al. 1988; McMartin et al. 1981; Wang et al. 1995). These observations are likely due to differential metabolism and detoxification in fasted/starvation states due to fasting-associated induction of hepatic cytochrome P450 enzymes coupled with decreased detoxification capacity due to decreased GSH content (McMartin et al. 1981; Wang et al. 1995).

Obese individuals and those with diseases that lead to fat accumulation in the liver such as alcoholic liver diseases or metabolism associated fatty liver disease may be at increased risk of toxicity since chloroform preferentially distributes to fat (see Section 3.1.2). The kinetics of exposure for lipophilic compounds will be altered in obese individuals, compared to lean individuals. Clearance from blood may be quicker, leading to lower blood levels due to increased uptake in body fat, resulting in an overall extension of half-life and thus increasing cumulative exposure potential (La Merrill and Birnbaum 2011). Increased activity of CYP2E1 has also been observed in obese individuals, especially those with type II diabetes (Brill et al. 2012; Wang et al. 2003), which could also contribute to increased susceptibility to chloroform toxicity in target organs with CYP2E1-mediated effects (e.g., liver and kidney).

Genetic Polymorphisms. Certain genetic polymorphisms may alter the risk of specific cancer types associated with exposure to chlorinated solvents. In a population-based, case-control study assessing women diagnosed with non-Hodgkin's lymphoma, single nucleotide polymorphisms in the DNA repair

genes *MGMT* and *NBS1* modified the association between occupational-exposure to chlorinated solvents and increased risk for non-Hodgkin's lymphoma (Jiao et al. 2012). Polymorphisms in the *GSTT1* and *CYP2E1* genes, which are thought to be involved in the metabolism and biotransformation of chloroform, may increase the risk of childhood acute lymphoblastic leukemia associated with exposure to trihalomethanes in drinking water (Infante-Rivard et al. 2002).

Luzhetskiy et al. (2015) suggested that polymorphisms in the serotonin receptor gene (*HTR2A*) may increase susceptibility to metabolic disorders with exposure to chloroform. Three variations exist, in order of decreasing percent detection: AA, AG, GG. Within the cohort of 212 children assessed, the children with the AG variant showed increased susceptibility to chloroform-associated over-eating and obesity.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 2006).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for chloroform from this report are discussed in Section 5.6, General Population Exposure.

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 2006). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to chloroform are discussed in Section 3.3.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 2006). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung

capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by chloroform are discussed in Section 3.3.2.

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

3.3.1 Biomarkers of Exposure

Chloroform levels can be measured in blood, tissue, urine, breast milk, and expired air; however, levels in blood and expired air have been validated to a higher degree. Since environmental exposure to chloroform likely represents a combination of inhalation (from the air polluted with volatile halogenated hydrocarbons; volatilization from chlorinated water sources), oral (from chlorinated water sources), and dermal (from showering, bathing, or swimming in chlorinated water) exposure routes, interpretation of biomarkers of exposure are challenging. Clewell et al. (2008) reviewed the utility of using a Monte Carlo approach to reconstruct exposure to chloroform from biomonitoring data using blood concentrations of chloroform and validated human PBPK models. They present an exposure conversion factor (ECF) distribution approach to reconstruct likely exposure scenarios via multiroute exposure to estimate chloroform levels in household drinking water. However, this model depends heavily on estimates of background chloroform levels in ambient air, drinking water intake, shower duration and flow rate, and shower stall dimensions. Additionally, the presence of chloroform or its metabolites in biological fluids and tissues needs to be interpreted with caution, as it may reflect exposure to chloroform or the metabolism of other chlorinated hydrocarbons. For example, chloroform also can be detected in the breath after exposure to carbon tetrachloride (CCl_4) and other chlorinated hydrocarbons (Butler 1961).

The relationship between chloroform concentration in inspired air and resulting blood chloroform levels is the most well-defined measure of exposure due to the extensive use of chloroform as a surgical anesthetic. A mean arterial blood concentration of 9.8 mg/dL (range 7–16.6 mg/dL) was observed among 10 patients receiving chloroform anesthesia at an inspired air concentration of 8,000–10,000 ppm (Smith et al. 1973). Monitoring of blood levels in workers experiencing toxic jaundice due to chloroform exposure revealed that when workroom air concentrations were estimated to be >400 ppm, the blood

samples of 13 workers with jaundice were 0.10–0.3 µg/l00 mL blood (Phoon et al. 1983). These data suggest an association between increased blood concentrations and increased exposure concentrations, but the blood levels varied too greatly to establish a direct quantitative relationship.

Chloroform is often used as a biomarker for exposure to total trihalomethanes, particularly from chlorinated drinking water and indoor swimming pools. Several studies have examined the increase of chloroform in bodily fluids and/or expired breath as a measure of exposure to trihalomethanes following swimming (Aggazzotti et al. 1995, 1998; Caro and Gallego 2008; Font-Ribera et al. 2010; Pleil and Lindstrom 1997), although these concentrations can vary in swimmers based on their age and physical intensity (Aggazzotti et al. 1995). Correlations have been observed between chloroform concentrations in the air around indoor pools and concentrations in alveolar air (Caro and Gallego 2008; Font-Ribera et al. 2010), and chloroform in alveolar air with concentrations in urine (Font-Ribera et al. 2010). Although chloroform levels in air and water appear to be representative of total trihalomethane exposure (Aggazzotti et al. 1998; King et al. 2004), it has not been proven to be a reliable biomarker of chloroform exposure as elevated tissue levels of chloroform or its metabolites may reflect exposure to other compounds.

3.3.2 Biomarkers of Effect

The primary targets of chloroform toxicity are the CNS, liver, and kidney. The signs and symptoms of CNS effects (e.g., dizziness, fatigue, headache) are easily recognized. Monitoring liver and kidney effects induced by exposure to low levels of chloroform requires the testing of organ functions. Liver effects are commonly detected by monitoring for elevated levels of liver enzymes in the serum or testing for bromosulfalein retention. Urinalysis and measurements of BUN and β-2-microglobin are used to detect abnormalities in kidney function. Because many toxic chemicals can cause adverse liver and kidney effects, these tests are not specific for chloroform. One study attempted to evaluate impaired respiratory health associated with exposure to chlorinated swimming pools using biomarkers of respiratory injury, but no associations were observed with chloroform concentrations in expelled air (Font-Ribera et al. 2010). No specific biomarkers used to characterize effects caused specifically by chloroform were located.

3.4 INTERACTIONS WITH OTHER CHEMICALS

The interactions of chloroform with other chemicals are of particular concern when considering exposure to chlorinated water, which usually contains other trihalomethanes and may contain other potential toxicants. Oral administration of chloroform with one or more trihalomethanes (bromodichloromethane, dibromochloromethane, or bromoform) resulted in higher blood concentrations of chloroform in rats compared to chloroform treatment alone (Da Silva et al. 1999, 2000). Studies on liver and kidney effects in mice for binary and complex mixtures using four disinfection byproducts (chloroform, bromoform, chlorodibromomethane, bromodichloromethane) suggest that dose-additivity is a reasonable assumption when assessing the toxicity of trihalomethane mixtures (Teuschler et al. 2000).

To further complicate assessment of potential interactions, exposure to disinfection byproducts is often multi-route, including ingestion of tap water as well as dermal and inhalation exposure from showering, bathing, and/or swimming. Florentin et al. (2011) discusses concerns for potential interactions between disinfection byproducts in chlorinated swimming pools, including organohalogens (trihalomethanes, haloacetic acids, halocetonitriles, chloral hydrate, chloropicrin, halophenols, N-chloramines, halofuranones, bromohydrins), non-halogenic organics (aldehydes, alkanic acids, benzene, carboxylic acids), and inorganics (chlorate), and the impact on human health risk assessment. The EPA has developed a cumulative risk assessment model for human exposure to disinfection byproduct mixtures using a cumulative relative potency factors approach, which accounts for both dose and response addition across multiple chemicals via multiple routes (Teuschler et al. 2004). This method groups compounds into subclasses based on common modes of action and selects an index compound for each subclass.

The role that dichloroacetate (DCA) and trichloroacetate (TCA) play in chloroform toxicity was studied in rats (Davis 1992). TCA and DCA are formed in conjunction with chloroform during the chlorination of drinking water; therefore, animals drinking chlorinated water may be exposed to all three compounds simultaneously. It was found that DCA increases the hepatotoxicity and nephrotoxicity of chloroform in rats, that TCA increases the nephrotoxicity of chloroform, and that these effects were gender-specific, occurring mainly in females. Another study found that exposure to chloroform inhibited liver tumor promotion in DCA-treated female mice, while increasing kidney tumor promotion in DCA-treated male mice (Pereira et al. 2001). Combinations of monochloroacetate (MCA) and chloroform toxicity have also shown to have toxic effects on the liver and kidneys of rats (Davis and Bemdt 1992). Additional studies are available that have investigated the mechanisms by which chloroacetic acids (TCA, DCA, MCA)

influence the metabolism and metabolic interactions of chloroform and other trihalomethanes (St-Pierre et al. 2003, 2005).

Hertzberg et al. (2024) used hepatotoxicity of chloroform and three other trihalomethanes (bromodichloromethane, chlorodibromomethane, bromoform) to evaluate the interaction-based hazard index (HI_{INT}) approach to evaluating mixture toxicity. These four trihalomethanes were selected as they are currently regulated under EPA's Stage 2 Disinfection/Disinfection Byproduct Rule. The HI_{INT} approach modifies the standard hazard index (HI), which is based on the assumption of addition, by considering all available evidence for pairwise toxicological interactions. Using this model for hepatotoxicity based on relative liver weight in mice, this model predicted greater-than-dose-additivity for binary, tertiary, and quaternary mixtures of trihalomethanes (using 1:1 ratios and various environmental ratios). This finding is consistent with findings from animal mixture studies. Using serum clinical chemistry data, HI_{INT} model predictions were mixed; however, most predictions were less-than-additive, which concurs with findings from animal mixture studies.

Several animal studies indicate that chloroform can interact with chemicals that induce CYP450 enzymes. The lethal and hepatotoxic effects of chloroform were increased by dicophane (DDT) (McLean 1970) and phenobarbital (a long-acting barbiturate) in rats (Ekstrom et al. 1988; McLean 1970; Scholler 1970). Increased hepatotoxic and nephrotoxic effects were observed after interaction with ketonic solvents and ketonic chemicals in rats (Hewitt and Brown 1984; Hewitt et al. 1990) and in mice (Cianflone et al. 1980; Hewitt et al. 1979). The hepatotoxicity of chloroform was also enhanced by co-exposure to CCl_4 in rats (Harris et al. 1982), co-exposure to various alcohols (allyl alcohol, methanol, ethanol, isopropanol, t-butanol, pentanol) in rats (Anand et al. 2003; Ray and Mehendale 1990), co-exposure to ethanol in mice (Kutob and Plaa 1962), or co-exposure to chlordecone (Kepone®) in mice and gerbils (Cai and Mehendale 1991; Purushotham et al. 1988). Furthermore, ethanol pretreatment in rats enhanced chloroform-induced hepatotoxicity (Wang et al. 1994) and increased the *in vitro* metabolism of chloroform (Sate et al. 1981).

A series of studies examined the hepatotoxic interaction between chloroform and CCl_4 in rats. Coadministration of chloroform and CCl_4 in ethanol-pretreated rats resulted in dose- and duration-dependent increases in CCl_4-induced hepatotoxicity (Ikatsu and Nakajima 1992). Further studies into the mechanism revealed alterations in ALT and CYP2E1 activity, which only occurred in ethanol-pretreated animals (Ikatsu et al. 1998), suggesting that persons with alcohol use disorder may be a particularly sensitive population to the hepatotoxic effects of chloroform and/or CCl_4 (Lionte 2010).

The potential interaction between chloroform and trichloroethylene has been evaluated with respect to hepatotoxicity in rats (Anand et al. 2005a, 2005b). When administered via intraperitoneal injection, measures of hepatotoxicity (plasma ALT) showed a less-than-additive effect for both compounds. Pharmacokinetic data suggested that chloroform acted in an antagonistic manner with respect to trichloroethylene via inhibition of trichloroethylene metabolism. This relationship was further described in a joint PBPK model for chloroform and trichloroethylene by Isaacs et al. (2004). Since both compounds require bioactivation by CYP2E1 for some toxic effects, less-than-additive toxicity could be explained by mutual inhibition of CYP2E1 metabolism. Based on this logic, other compounds with toxicity mediated via CYP2E1 metabolism may also show interactions with chloroform (e.g., 1,1-dichloroethene; ATSDR 2022a).

Anand et al. (2004, 2005b) proposed that trichloroethylene-induced liver injury was also reduced by co-exposure with chloroform due to increased compensatory liver tissue repair. Several other studies have shown increased compensatory liver tissue repair in rats exposed to binary chemical mixtures of chloroform and other known hepatotoxicants, despite varying mechanisms of toxicity, including allyl alcohol, thioacetamide, and chlordecone (Anand et al. 2003, 2004; Mehendale 1991; Mehendale et al. 1989). As seen in chloroform-only studies (Section 2.9), the capacity for regenerative repair in binary mixture studies resulted in a halted progression of hepatotoxicity and reduction in lethality.

A mixture of cadmium and chloroform potentiated the cytotoxicity of each in *in vitro* experiments in rat hepatocytes (Stacey 1987a, 1987b). In contrast, mirex did not increase chloroform toxicity in mice (Hewitt et al. 1979). Disulfiram, an inhibitor of microsomal enzymes, decreases the hepatotoxicity of chloroform (Masuda and Nakayama 1982; Scholler 1970). Diethyldithiocarbamate and carbon disulfide pretreatment also protect against chloroform hepatotoxicity (Gopinath and Ford 1975; Masuda and Nakayama 1982, 1983), presumably by inhibiting microsomal enzymes. In general, chloroform toxicity can be influenced by chemicals that alter microsomal enzyme activity or hepatic GSH levels. Dimethyl sulfoxide (DMSO) was shown to decrease chloroform-induced hepatotoxicity and nephrotoxicity in male rats, although the mechanism of action has yet to be determined (Lind and Gandolfi 1997; Lind et al. 2000).

Clinical reports of patients who underwent chloroform anesthesia indicated that premedication with morphine caused serious respiratory depression when chloroform was co-administered (Whitaker and Jones 1965). Additionally, thiopentone (thiopental Na, an ultra-short-acting barbiturate anesthetic) was

associated with increased incidences of hypotension in chloroform-anesthetized patients (Whitaker and Jones 1965).

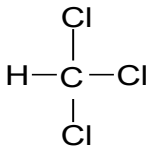
The potential neurobehavioral effects following simultaneous exposures to chloroform and trimethylsilanol in U.S. Navy aircraft were evaluated in male Sprague-Dawley rats (DHA 2022). The animals were initially exposed to escalating concentrations of chloroform or trimethylsilanol alone and evaluated for motor activity and motor coordination in a rotarod test. The 401 and 134 ppm exposure concentrations were identified as NOAELs for chloroform and trimethylsilanol, respectively, when the rats were exposed to each chemical singly. When male rats were exposed concurrently to 401 ppm chloroform and 134 ppm trimethylsilanol, neurological effects were observed. The combined exposure caused reductions in total distance traveled, movement time, ambulatory activity count, "stereotypic activity" count, and vertical activity count in the rats. In contrast, no statistically significant deficits were noted for the duration of time on the rotarod or rod distance traveled. The study authors indicated that these results suggest that combined exposures to chloroform and trimethylsilanol impair motor activity in an additive or synergistic manner.

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of chloroform is presented in Table 4-1.

Table 4-1. Chemical Identity of Chloroform

Characteristic	Information	Reference
Chemical name	Chloroform, trichloromethane	NLM 2023
Synonym(s) and registered trade name(s)	Chloroform, methenyl trichloride, methane trichloride, methyl trichloride, Freon 20, R 20, R 20 refrigerant	
Chemical formula	$CHCl_3$	
SMILES	C(Cl)(Cl)Cl	
Chemical structure		
CAS Registry Number	67-66-3	

CAS = Chemical Abstracts Service; SMILES = Simplified Molecular Input Line Entry System

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of chloroform is presented in Table 4-2.

Table 4-2. Physical and Chemical Properties of Chloroform

Property	Information	Reference
Molecular weight	119.37 g/mol	NLM 2023
Color	Colorless	NLM 2023
Physical state	Liquid	NLM 2023
Melting point	-63.2°C -63.47°C	NLM 2023
Boiling point	61.12°C	NLM 2023
Density at 20°C	1.484 g/cm^3 1.4832 g/cm^3	NLM 2023
Density at 25°C	1.4778 g/cm^3	
Vapor density (air = 1)	4.12	NLM 2023
Odor	Pleasant, ethereal, nonirritating, sweet	NLM 2023
Odor threshold:		
Water	2.4 ppm (w/v)	NLM 2023
Air	51–307 ppm (w/v)	NLM 2023
Solubility:		
Water at 25°C	7,950 mg/L	NLM 2023
Organic solvents	Miscible with principal organic solvents, alcohol, benzene, ether, petroleum ether, carbon tetrachloride, carbon disulfide, oils	NLM 2023
Partition coefficients:		
Log K_{ow}	1.97	NLM 2023
Log K_{oc}	1.5–2.29	NLM 2023
Vapor pressure:		
at 20°C	159 mm Hg	Boublik et al. 1984
at 25°C	197 mm Hg	NLM 2023
Henry's law constant:		
at 20°C	3.00x10^{-3} atm-m^3/mol	Nicholson et al. 1984
at 24.8°C	3.67x10^{-3} atm-m^3/mol	Gossett 1987; NLM 2023
Autoignition temperature	>1,000°C Not flammable	Deshon 1979 NLM 2023
Flashpoint	None	Deshon 1979
Flammability limits	No data	
Conversion factors[a]	1 ppm (v/v)=4.95 mg/m^3 1 mg/m^3=0.20 ppm (v/w)	
Explosive limits	No data	

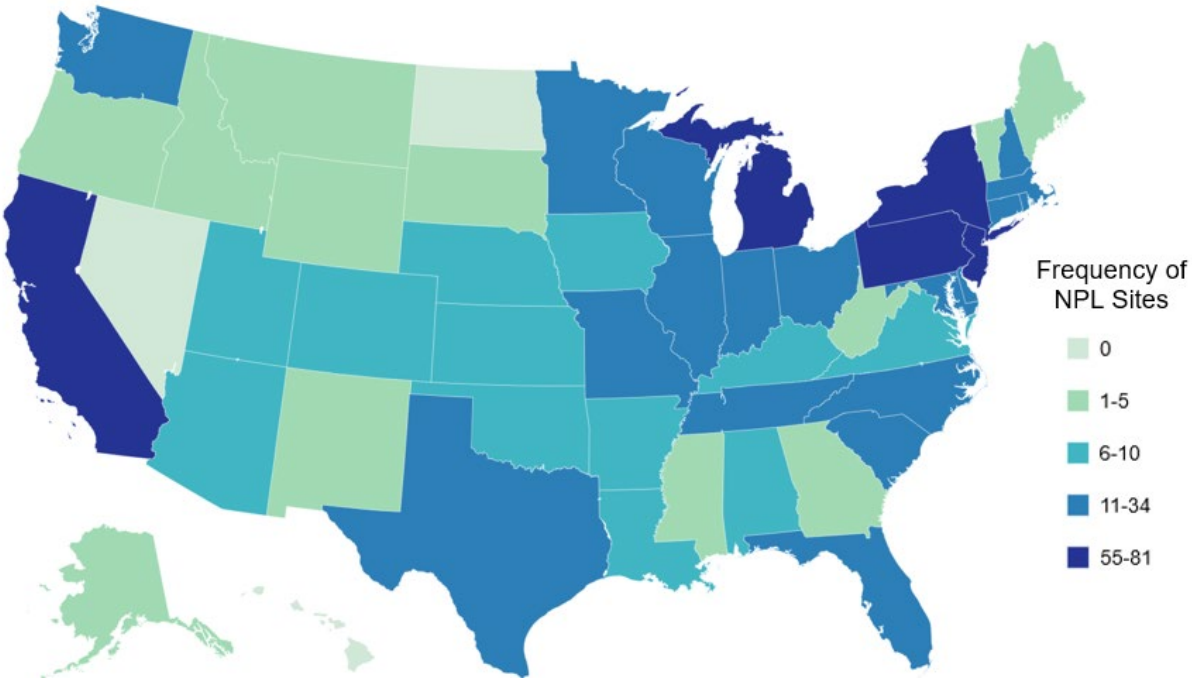
[a]An atmospheric pressure of 1 atm is assumed and a temperature of 20°C.

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Chloroform has been identified in at least 792 of the 1,868 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2022c). However, the number of sites in which chloroform has been evaluated is not known. The number of sites in each state is shown in Figure 5-1. Of these sites, 783 are located within the United States, 1 is located in the Virgin Islands, and 8 are located in Puerto Rico (not shown).

Figure 5-1. Number of NPL Sites with Chloroform Contamination



Source: ATSDR 2022c

- The general public is most likely exposed to chloroform through ingesting food and water containing chloroform, inhaling contaminated air, and dermal contact with chloroform-containing water.
- The primary route of exposure is ingestion from the small amount of chloroform produced in drinking water as a byproduct of chlorination.
- Populations working and/or living near industries that use or create chloroform or hazardous waste sites may have an increased risk of exposure.

- Chloroform is released into the environment from industrial facility waste streams, primarily those that manufacture hydrochlorofluorocarbon-22 (HCFC-22), and as a byproduct of water disinfection.

- Chloroform is produced naturally through biotic and abiotic processes in terrestrial and aquatic environments, and as a result, is often detected in small amounts in remote environments.

- Chloroform is expected to exist almost entirely in the vapor phase in the atmosphere and is expected to volatize rapidly from surface water. Chloroform does not adsorb significantly to soil or sediment and therefore may migrate to groundwater. Chloroform does not significantly bioconcentrate in aquatic environments.

- The dominant degradation process of chloroform in the environment is the reaction of chloroform with free radicals in the atmosphere. At low concentrations and anaerobic conditions, microbial degradation of chloroform can also occur.

Chloroform is a dense liquid with a low boiling point, existing in its vapor form at temperatures above approximately 61°C. In liquid form, it is used primarily in the production of chlorodifluoromethane (HCFC-22). HCFC-22 was previously used as a refrigerant for home air conditioners or large supermarket freezers. However, as a result of the Montreal protocol and the phaseout of HCFC-22 as a refrigerant between 2010 and 2020, the demand for chloroform in the United States as a refrigerant has declined. Despite this phaseout, demand for chloroform remains high due to the use of HCFC-22 as an intermediate for fluoropolymers. Chloroform has also been used as a solvent in the pharmaceutical industry, as a heat transfer medium in fire extinguishers, as an intermediate in the preparation of dyes and pesticides, as well as in various other applications. Chloroform was previously used as a medical anesthetic, but medical use was largely phased out with availability of safer alternatives. It may still have limited medical uses in some dental procedures and in the administration of drugs for the treatment of certain diseases. Chloroform is not currently reported as an active or inert ingredient in registered pesticide products, and its use in drug, cosmetic, and food packaging products has been discontinued.

Chloroform is both a synthetic and naturally occurring compound, and natural formation may contribute a significant portion of emissions. Chloroform is released into the environment from manufacture and use. Chloroform is formed when drinking water, municipal and industrial wastewater, or swimming pool and spa water are chlorinated, or when other water treatment processes involve chlorination. Most of the chloroform released into the environment will eventually enter the atmosphere. In the atmosphere, chloroform may be transported long distances before ultimately being degraded by indirect photochemical reactions with free radicals such as hydroxyl radicals, with a half-life on the order of months. The compound has been detected in ambient air in locations that are remote from anthropogenic sources,

possibly due to natural formation via abiotic or biotic processes. Chemical hydrolysis is not a significant removal process. While microbial biodegradation can take place, such reactions are generally possible only at fairly low concentration levels due to chloroform's toxicity. Microbial biodegradation of chloroform may also be inhibited due to high levels of aromatics (e.g., toluene), chlorinated hydrocarbons (e.g., trichloroethylene [TCE]), or heavy metals (e.g., zinc). Because of its low soil adsorption and water solubility, chloroform will readily leach from soil into groundwater. In groundwater, chloroform is expected to be persistent when oxygenated conditions are present.

The general population is exposed to chloroform by ingesting water and food, inhaling contaminated air, and through dermal contact with chloroform-containing water. Generalizations can be made concerning the chloroform concentrations in the environment. Background air concentrations appear to be in the parts per trillion (ppt) range, but certain urban, indoor, and source-dominated areas may show elevated concentrations when compared to background concentrations. Chlorine is the most commonly used disinfectant for drinking water treatment in the United States, reportedly used by 70% of water systems (AWWA Disinfection Committee 2021); as a result, chloroform is prevalent in tap water throughout the country. The EPA designated a maximum contaminant level goal of <70 ppb (0.07 mg/L) for chloroform (EPA 2022). Drinking water levels as high as 75 ppb have been reported in public water supplies (USGS 2006), although most of the reported concentrations are <25 ppb, typically ranging between <0.2 and 23 ppb. Levels in drinking water derived from groundwater contaminated with leachate from landfills and hazardous waste sites can sometimes be much higher. Except for a few special surveys, regular testing for chloroform or other trihalomethanes has focused on larger community water treatment systems serving at least 10,000 people. Very limited information was located regarding the concentrations found in ambient soil. Chloroform has also been detected in the ppb range in certain foods.

Occupational exposure to higher than background levels of chloroform can be expected to occur in some occupations although few quantitative exposure data were located. Populations with the highest potential exposures appear to be workers who manufacture or use chloroform and operators at incinerators, wastewater facilities, paper, or pulp plants. People who live near these facilities or contaminated hazardous waste sites may also have increased potential for exposure. Persons who derive their drinking water from groundwater sources contaminated with leachate from hazardous waste sites may also have increased exposure to chloroform, compared to the general population.

5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.2.1 Production

Chloroform can form naturally through biotic and abiotic mechanisms in aquatic and terrestrial environments, such as oceans, volcanoes, forest soils, grasslands, dry swamplands, peat moorland, and rice fields (Gron et al. 2012; Hoekstra et al. 2001; Laturnus et al. 2002). Chloroform flux from termite mounds has also been measured, and the concentration of chloroform within the mounds were 1,000 times greater than levels in the ambient air (Laturnus et al. 2002). Chloroform formation in soils can occur through biologically mediated chlorination of natural organic material with hypochlorous acid or other oxidized chlorine species, to an intermediate trichloroacetyl-containing compound, which is then hydrolyzed to chloroform (Breider et al. 2013). Fungi have also been shown to play a part in natural chloroform formation, which may be due to their chloroperoxidase enzymes, which catalyze the production of hypochlorous acid from chloride ions and hydrogen peroxide (Hoekstra et al. 2001; USGS 2004).

Chloroform can be formed abiotically via the decarboxylation of trichloroacetic acid by reduction of tetrachloromethane in iron-reducing environments, which may be mineral-mediated, or by the oxidation of organic matter by an electron acceptor like iron (III) (Laturnus et al. 2002). Natural formation of chloroform in water occurs through reaction of dissolved chlorine with sediment and other materials, from biological production by marine algae, and by the reaction of chlorinated pollutants with humic materials (EPA 1985a; Laturnus et al. 2002).

Chloroform is also formed in the process of making paper and as a disinfection byproduct of chlorination, a process that is used to produce potable water or treat wastewater (Ohligschläger et al. 2019). Chloroform has also been detected as a byproduct of chloride-containing cleaning products, such as hypochlorite (bleach), and can be generated when bleach is mixed with other common household chemicals, such as isopropyl alcohol (Bruchard et al. 2023; Odabasi 2008; Lin et al. 2022). Chloroform forms through the oxidation of dissolved organic material by chlorine, hypochlorite, or ozone (in the presence of chloride ions). Formation is increased when there are increased levels of free available chlorine, increased pH, increased temperature, and increased presence of organic matter (Kanan et al. 2015).

Industrially, chloroform can be produced from the chlorination of methane, methyl chloride, or methylene chloride, or from the hydrodechlorination of carbon tetrachloride (Holbrook 2003). Compounds with ketone or alcohol groups produce chloroform upon reaction with chlorine and an alkali, or hypochlorite. Methyl chloride chlorination has been reported as the most common commercial process for chloroform production (Holbrook 2003). This process is carried out in the gas phase at 400–500°C, with methyl chloride and gaseous chlorine, through free-radical reactions. Novel methods such as a liquid-phase process, light initiated processes, or the use of fluidized beds with a catalyst have been proposed for increased selectivity, although it is not known if these are commonly used in industry today (Holbrook 2003).

The nationally aggregated production volume reported to the EPA Chemical Data Reporting (CDR) database for 2019 was between 250 and 500 million pounds (EPA 2023a). This quantity has remained consistent between 2016 and 2019. Four companies reported manufacturing chloroform at a total of six sites to the 2020 CDR database, covering the years 2016–2019 (EPA 2023a). These include Shintech Louisiana LLC (Plaquemine, Louisiana), EMD Holding Corporation (Rockland, Massachusetts); Olin Corporation (Freeport, Texas), and Occidental Petroleum Corporation (Wichita, Kansas; Geismar, Louisiana; and La Porte, Texas). This may not be an exhaustive list of producers; companies must meet a volume threshold to trigger reporting to the EPA CDR database, and some companies' site activities were not available in the public dataset.

Table 5-1 summarizes information on companies that reported the production, import, or use of chloroform for the Toxics Release Inventory (TRI) in 2022 (TRI22 2024). TRI data should be used with caution since only certain types of industrial facilities are required to report. This is not an exhaustive list.

Table 5-1. Facilities that Produce, Process, or Use Chloroform

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AL	5	0	9,999,999	1, 5, 6, 10
AR	1	10,000	99,999	1, 2, 3, 5, 9, 12
CO	1	100,000	999,999	6, 10, 14
CT	1	10,000	99,999	9
FL	1	100	999	1, 5
IL	3	10,000	99,999	1, 5, 10, 12
IN	1	1,000	9,999	9, 12
KS	2	100	9,999,999	1, 4, 12

Table 5-1. Facilities that Produce, Process, or Use Chloroform

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
KY	3	10,000	9,999,999	1, 2, 3, 4, 6, 9
LA	12	0	99,999,999	1, 3, 4, 5, 6, 9, 12, 13
MA	2	10,000	99,999	10, 11
MI	1	100,000	999,999	7, 9, 12
MO	4	0	999,999	1, 5, 7, 9, 10, 12
MT	1	10,000	99,999	10
NC	2	100	99,999	1, 5, 12
NE	1	10,000	99,999	9, 12
NJ	2	1,000	999,999	9, 10
NY	1	10,000	99,999	12
OH	9	100	999,999	7, 8, 9, 12
OR	1	1,000	9,999	10
PA	1	10,000	99,999	1, 5
SC	3	100	9,999	1, 5, 12
TX	14	0	49,999,999	1, 2, 3, 4, 5, 6, 9, 10, 12, 13, 14
UT	2	100	99,999	1, 5, 9, 10, 12
VA	1	1,000,000	9,999,999	10
WI	1	10,000	99,999	7, 9, 10
WV	2	1,000	99,999	1, 6, 10, 13

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/uses:

1. Produce
2. Import
3. Used Processing
4. Sale/Distribution
5. Byproduct
6. Reactant
7. Formulation Component
8. Article Component
9. Repackaging
10. Chemical Processing Aid
11. Manufacture Aid
12. Ancillary
13. Manufacture Impurity
14. Process Impurity

Source: TRI22 2024 (Data are from 2022)

5.2.2 Import/Export

Three companies reported importing chloroform to the 2020 CDR database between 2016 and 2019: ICC Industries, Inc., EMD Holding Corporation, and INEOS Chlor Americas Inc (EPA 2023a). Import volumes were not reported. No companies reported exportation of chloroform. This may not be complete information, as volume thresholds must be met to trigger reporting to the CDR database, and some data were not available in the public dataset. The U.S. International Trade Commission reported a total import volume of 357,517 kg for chloroform in 2022 (USITC 2023).

5.2.3 Use

The major use for chloroform is in the manufacture of the refrigerant HCFC-22, also known as R-22 (Ohligschläger et al. 2019). Despite the phase out of HCFC-22 as a refrigerant, chloroform demand has remained stable due to the use of chlorodifluoromethane as a precursor to feedstocks for fluoropolymers, such as polytetrafluoroethylene. These polymers are used as corrosion-resistant liners in steel pipes and reactors for the chemical and pharmaceutical industry and in electronics and medical equipment. Additionally, the polymers are used as coatings for nonstick cookware and waterproof, breathable fabrics. The polymers are also used as lubricators for sprays and greases and are in specialty roofing and gliding materials (Ohligschläger et al. 2019). Chemical manufacturing companies reported industrial usage of chloroform as an intermediate, solvent, and laboratory chemical in chemical manufacturing (EPA 2023a). There were no details on consumer product usage reported (EPA 2023a).

Chloroform has been used in the past as a solvent or an extraction solvent for fats, oils, greases, resins, lacquers, rubber, alkaloids, gums, waxes, gutta-percha, penicillin, vitamins, flavors, floor polishes, and adhesives in artificial silk manufacture. It has also been used as a dry-cleaning spot remover, in fire extinguishers, and as an intermediate in the manufacture of dyes and pesticides (Deshon 1979). Chloroform has been used as a fumigant and insecticide (Holbrook 2003); however, there are no currently active pesticide products containing chloroform as an active or inert ingredient (EPA 2023b, 2023c).

Chloroform was previously used as an anesthetic, but it has been replaced by safer and more versatile materials (Deshon 1979). The U.S. Food and Drug Administration (FDA) banned chloroform use in drug, cosmetic, and food packaging products in 1976 (IARC 1979). However, since the ban did not include drug products that contain chloroform in residual amounts, it may still be used as a solvent in the pharmaceutical industry and may be present as a byproduct from the synthesis of drug ingredients (IARC 1979). Federal regulations for indirect food additives currently allow chloroform use as a processing aid in polymers, adhesives, and as components of coatings (FDA 2024a, 2024b, 2024c, 2024d). In the early 1990s, chloroform was still reportedly used as a local anesthetic and solvent in certain dental endodontic (gutta-percha root canal) surgery procedures and in topically applied aspirin-chloroform mixtures for pain relief in severe cases of herpes zoster (shingles) or post-therapeutic neuralgia (King 1993; McDonald and Vire 1992); however, it is uncertain if these uses still occur today.

5.2.4 Disposal

Chloroform has been identified as a hazardous waste by EPA, and disposal of this waste is regulated under the Federal Resource Conservation and Recovery Act (RCRA) (EPA 1988a, 1989). Specific information regarding federal regulations on chloroform disposal on land is available in the Code of Federal Regulations (EPA 1988a, 1989). Ultimate disposal of chloroform, preferably mixed with another combustible fuel, can be accomplished by controlled incineration. Complete combustion must be ensured to prevent phosgene formation, and an acid scrubber should be used to remove the haloacids produced. Chloroform may also be disposed of by liquid injection incineration, although the use of this method has not been verified. Chloroform has been previously used in some pesticides, so the disposal of old pesticide containers may be relevant. Combustible containers from organic or many metallo-organic pesticides could be disposed of in pesticide incinerators or in specified landfill sites. Except for the TRI statistics, no data were located regarding the approximate amounts of chloroform disposed or released to environmental media.

Wastewater treatment methods that limit chloroform formation as a disinfection byproduct have been developed. In a study comparing disinfection byproduct formation following wastewater treatment by ultraviolent irradiation (UV), vacuum ultraviolet radiation (VUV), and chlorination, products were limited when under 40 mJ/cm^2 UV and 3 mg/L chlorine were applied at the same time (Du et al. 2024). Applying VUV at the same time as the UV/chlorine treatment resulted in accelerated disinfection with less formation of chloroform. Conversely, when these treatment methods were applied sequentially, an increase in formation of disinfection byproducts, including chloroform, was observed. In another study, UV/peroxymonosulfate treatment was the most effecting post treatment in reducing chloroform formation, followed by UV and UV/H_2O_2 (Huang et al. 2022).

5.3 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2022). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ ≥10 full-time employees; if their facility's North American Industry Classification System (NAICS) codes is covered under EPCRA Section 313 or is a federal facility; and if their facility manufactures (defined to include importing) or processes any TRI chemical in excess of 25,000 pounds, or otherwise uses any TRI chemical in excess of 10,000 pounds, in a calendar year (EPA 2022).

5.3.1 Air

Estimated releases of 258,677 pounds (~117 metric tons) of chloroform to the atmosphere from 78 domestic manufacturing and processing facilities in 2022, accounted for about 59% of the estimated total environmental releases from facilities required to report to the TRI (TRI22 2024). These releases are summarized in Table 5-2.

Table 5-2. Releases to the Environment from Facilities that Produce, Process, or Use Chloroform[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AL	5	4,524	10	1,134	0	0	4,534	1,134	5,668
AR	1	3	0	0	28	0	3	28	31
CO	1	85	0	0	0	0	85	0	85
CT	1	0	0	0	0	0	0	0	0
FL	1	31,400	1,280	0	42	0	32,722	0	32,722
IL	3	1,280	5	0	14	0	1,280	19	1,299
IN	1	169	0	0	0	0	169	0	169
KS	2	4,153	0	12,137	0	0	16,290	0	16,290
KY	3	829	56	0	0	0	834	52	886
LA	12	85,215	2,456	0	142	391	87,671	533	88,203
MA	2	833	1	0	0	88,894	833	88,895	89,728
MI	1	124	1	0	0	0	124	1	125
MO	4	2,199	3,605	0	0	1,345	5,799	1,350	7,149
MT	1	1,901	3	0	0	0	1,901	3	1,904
NE	1	252	0	0	0	0	252	0	252
NJ	2	69	55	0	0	0	69	55	124
NY	1	174	0	0	0	0	174	0	174
NC	2	36,171	15	0	37	0	36,223	0	36,223
OH	9	665	159	0	207	68,987	810	69,208	70,018
OR	1	38	0	0	0	0	38	0	38
PA	1	4,716	1	0	0	0	4,716	1	4,717
SC	3	6,183	12	0	0	0	6,195	0	6,195
TX	14	67,265	675	2	28	37	67,960	46	68,006
UT	2	0	0	0	14	0	0	14	14
VA	1	10,094	27	0	0	0	10,121	0	10,121
WV	2	255	0	0	0	0	255	0	255

Table 5-2. Releases to the Environment from Facilities that Produce, Process, or Use Chloroform[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
WI	1	80	34	0	0	0	80	34	114
Total	78	258,677	8,395	13,273	511	159,654	279,138	161,372	440,510

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, wastewater treatment (metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown.
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI22 2024 (Data are from 2022)

EPA's National Emission Inventory (NEI) database contains information regarding sources that emit criteria air pollutants (CAPs) and their precursors, and hazardous air pollutants (HAPs) for the 50 United States, Washington DC, Puerto Rico, and the U.S. Virgin Islands. Emissions are estimated from multiple sources, including state and local environmental agencies; the TRI database; computer models for on- and off-road emissions; and databases related to EPA's Maximum Achievable Control Technology (MACT) programs to reduce emissions of HAPs. Chloroform emissions estimated from the 2017 inventory are summarized in Table 5-3.

Table 5-3. National Emission Inventory (NEI) Total National Emissions for Chloroform Estimated by Sector, 2020

Sector	Emissions (pounds)
Industrial processes; pulp and paper	355,872
Industrial processes; cement manufacturing	254,170
Industrial processes; chemical manufacturing	208,313
Agriculture; livestock waste	203,872

Table 5-3. National Emission Inventory (NEI) Total National Emissions for Chloroform Estimated by Sector, 2020

Sector	Emissions (pounds)
Industrial Processes; NEC	174,915
Waste disposal	133,052
Industrial processes; petroleum refineries	52,928
Industrial processes; oil and gas production	27,518
Fuel combustion; industrial boilers, ICEs; natural gas	11,315
Solvent; industrial surface coating and solvent use	10,098
Fuel combustion; industrial boilers, ICEs; biomass	9,523
Industrial processes; storage and transfer	8,499
Fuel combustion; comm/institutional; biomass	6,033
Fuel combustion; electric generation; coal	5,749
Fuel combustion; electric generation; biomass	1,568
Fuel combustion; industrial boilers, ICEs; coal	722
Industrial processes; non-ferrous metals	402
Solvent; graphic arts	320
Fuel combustion; electric generation; natural gas	288
Fuel combustion; commercial/institutional; natural gas	266
Solvent; dry cleaning	261
Fuel combustion; industrial boilers, ICEs; oil	252
Industrial processes; ferrous metals	203
Fuel combustion; industrial boilers, ICEs; other	201
Fuel combustion; electric generation; other	122
Fuel combustion; commercial/institutional; other	116
Industrial processes; mining	110
Solvent; degreasing	66
Fuel combustion; commercial/institutional; coal	10
Bulk gasoline terminals	0
Fuel combustion; comm/institutional; oil	0
Fuel combustion; residential; other	0
Fuel combustion; electric generation; oil	0

ICE = internal combustion engine; NEC = not elsewhere classified

Source: EPA 2023f

Direct releases to the atmosphere are expected to occur during the manufacture, loading, and transport of chloroform (EPA 1985a, 1985b). The current largest estimated anthropogenic sources of atmospheric emissions are from the manufacturing of paper and pulp, cement, and chemicals (EPA 2023f). Indirect chloroform releases have resulted from its use in the manufacture of HCFC-22, fluoropolymers,

pharmaceuticals, ethylene dichloride, dyes, and fumigants (Deshon 1979; EPA 1985a, 1985b; Holbrook 2003). By one estimate for the state of Minnesota, point sources were estimated to be the greatest contribution to chloroform emissions (~80%) (Pratt et al. 2000). Global emissions of chloroform were estimated to be between 260 and 400 giga grams (~570–880 million pounds) per year for 2016, an estimated 20% increase from 2011 (WMO 2018).

Chloroform releases can also result from its formation and subsequent volatilization from chlorinated waters including drinking water, domestic water use, municipal and industrial wastewaters, process waters and effluent from the bleaching of pulp in pulp and paper mills, cooling tower water, and swimming pool water, and whirlpool spa water (Benoit and Jackson 1987; EPA 1985a, 1985b; Hoigne and Bader 1988; Scott et al. 2020; Shepherd et al. 1996). Volatilization of chloroform formed during wastewater treatment has been estimated to be contributing 55,000 tons/year to the atmosphere (Ohligschläger et al. 2019). Ranges of 1.27–155 $\mu g/m^3$ chloroform were detected in samples collected in the headspace of primary sedimentation and secondary treatment tanks at an industrial wastewater treatment plant in Spain (Ramírez et al. 2011). Previous estimates of chloroform released were 183 mg/person/year from showering, and 120–140 mg chloroform/person/year from laundry loads (Shepherd et al. 1996). Domestic water usage is expected to be the main source of chloroform emissions to indoor air. Increased release rates of the chloroform in water can be expected from chloroform-containing water that is heated (e.g., water used for cooking, showers, swimming pools, and spas). Aeration and use of groundwater contaminated with chloroform are also potential sources of emission to the atmosphere (Crume et al. 1990).

Chloroform is released as a result of hazardous and municipal waste treatment processes. The chloroform released may have initially been present in the waste or possibly formed during chlorination treatment (Corsi et al. 1987; EPA 1990b; Namkung and Rittmann 1987). Releases may also occur from hazardous waste sites and sanitary landfills where chloroform was disposed, and from municipal and hazardous waste incinerators that burn chloroform-containing wastes or produce chloroform during the combustion process (LaRegina et al. 1986; Travis et al. 1986).

In the past, minor releases may have resulted from the use of consumer products (e.g., certain air deodorizers and cleaning products) that contained chloroform as a component or residual product (Bayer et al. 1988; Wallace et al. 1987a). Chloroform is widely used in laboratory work as an extractant, and the deuterated form of chloroform is used as a solvent in nuclear magnetic resonance spectroscopy.

Some studies estimated that only 9.5–10% of chloroform released to the atmosphere is anthropogenic (Laturnus et al. 2002; USGS 2004); however, others suggested that up to 50% of the total global emission is attributable to man-made sources (Sekar et al. 2022). Natural sources of chloroform include volcanic emissions and biomass burnings, and fluxes of chloroform to the atmosphere have been measured from marine and terrestrial environments, such as tropical oceans, forest soil, rice fields, and peatland. Previously estimated emissions for these sources were 360,000 tons/year from oceans, 220,000 tons/year from soil, and 15 tons/year from other natural processes (Ohligschläger et al. 2019).

5.3.2 Water

Estimated releases of 8,395 pounds (~3.8 metric tons) of chloroform to surface water from 78 domestic manufacturing and processing facilities in 2022, accounted for 2% of the estimated total environmental releases from facilities required to report to the TRI (TRI22 2024). This estimate includes releases to wastewater treatment and publicly owned treatment works (POTWs) (TRI22 2024). These releases are summarized in Table 5-2.

Other than TRI data, current, more comprehensive quantitative data or estimates of chloroform releases to natural waters are lacking. Disinfection of water supplies by chlorination is nearly universal; thus, residual chloroform is found in most drinking water as a disinfection byproduct. This widespread chloroform contamination makes direct releases challenging to measure.

Chlorination of municipal and industrial wastewaters at wastewater treatment plants, process waters, and effluent from the bleaching of pulp in pulp and paper mills, cooling-tower water, and swimming-pool and whirlpool-spa water will also result in chloroform formation (Benoit and Jackson 1987; Comba et al. 1994; EPA 1985a, 1985b, 1990a; Hoigne and Bader 1988). Maximum chloroform formation under simulated chlorination treatment of raw water was 11–13 mg/L (Chaidou et al. 1999). The use of modern treatment facilities may reduce the amounts of chloroform released to environmental waters. This has been demonstrated at a modern kraft pulp mill (Paasivirta et al. 1988); however, much of the chloroform removed from the wastewater may be released to the atmosphere by volatilization. Release of chloroform to groundwater has resulted from improper disposal of chloroform-containing waste at hazardous waste sites (Clark et al. 1982; Dewalle and Chian 1981; Harris et al. 1984; Sawhney 1989).

Microplastics in wastewater have been shown to contribute to disinfection byproduct formation. As the microplastics degrade under UV or natural sunlight, dissolved organic matter (DOM) is released, which is

used as substrate for chloroform formation during chlorination (Chen et al. 2024). Chloroform formation potentials determined for DOM leached from polystyrene and polypropylene microplastics under simulated natural water and chlorination conditions were 60.3±7.8 and 73.7±9.8 µg/g respectively (Yan et al. 2024).

An additional minor source of water contamination may be atmospheric rainout since chloroform has been found in rainwater (Kawamura and Kaplan 1983). Chloroform has been detected in urban stormwater (Lopes and Bender 1998). Other sources of chloroform release to surface water include breweries and thermal combustion of plastics (EPA 1985a).

Direct releases to water are expected via wastewaters generated during chloroform manufacture and its use in the manufacture of other chemicals and materials (EPA 1985a). Direct discharge sources are expected to be relatively minor contributors to total chloroform emissions to water relative to the formation of chloroform resulting from the chlorination of drinking water or chlorination to eliminate pathogens in discharged wastes or other process waters (EPA 1985a).

Natural formation of chloroform in water occurs through abiotic and biotic processes, most of which are reported in marine environments (EPA 1985a; Laturnus et al. 2002). One estimate reported production of 350 giga gram (~770 million pounds) per year in the ocean (USGS 2004). Due to its volatility, chloroform formed in surface water is expected to emit to the atmosphere. Chloroform is also found in groundwater that originates from its natural production in soil (Gron et al. 2012) and is persistent in environments where oxygen is present (Hunkeler et al. 2012).

5.3.3 Soil

Estimated releases of 551 pounds (~0.23 metric tons) of chloroform to soil from 87 domestic manufacturing and processing facilities in 2022, accounted for about <1% of the estimated total environmental releases from facilities required to report to the TRI (TRI22 2024). An additional 13,273 pounds (~6.0 metric tons), accounted for about 3% of the total environmental emissions, were released via underground injection (TRI22 2024). These releases are summarized in Table 5-2.

Other than TRI data, current comprehensive quantitative data or estimates of chloroform releases to soil are lacking. Chloroform releases to soil have occurred at hazardous waste sites containing improperly disposed wastes where chloroform has leached through soil to groundwater (Clark et al. 1982; Dewalle

and Chian 1981; Harris et al. 1984; Sawhney 1989). Land disposal of sludge from municipal and industrial wastewater-treatment plants may also result in chloroform releases to soil (EPA 1990a). Direct land disposal of chloroform-containing wastes may have occurred in the past, but land disposal of chloroform wastes is currently subject to restrictive regulations (EPA 1988a, 1989). An additional minor source of soil contamination may be atmospheric rainout since chloroform has been found in rainwater (Kawamura and Kaplan 1983).

Chloroform is produced in terrestrial environments by biomediated processes; one of the more significant sources seems to be forest soil, although fluxes from grasslands, dry swamplands, and peat moorland have also been detected (Hoekstra et al. 2001; Laturnus et al. 2002). Chloroform has also been detected in the emissions of aerobic composting of vegetable waste and cow manure (Qu et al. 2022). Chloroform emissions from aerobic composting varied temporally and ranged from not detected (detection limit to reported) to 20 μg/m^3. However, chloroform formed in terrestrial environments may not remain there; it is expected to volatilize to the atmosphere or migrate to groundwater. The volatilization flux of chloroform from the soil of a Douglas fir forest was 1,000 ng/m^2/hour (Hoekstra et al. 2001). Fluxes from rice fields have been measured in the range of 1.4×10^4–9.6×10^4 ng/m^2/day (Laturnus et al. 2002). Fluxes from termite mounds have also been detected at 0.0002 ng/cm^2/second (Laturnus et al. 2002).

5.4 ENVIRONMENTAL FATE

5.4.1 Transport and Partitioning

Air. Based on vapor pressures of 159–197 mm Hg at 20–25°C, chloroform is expected to exist almost entirely in the vapor phase in the atmosphere (Boublik et al. 1984; Eisenreich et al. 1981). Large amounts of chloroform in the atmosphere may be removed by wet deposition since chloroform has significant solubility in water (Table 4-2). This is confirmed by its detection in rainwater (Kawamura and Kaplan 1983). Most chloroform is removed from the atmosphere in precipitation and is likely to re-enter the atmosphere by volatilization. Since chloroform has a relatively long half-life in the atmosphere, long-range transport is possible. Trace amounts of chloroform have been documented in air samples from remote, often relatively pristine, areas of the world (Class and Ballschmidter 1986). This may also be due to natural chloroform formation via reaction of naturally generated chlorinated oxidants with organic matter (Laturnus et al. 2000; Laturnus et al. 2002).

Water. Based on the measured Henry's law constant of 3.00–3.67x10^{-3} atm-m^3/mol, the dominant fate process for chloroform in surface waters is volatilization (Gossett 1987; Nicholson et al. 1984). Chloroform present in surface water is expected to volatilize rapidly to the atmosphere. An experimental half-life of 18–25 minutes has been measured for volatilization of chloroform from a 1-ppm solution with a depth of 6.5 cm that was stirred with a shallow pitch propeller at 200 rotations per minute at 25°C under still air (≈0.2 mph air currents) (Dilling 1977; Dilling et al. 1975). Using the Henry's law constant, a half-life of 3.5 hours was calculated for volatilization from a model river that is 1 meter deep flowing at 1 m/second, with a wind velocity of 3 m/second, and neglecting adsorption to sediment (Lyman et al. 1982).

Sediment and Soil. In soil, the dominant transport mechanism for chloroform near the surface will likely be volatilization. A chloroform soil-air partition coefficient (K_{SA}) of 43.8 in soil with 67% water content at 25 °C was estimated based on the air-octanol partition coefficient (log K_{oa}) of 2.79 L/kg (Ahn et al. 2020). Volatilization rates were comparable over a wide variety of soil types and were not concentration dependent (Park et al. 1988). However, volatilization may be impacted by flow rate in soil. In soil column studies using fine sandy soil, 75% of the chloroform initially present in water volatilized when applied at a slow flow rate compared to 54% volatilization when applied at a faster flow rate (Piwoni et al. 1986; Wilson et al. 1981). All, or nearly all, of the remaining chloroform traveled through the soil because of its low adsorption onto soil. Another laboratory study of 15 common volatile or semi-volatile organic chemicals reported a half-life for chloroform of 4.1 days, which assumed first-order kinetic decay (Anderson et al. 1991).

The leaching potential of chloroform is confirmed by the detection of chloroform in groundwater, especially at hazardous waste sites (Clark et al. 1982; Dewalle and Chian 1981; Harris et al. 1984; Hunkeler et al. 2012; Sawhney 1989). Measured log K_{oc} values of 1.5–2.4 support the low sorption observed in laboratory studies (Sabljic 1984). Little or no chloroform concentration was observed on peat moss, clay, dolomite limestone, or sand added to water (Dilling et al. 1975). Chloroform slightly adsorbed to aquifer solids in laboratory studies utilizing different amounts of two different aquifer materials, with K_{oc} values ranging from 63.4 to 398 (log K_{oc}=1.80–2.59). The study authors reported higher adsorption with increasing organic content of the solids (Uchrin and Mangels 1986). Another study measured K_{oc} values ranging from 45 to 80 (log K_{oc}=1.65–190) in soil (Sabljic 1984; Wilson et al. 1981).

Other Media. Chloroform does not appear to bioconcentrate in higher aquatic organisms, based upon measured bioconcentration factors (BCF) of 6 and 8 for bluegill sunfish (*Lepomis macrochirus*) (Barrows et al. 1980; Veith et al. 1980) and of 13 for the common carp (*Cyprinus carpio*) (NITE 1980). A BCF of 690 experimentally determined in green algae, *Selenastrum capricornutum*, suggests that the compound has some tendency to concentrate in nonvascular aquatic plants (Mailhot 1987). No data regarding the biomagnification potential of chloroform were found. Based upon the observed BCF, however, significant biomagnification of chloroform is apparently unlikely.

5.4.2 Transformation and Degradation

Air. The vapor-phase reaction of chloroform with photochemically generated hydroxyl radicals is the dominant degradation process in the atmosphere. The rate constant for this process at 25°C has been experimentally determined as 1.05×10^{-13} cm^3/molecule-second, which corresponds to a half-life of ≈102 days based upon a 12-hour sunlit day in a typical atmosphere containing 1.5×10^6 hydroxyl radicals/cm^3 (DOT 1980; Singh et al. 1981). Chlorinated degradation products from reaction with hydroxyl radicals include inorganic chlorine, hydrogen chloride, formyl chloride, and phosgene (Holbrook 2003; Tsai 2017).

Chloroform is more reactive in photochemical smog conditions, where the approximate half-life is 11 days (Dimitriades and Joshi 1977). Direct photolysis of chloroform will not be a significant degradation process in the atmosphere. Chloroform solutions sealed in quartz tubes and exposed to sunlight for 1 year degraded at almost the same rate as solutions in sealed tubes stored in the dark, indicating that little or no photodegradation of the compound had occurred (Dilling et al. 1975). This is expected because chloroform does not show significant light absorbance at wavelengths >290 nm (Hubrich and Stuhl 1980).

Water. Hydrolysis will not be a significant degradation process in water based upon rate constants experimentally determined at 25°C that correspond to half-lives ranging from 1,850 to 3,650 years at pH 7, and from 25 to 37 years at pH 9 (Jeffers et al. 1989; Mabey and Mill 1978). Direct photolysis of chloroform will not be a significant degradation process in surface waters because, as noted above, the compound does not absorb light at wavelengths >290 nm (Hubrich and Stuhl 1980). The reaction rate of chloroform with hydrated electrons photochemically produced from dissolved organic matter has been predicted to correspond to a near-surface half-life of ≈44 days based upon an experimentally determined rate constant and a hydrated electron concentration of 1.2×10^{-17} mol of hydrolyzed electrons/L (Zepp et al.

1987). This latter process is probably too slow to effectively compete with volatilization as a removal process from surface waters. Under iron- or sulfate-reducing conditions, chloroform can be reduced to dichloromethane (USGS 2004).

Biological degradation of chloroform has been studied primarily under conditions of batch process operations at wastewater treatment plants or as a remediation option at hazardous waste disposal sites. Above certain dosage levels, chloroform becomes toxic to anaerobic and aerobic microorganisms. This is especially noticeable for biological treatment facilities that use anaerobic digestion systems, where sustained inputs with chloroform concentrations approaching 100 mg/L can all but eliminate methanogenic (methane-fermenting) bacteria (Rhee and Speece 1992). Other studies have shown appreciable inhibition of methanogenesis, with levels of chloroform of 1 mg/L (Hickey et al. 1987). Other chlorinated hydrocarbons, and particularly such common 2-carbon chlorinated aliphatics as TCE, can similarly inhibit bacteria found in sewage sludges (Long et al. 1993; Rhee and Speece 1992). Similar inhibition effects can be the result of heavy metal toxics, zinc being particularly stressful to methanogenic bacteria (van Beelen et al. 1994; van Vlaardingen and van Beelen 1992).

Studies of natural waters or wastewaters, where it is difficult to control the levels of specific chemicals or preclude inputs of other toxicants, yield a wide variety of results on the efficiencies of chloroform biodegradation. For instance, little or no degradation was observed during 25 weeks in aqueous aerobic screening tests utilizing primary sewage effluent inocula (Bouwer et al. 1981a), or in 2 weeks following a standard readily biodegradability screening test (Organization for Economic Co-operation and Development [OECD] 301C) in aerobic inoculum (NITE 2010). No chloroform degradation was observed in aerobic biofilm column studies (Bouwer et al. 1981b). Aerobic screening tests utilizing settled domestic wastewater as inoculum reported significant loss of chloroform, 46–49% loss in 7 days, indicating degradation; however, at least some of the loss was apparently attributable to volatilization (Tabak et al. 1981).

Under the proper conditions, chloroform appears to be much more susceptible to anaerobic biodegradation. Bouwer et al. (1981a) determined that degradation of chloroform under anaerobic conditions was more rapid at lower chloroform concentrations (81 and 99% degradation after 2 and 16 weeks, respectively, at 16 ppb) compared to higher concentrations (78% degradation after 16 weeks, at 157 ppb). Reported anaerobic degradation products were dichloromethane and carbon dioxide (Vickstrom et al. 2017). No degradation was observed when chloroform was incubated with aquifer material under anaerobic conditions for 27 weeks (Wilson et al. 1981). Several studies have indicated

that in the presence of acid, chloroform will more easily undergo anaerobic degradation (Gupta et al. 1996). Up to 96% chloroform removal was achieved with concentrations up to 16.74 µM (2,000 ppb) with acetic acid as the primary substrate. Dichloromethane was identified as the primary transformation product via reductive dehalogenation.

In the absence of toxicity from other solvents, chlorinated hydrocarbons, or heavy metals, and where chloroform concentrations can be held below approximately 100 ppb, both aerobic and anaerobic bacteria can biodegrade chloroform, with removal rates well over 80% in a period of 10 days (Long et al. 1993). Deviations from these ideal conditions can lead to lower removal efficiencies. These biodegradation reactions generally lead to the mineralization of the chloroform to chlorides and carbon dioxide (Bouwer and McCarty 1983; Rhee and Speece 1992). One study, however, documented the production of the toxicant methylene chloride (dichloromethane) from the breakdown of chloroform containing wastes in a mixed culture of bacteria from sewage sludge (Rhee and Speece 1992, citing results from work at Tyndall Air Force Base, Florida). However, caution should be exercised in generalizing without site-specific evidence since commercial grades of chloroform will often contain methylene chloride as an impurity. In waters containing mixtures of different chlorinated aliphatics, biodegradation may produce new chloroform, at least as a temporary byproduct, the breakdown of carbon tetrachloride into chloroform having been confirmed in laboratory studies (de Best et al. 1998; Long et al. 1993; Picardal et al. 1993).

Sediment and Soil. Little information was located regarding the degradation of chloroform in soil. Based on data for degradation in water, chemical degradation in soil is not expected to be significant. Under proper redox (iron- or sulfate-reducing) conditions, chloroform can be abiotically reduced to dichloromethane (USGS 2004). The available soil data suggest that chloroform biodegradation rates in soil may vary, depending upon conditions.

In soil column studies, the chloroform present in treated wastewater appeared to pass through the column nearly unchanged even though some of the other organic compounds present were apparently biodegraded, which indicated that the wastewater was not too toxic to the microorganisms in the soil (Bouwer et al. 1981b). In contrast to these studies, significant degradation of chloroform (33% removed in 6 days) was observed in fine sandy soil in sealed bottles; however, the chloroform may have been co-metabolized by methylotrophic bacteria already present in the soil. (Henson et al. 1988). In this study, the aerobic degradation was even faster in methane-enriched soil. Such bio-oxidation of chloroform was also observed under methanogenic conditions in batch experiments using an inoculum derived from activated sludge and in a continuous-flow laboratory scale column, using a methanogenic fixed film derived from

primary sewage effluent (Bouwer and McCarty 1983). Overall, biodegradation in soil is not expected to compete with the predicted rapid rate of volatilization from soil (Park et al. 1988). As with biodegradation in water, concentrations of chloroform above certain threshold levels may inhibit many bacteria, especially methane-fermenting bacteria under anaerobic or near-anaerobic conditions (Hickey et al. 1987).

Other Media. No studies on the transformation and degradation of chloroform in biological or other systems were located.

5.5 LEVELS IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to chloroform depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of chloroform in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on chloroform levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

Table 5-4 shows the reported lowest limit of detections that are achieved by standard analytical analysis in environmental media. An overview summary of the range of concentrations detected in environmental media is presented in Table 5-5.

Table 5-4. Lowest Limit of Detection Based on Standards[a]

Media	Detection limit	Reference
Air (ppbv)	0.020	WHO 2004
	0.01–1.8	EPA 2023d
	300	NIOSH 2018
Drinking water (ppb)	0.001	WHO 2004
	0.055	EPA 1995
Surface water and groundwater (ppb)	0.001	WHO 2004
	1	EPA 2014
	30–900	EPA 2018a
Soil (ppb)	0.0015	Laturnus et al. 2000
	1	EPA 2014
	30–900	EPA 2018a

Table 5-4. Lowest Limit of Detection Based on Standards[a]

Media	Detection limit	Reference
Whole blood (ppb)	0.0021–0.008	CDC 2022a, 2022b

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

Table 5-5. Summary of Environmental Levels of Chloroform

Media	Low	High	For more information
Outdoor air (ppbv)	0.008	27.2	Section 5.5.1
Indoor air (ppbv)	0.04	93.60	Section 5.5.1
Surface water (ppb)	0.26	85	Section 5.5.2
Groundwater (ppb)	<0.2	324	Section 5.5.2
Groundwater (ppb)	<0.2	120	Section 5.5.2
Drinking water (ppb)	<0.2	75	Section 5.5.2
Sediment (ppb)	0.0017	539	Section 5.5.3
Soil (ppb)	–	–	Section 5.5.3
Food (ppb)	0	176	Section 5.5.4

Detections of chloroform in air, water, and soil at NPL sites are summarized in Table 5-6.

Table 5-6. Chloroform Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
Water (ppb)	22	29.9	17.0	474	274
Soil (ppb)	77	250	57.1	115	82
Air (ppbv)	0.881	1.32	28.9	78	56

[a]Concentrations found in ATSDR site documents from 1981 to 2022 for 1,868 NPL sites (ATSDR 2022c). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

5.5.1 Air

While chloroform is found abundantly in the environment due to both natural and anthropogenic sources, it is infrequently monitored (Sekar et al. 2022). Fairly stable global averages of 7.3–7.7 parts per trillion by volume (pptv) chloroform in air were reported based on measurements between 1997 and 2010; an

increase to 8.9 pptv was reported in 2016, likely based on increased anthropogenic inputs (WMO 2018). The Air Quality System (AQS) reports the average ambient chloroform concentrations in air from hazardous air pollutant monitors across the United States; levels from 2018 to 2022 have remained relatively constant between 0.018 and 0.04 ppbv (EPA 2023d). The data are summarized in Table 5-7. In an assessment of 3,650 urban and rural locations in Minnesota, chloroform was detected (>0.023 ppbv) in 1,445 samples, the mean concentration was 0.027 ppbv, with a maximum of 1.41 ppbv (Pratt et al. 2000).

Table 5-7. Summary of Annual Concentrations of Chloroform (ppbv) Measured in Ambient Air at Locations Across the United States[a,b]

Year	Number of monitoring locations	Number of samples	Average	Maximum
2018	199	9,900	0.018	9.1
2019	129	7,053	0.025	27.2
2020	166	8,461	0.04	8.8
2021	170	11,466	0.03	7.7
2022	125	2,988	0.032	10.8
2023[c]	115	2,443	0.025	5.5

[a]Values were originally reported in parts per billion carbon (ppbC) and converted to ppbv.
[b]24-hour sampling period.
[c]As of October 26, 2023.

Source: EPA 2023d

Chloroform levels in air can be much higher in areas near hazardous waste sites (Stephens et al. 1986). The median concentration for source-dominated areas in the United States was 0.82 ppbv for data reported between 1977 and 1980, and 0.51 ppbv for data reported in 1987 (EPA 1982, 1988b). Certain source-dominated areas contained much higher chloroform levels. The ambient air concentrations outside homes in Love Canal, New York, in 1978, were 2–22 ppbv, and the maximum concentration found in ambient air at 20 California municipal landfills was 610 ppbv (Barkley et al. 1980; Wood and Porter 1987). Concentrations of 0.29–6 ppbv were found in air samples taken from five hazardous waste sites in New Jersey (LaRegina et al. 1986). Ambient air samples measured near a hazardous waste landfill contained ≤1 ppbv chloroform. Other source-dominated areas that may have ambient air chloroform concentrations significantly higher than background levels include areas near facilities that treat hazardous and municipal waste, as well as areas near contaminated groundwater, and municipal and hazardous waste incinerators (Corsi et al. 1987; EPA 1990a; LaRegina et al. 1986; Namkung and Rittmann 1987; Travis et al. 1986).

Elevated chloroform concentrations in air are present near some industrial sites, such as paper mills. Air samples were collected from 2017 to 2018 from the Lewis-Clark Valley in Washington, where a pulp paper mill operates (Scott et al. 2020). Annual chloroform averages were 0.15±0.19 μg/m^3 (range: 0.02–1.55 μg/m^3) for 2017 and 0.26±0.34 μg/m^3 (range: 0.03–3.33) for 2018.

The EPA (2016) includes chloroform in its Vapor Intrusion Screening Levels (VISL) Calculator, indicating that it is sufficiently volatile and sufficiently toxic to be considered a concern for vapor intrusion from soil water and groundwater. Accordingly, ATSDR (2016) recommends that health assessors should evaluate potential health implications of vapor intrusion for chloroform during site risk assessments.

A review of ATSDR public health assessments completed between 1994 and 2009 identified 33 sites with chloroform detected in soil gas, crawl space, indoor air, or outdoor air (Burk and Zarus 2013). Indoor air was sampled at 15 of the sites with chloroform detected from 0.03 to 23 μg/m^3 (0.01–4.7 ppbv). Chloroform was detected in soil gas at the 15 sites ranging from 2.4 to 146 μg/m^3 (0.24–29 ppbv) and in outdoor air ranging from 0.29 to 2.2 μg/m^3 (0.06–0.45 ppbv).

Data from the EPA vapor intrusion database found that chloroform was detected in 68.5% of 2,278 indoor air samples collected from 1990 to 2005 (EPA 2012). The maximum concentration of chloroform detected in indoor air at residential sites included in the vapor intrusion database was reported as 1.4 μg/m^3 (0.31 ppbv) (EPA 2012). In a study of chloroform vapor intrusion, the source was hypothesized to be chlorinated water in sewer lines below the impacted residence (McHugh et al. 2017).

One of the most significant indoor sources of chloroform is chlorinated tap water, and taking showers is expected to contribute a substantial amount to the indoor chloroform levels (Andelman 1985a, 1985b; EPA 1987; Kerger et al. 2005; Wallace 1997). In 100 residences monitored in suburban and rural areas of New Jersey, concentrations of chloroform detected in indoor air ranged from 0.20 to 1.2 ppbv (Weisel et al. 2008). Only 29% of samples were above the detection limit; however, the high limit of detection for about half of the samples (0.49 ppbv for 48 samples and 0.20 ppbv for 52 samples) limits the usefulness of this study. In portable classrooms used in kindergarten through grade 12 public schools in Los Angeles, California, daily average chloroform concentrations ranged from 0.02 to 0.06 ppbv (Shendell et al. 2004). Median indoor chloroform levels were 0.39 ppbv in 99 private residences monitored in southeast Louisiana between 2013 and 2015 (Wickliffe et al. 2020). Chloroform was not detected in the

main school building classroom during June but was detected between <0.027 and 0.08 ppbv during the winter and fall months.

The air around swimming pools may also contain chloroform. This is especially likely in heated, indoor pools, which can approximate the conditions found in shower stalls. Concentrations ranging from 3.99 to 205.6 ppbv have been measured in air at indoor swimming pools (Ahmadpour et al. 2022; Font-Ribera et al. 2010; Nitter and Svendsen 2019; Sa et al. 2011). In one study of four Canadian indoor swimming pools, there was not a significant difference between chloroform concentrations in air samples collected at 0.5 or 1.5 m above the water surface (Ahmadpour et al. 2022). Temporal variation in chloroform concentrations led the study authors to recommend an 8-hour sampling strategy instead of using 2-hour samples for assessing worker chloroform exposures (Ahmadpour et al. 2022).

Due to chloroform's potential to be transported long distances in air and production in the environment, chloroform has been detected in many remote locations. In 2003, the concentration of chloroform in air in remote areas in the United States ranged from 0.008 to 0.0098 ppbv (McCarthy et al. 2006). It is noted that these reported concentrations are below the reported limits of detection for standard analytical methods shown in Table 5-4. McCarthy et al. (2006) does not report the limit of detection for the analytical method used in their analysis.

5.5.2 Water

The EPA maintains a Water Quality Portal (WQP) database, which aggregates environmental monitoring data from the National Water Information System (NWIS) and STORage and RETrieval (STORET) systems. A summary of the data for ambient surface and groundwater from recent years is provided in Table 5-8 (WQP 2024). Chloroform has been detected in surface water and groundwater. Concentrations were generally at trace levels, with chloroform detected more frequently and at higher concentrations in groundwater. Chloroform has been detected in surface waters at levels as high as 85 ppb (USGS 2003). In another survey, chloroform was detected in surface waters of three rivers in Arizona at 5.4, 3.8, and 2.3 ppb (Rostad et al. 2000).

Table 5-8. Summary of Concentrations of Chloroform (ppb) Measured in Surface and Groundwater Across the United States

Year	Average	Maximum	Number of samples	Percent detected
Surface water				
2018	0.68	4.5	510	2.2
2019	0.29	1.6	459	3.1
2020	0.58	2.6	198	5.1
2021	0.71	5.7	499	3.0
2022	1.24	4.3	351	2.6
2023	0.15	0.49	71	14
Groundwater				
2018	4.2	120	1,323	21
2019	1.4	29	2,022	21
2020	1.5	25	1,689	34
2021	1.6	43.6	2,142	36
2022	1.4	38	3,020	22
2023	2.4	324	1,970	20

Source: WQP 2024

In a survey of principal aquifers in the United States between 1991 and 2010, the U.S. Geological Survey (USGS 2015a, 2015b) reported the number of aquifers in which volatile chemicals, including chloroform, were detected at a preselected benchmark concentration of >0.2 ppb. Multiple samples were taken per aquifer with the intention to determine the frequency and variation of chloroform detection in each aquifer. During this period, chloroform was detected at >0.2 ppb in 10 of the 17 shallow aquifers beneath agricultural land, and in 19 of the 22 aquifers beneath urban land (USGS 2015a, 2015b). The number of samples per site with chloroform present at concentrations above the benchmark of 0.2 ppb ranged from 0.75 to 8.11% for agricultural aquifers and from 2.27 to 55.00% beneath urban land. In another study, chloroform was detected in 48.1% of aquifers sampled below urban areas; 13.9% had concentrations above the benchmark of 0.2 ppb (Squillace et al. 2004). For urban and untreated rural wells, samples with concentrations >0.2 ppb were 26.4 and 7.3%, respectively (Squillace et al. 1999, 2004). Detection frequency was associated with redox conditions of the groundwater, with greater chloroform concentrations associated with increased levels of oxygen in the water. This is due to increased stability of chloroform in water with larger concentrations of oxygen, compared to low-oxygen conditions.

Between 1991 and 2010, chloroform was detected above the benchmark of 0.2 ppb in 28 of the 40 areas of principal aquifers in the United States used for drinking water (USGS 2015a, 2015b). The number of

samples with concentrations above 0.2 ppb within these areas were relatively low, ranging from 0.73 to 23.68%. Between 1986 and 2001, chloroform was detected at >0.2 ppb in 11.4% of 1,092 public wells and 5.2% of 2,400 domestic (private) wells used for drinking water across the United States (USGS 2006). Measured concentrations ranged from approximately 0.008 to 23 ppb chloroform in public wells and from approximately 0.002 to 75 ppb chloroform in domestic wells. An independent analysis of these data reported that detections in domestic wells were associated with dissolved oxygen content, with higher probability of detecting chloroform associated with higher dissolved oxygen (Rowe et al. 2007). Consistent with USGS data, another more recent large-scale study by Bexfield et al. (2022a) detected chloroform at >0.2 ppb in 93 of 1,537 (6.1%) of samples collected between 2013 and 2019 from 1,531 wells and 6 springs used for drinking water in the United States (calculated from supplemental data presented in Bexfield et al. (2022b)). In total, chloroform was only detected above the limit of detection (0.015 ppb) in 25% of wells, with concentrations ranging from <0.03 to 46.82 ppb (Bexfield et al. 2022b).

Other U.S. studies have also detected chloroform in drinking water. In Florida, 7.1 ppb chloroform was detected in tap water in Dade County and 14.8 ppb was detected in tap water from Broward County (Gibbons and Laha 1999). Households in the Lower Rio Grande valley had median levels of 5.0 and 4.1 ppb chloroform detected in spring and summer of 1993, respectively (Berry et al. 1997). Concentration ranges for these two time periods were 1.1–26.1 and 2.0–18.2 ppb, respectively. Of the 70 residential wells sampled in the Piedmont and Upper Coastal Plain regions of South Carolina, only three wells had detectable chloroform: at 0.9, 1.3, and 7.5 ppb (Aelion and Conte 2004). In a statewide study of Arizona residential drinking water, chloroform was detected at a mean of 2.60 ppb in tap water and 1.30 ppb in nontap water (Sofuoglu et al. 2003). For populations on the Arizona-Mexican border, 0.39 ppb mean chloroform was detected in tap water and 0.74 ppb was detected in non-tap water (Sofuoglu et al. 2003).

Limited recent data are available for chloroform in water near hazardous waste sites. Chloroform was not detected in surface or groundwater collected during a 2013 sampling campaign at Palermo Wellfield Superfund Site (WQP 2024). Chloroform was also not detected in groundwater during a 2011 sampling campaign of the Boomsnub Superfund Site (WQP 2024). Historical levels of chloroform in drinking water derived from wells near hazardous waste dumps in the 1980s ranged from 0.3 to 1,890 ppb (Clark et al. 1982; Dewalle and Chian 1981).

A review of ATSDR public health assessments completed between 1994 and 2009 identified 33 assessments with chloroform detected on site (Burk and Zarus 2013). Chloroform was detected in groundwater at 15 sites ranging from 0.3 to 134 μg/L.

In addition to drinking water, chlorinated oxidants reacting with organic materials will lead to the formation of chloroform in swimming pools. Reported concentrations in samples from public pools fall in a range of 32–207 ppb (Kanan et al. 2015). In poorly tended or very crowded pools, where there are large inputs of organic materials or heavy use of chlorinating agents, chloroform formation increases (Kanan et al. 2015). Chloroform production in swimming pools can be increased where the pools are treated with copper-containing algicides. In tests on chlorinated water using various doses of chlorine, cupric salts (with various anions), and varying levels of humic acid (Barnes et al. 1989), chloroform concentrations after a given reaction time were generally ≥50% higher in samples treated with copper, which acts as a catalyst in the reactions with the humic acids.

Chloroform at 0.25 ppb has been found in rainwater collected in Los Angeles, California, in 1982 (Kawamura and Kaplan 1983). Chloroform has been detected in seawater between 0.0016 and 1.090 ppb (Ohligschläger et al. 2019).

5.5.3 Sediment and Soil

Very limited recent soil and sediment analysis for chloroform has been conducted. Chloroform was detected in 4 of 365 sediment samples collected from the Great Lakes in 2020–2021; the maximum concentration was 72 ppb (WQP 2024). However, no chloroform was detected in 28 sediment samples collected in Texas in 2019. Historically, chloroform has been found in sediment samples from the three passes of Lake Pontchartrain, Louisiana, at concentrations ranging from 0.0017 to 0.018 ppb (wet weight basis) (Ferrario et al. 1985).

Chloroform was found at concentrations ranging from 0.030 to 0.080 ppb (dry weight basis) in sediment samples exposed to chlorinated electrical power plant cooling water; the control samples that were not exposed to cooling water contained nearly the same amounts of chloroform (Bean et al. 1985). Chloroform was detected in sediments collected in 2010 at the BP Deep Water Horizon oil spill, between 5.33 and 44.3 ppb (WQP 2024). Chloroform was not detected in sediment samples collected in 2021 at the Palermo Wellfield Superfund Site (WQP 2024).

Soil data are primarily limited to industrial sites. The only ambient soil sampling data available were seven samples collected in Montana in 2023; chloroform was not detected (detection limits are 0.087–0.13 mg/kg). Chloroform was detected in four soil samples collected in 2011 from the Salt Chuck Mine in Alaska at 12–48 ppb (WQP 2024). Chloroform was not detected in soil samples collected in 2015 from the Gay Mine Superfund Site (WQP 2024). It can be predicted that chloroform contamination occurs at hazardous waste sites where chloroform-containing leachate moves through the soil to groundwater. An explanation of the lack of data results from the fact that any chloroform in the soil is expected to either rapidly volatilize or leach. Laboratory studies using a variety of different soil types document the effectiveness of volatilization in removing chloroform from soils (Park et al. 1988).

5.5.4 Other Media

Chloroform has been detected in various foods and beverages at trace concentrations (in the ppb range). A summary of the available data is provided in Table 5-9. The most recent data are from a Canadian market study conducted in 2015 (Cao et al. 2024). Chloroform was detected in 37 of the 159 food and beverage samples, with butter, tap water, baking powder, cream, and cheese having the highest chloroform concentrations. In an older American study, dairy products also had the highest chloroform concentrations (Fleming-Jones and Smith 2003). This was likely due to the use of cleaning and disinfecting solvents containing chlorine, which form chloroform when residual cleaning products used on the processing equipment come in contact with the organic material in the dairy products. Rinsing equipment pre- and post-cleaning reduces this chloroform contamination source (Fleming-Jones and Smith 2003).

Fleming-Jones and Smith (2003) reported chloroform in foods such as produce and meat that were >100 ppb in total VOCs. Pork bacon, beef frankfurters, fast food chicken nuggets, and avocado were some of the foods that met the total VOC criteria and contained chloroform. Additionally, chloroform was detected in butter and cheese samples, which is in accord with reporting by Cao et al. (2024). Both studies reported chloroform levels (3–14 ppb) in beef frankfurters or wieners and sausages (Cao et al. 2024, Fleming-Jones and Smith 2003). There are several reasons why the studies may differ in the samples containing chloroform, especially as Fleming-Jones and Smith (2003) only reported chloroform levels in foods that met the VOC criteria. In addition, the studies prepared food samples differently, with Fleming-Jones and Smith (2003) cooking foods that are normally cooked and Cao et al. (2024) using a mixture of raw and cooked foods in their samples. It is possible that different food preparation methods may have contributed to the difference in results.

Residual chlorine in potable water can react with organic material to form chloroform, and chloroform has been observed to contaminate food and beverages during preparation (e.g., brewing tea or coffee, cooking soups, boiling vegetables or meat) and rinsing with chlorinated water (Huang and Batterman 2009, 2010). Chloroform may be introduced into commercial foodstuff and beverages via use of chlorinated water during food or beverage processing (Huang and Batterman 2009).

Table 5-9. Summary of Chloroform Measured in Food and Beverages in the United States (ppb)

Product	Average	Range	Source
Milk, whole	5.6		Cao et al. 2024[a]
Milk, 2%	1.9		
Milk, 1%	1.3		
Milk, skim	0.5		
Cream	26		
Ice cream	10		
Yogurt	3.5		
Cheese	21		
Cheese, cottage	13		
Cheese, processed	12		
Butter	58		
Chocolate milk, 1%	2.3		
Butter milk, 1%	0.6		
Soups, creamed, canned	4.1		
Apple juice, canned	3.6		
Citrus juice, frozen	0.6		
Citrus juice, canned	7.6		
Grape juice, bottled	2.4		
Fruit drinks (cocktails)	4.1		
Alcoholic drinks, beer	0.24		
Soft drinks, canned	2.9		
Soy beverage, fortified	1.6		
Tap water, kitchen	29		
Tap water, sample area	23		
Salad dressing	3.5		
Baby formula, milk base	2.8		
Syrup	2.2		
Nuts	3.9		
Condiments	0.6		
Salt	4		
Baking powder	26		

Table 5-9. Summary of Chloroform Measured in Food and Beverages in the United States (ppb)

Product	Average	Range	Source
Vanilla extract	1.7		
Soy sauce	0.8		
American cheese	–	11–54	Fleming-Jones and Smith 2003[b]
Cheddar cheese	–	3–107	
Cream cheese	–	38–100	
Margarine	–	7–14	
Butter	–	35–83	
Sour cream	–	14–176	
Cheese pizza	–	3–11	
Cheese and pepperoni pizza	–	2–6	
Ground beef	–	2–6	
Pork bacon	–	2–12	
Beef frankfurters	–	3–14	
Tuna, canned in oil	–	4	
Eggs, scrambled	–	5–13	
Quarter pound hamburger, cooked	–	2–14	
Chicken nuggets, fast food	–	2–16	
Cheeseburger, quarter pound	–	2–15	
Bologna	–	5–15	
Banana	–	8	
Peanut butter	–	2–8	
Raw avocado	–	3–15	
Popcorn, popped in oil	–	2–15	
Blueberry muffin	–	8–15	
Raw orange	–	4–6	
Potato chips	–	3–12	
Apple pie, fresh/frozen	–	9–19	
French fries, fast food	–	2–3	
Olive/safflower oil	–	4	
Mixed nuts	–	4–5	
Chocolate cake with icing	–	3–16	
Fruit-flavored cereal	–	2	
Cola, carbonated beverage	–	11–27	
Sweet roll/danish	–	2–12	
Fruit-flavored sherbet	–	0–27	
Popsicle	–	6–18	
Sandwich cookies	–	2–14	

Table 5-9. Summary of Chloroform Measured in Food and Beverages in the United States (ppb)

Product	Average	Range	Source
Chocolate chip cookies	–	3–4	
Graham crackers	–	5–12	
Sugar cookies	–	3–10	
Cake doughnuts with icing	–	2–6	
Natural spring bottled water (two brands sampled)	4.0; 3.8	–	Gibbons and Laha 1999

[a]Only positive detections were reported.
[b]Only foods with at least 100 ppb of any of the studied volatile organic compounds (VOCs) were reported.

Chloroform was not reported by the FDA's Total Diet Study in recent years. Previously, 41% of 231 samples of various foods contained chloroform at levels ranging from 4 to 312 ppb and 55% of 549 samples contained between 2 and 830 ppb chloroform (Daft 1988, 1989).

Chloroform was detected in volatile emissions at trace levels in baby wipes (56% of samples), baby diapers (67% samples), and adult diapers (50% of samples) (Lin et al. 2023). The maximum detection was 12 ng/g in a baby diaper. Chloroform may be present as an inadvertent contaminant or byproduct from manufacturing (Lin et al. 2023).

Since chloroform is highly volatile and shows little tendency to bioconcentrate or bioaccumulate in higher life forms such as fish, it is not ordinarily included in the types of persistent pollutants that are the focus of state fish consumption advisory programs. In a limited sampling survey conducted by the city and county of Honolulu between 2010 and 2014, chloroform was not detected in three species of marine fish: *Myripristis berndti*, *Selar crumenophthalmus*, and *Lutjanus kasmira* (WQP 2024).

5.6 GENERAL POPULATION EXPOSURE

The general population is likely to be exposed to chloroform through drinking water and beverages, eating food, inhaling contaminated air, and through dermal contact with water containing chloroform as a disinfection byproduct (e.g., while showering, bathing, cleaning, washing, swimming). All humans are expected to be exposed to at least low levels of chloroform. The most common chloroform exposures relate to chloroform generated when organic materials interact with chlorinated oxidants (e.g., chlorine or hypochlorous acid) widely used to purify water or remove pathogens from waste materials. Exposure to commercially produced chloroform is expected to be less common.

Accurate, current estimates of the daily intake of chloroform by various exposure routes are not available, or possible, due to the lack of appropriate monitoring data. Typical levels of atmospheric chloroform in remote, ambient, and source-dominated areas are 0.008–0.0098, 0.018–0.04, and 0.51–0.81 ppbv, respectively (EPA 1982, 1988b; McCarthy et al. 2006). Exposure via ingestion of contaminated drinking water is expected to be extensive since most U.S. community drinking-water supplies are chlorinated. Typical levels in drinking water range from 0.002 to 75 ppb (Aelion and Conte 2004; Berry et al. 1997; Gibbons and Laha 1999; Sofuoglu et al. 2003; USGS 2006). Levels in food have been well characterized, although data are not recent; the foods with the highest levels of chloroform are typically dairy products (Cao et al. 2024; Fleming-Jones and Smith 2003). Chloroform contamination is believed to be from water used for cooking and rinsing during food preparation, such as beverages and foods prepared by boiling water, including tea (3–67 ppb), coffee (3–13 ppb), rice (9 ppb), and soup (0.4–3 ppb) (Huang and Batterman 2009, 2010).

Generally low personal exposures are expected when not near a chloroform source. Personal air monitoring studies of married women in Pennsylvania and New Jersey were conducted for those who worked in smoking or nonsmoking environments and were married to a smoker or nonsmoker (Heavner et al. 1996). Mean personal air concentrations were comparable in nonsmoking homes (0.60 $\mu g/m^3$) and smoking homes (0.85 $\mu g/m^3$). Similarly, mean personal air concentrations in workplaces were comparable in nonsmoking (0.88 $\mu g/m^3$) and smoking environments (0.91 $\mu g/m^3$). Based on these measurements, daily median exposures to chloroform were calculated to be between 0.06 and 0.30 $\mu g/m^3$ for the different combinations of smoking and nonsmoking work and home environments (Heavner et al. 1996). In another personal air monitoring study of graduate students in a mixed-use university art building (mainly in areas where silk screen printing and cleaning occurred), median concentrations over 3-hour sampling periods were below the detection limits, which were reportedly between 0.5 and 1.5 ppb (Ryan et al. 2002).

Chloroform can be expected to exist in virtually all chlorinated drinking-water supplies. The main source of chloroform found in municipal drinking water is the chlorination of naturally occurring humic materials found in raw-water supplies (Ohligschläger et al. 2019). Factors that can increase the amount of chloroform in drinking water include seasonal effects (high summer values) and increased contact time between chlorine and humic material. Sources of water with high humic material content will contain higher levels of chloroform. The chloroform concentration in drinking water plants increases as the treated water moves through the distribution system, which contains humic or other organic material that

reacts with the chlorine used for disinfection (Kasso and Wells 1981). Drinking water derived from groundwater may contain higher levels of chloroform than normally encountered in drinking water derived from surface water. Microplastics in water may leach DOM, which promotes chloroform formation during water treatment (Chen et al. 2024; Yan et al. 2024). Chloroform ingestion from drinking water may be decreased in homes which utilize household reverse osmosis filtering systems or pitchers with activated carbon and ion exchange resin filters (Carrasco-Turigas et al. 2013). Reductions ranged from 56 to 91%, with greater removal observed for the pitcher filters.

Chloroform exposure in air may occur from chlorinated water, as chloroform is readily volatilized. Upper bound normalized daily inhalation from bathroom air during a 12-minute shower scenario was calculated to be 0.63–0.50 µg chloroform/day/µg chloroform in a liter of water (Kerger et al. 2005). Inhalation of chloroform during the 20-minute period immediately after the shower was calculated to be 0.56–0.58 µg chloroform/day/µg chloroform in a liter of water. Likewise, chloroform may volatilize during other domestic activities which utilize chlorinated water. Approximately two-thirds of chloroform formed during simulated normal dishwasher usage were released to the air (Olson and Corsi 2004). During the last 40 minutes of the 50-minute dishwasher cycle, chloroform was detected in the headspace air of all tested dishwashers at concentrations ranging from 0.01 to 36.1 µg/L. In the first 10 minutes, concentrations ranged from 0.04 to 6.3 µg/L in 8/10 dishwashers; chloroform levels were below the limit of detection (0.008 µg/L) for the other 2 dishwashers.

ATSDR's three-compartment Shower and Household-Use Exposure (SHOWER) model predicts air concentrations in the shower stall, bathroom, and main house throughout the day by estimating the contribution from showering or bathing and the contribution from other water sources in the house, such as the dishwasher, clothes washer, and faucets. This information, along with human activity patterns, is used to calculate a daily time-weighted average exposure concentration via inhalation exposure and from dermal uptake from skin contact. ATSDR's SHOWER model is available by sending a request to showermodel@cdc.gov. Using median treated water levels as discussed in Section 5.5.2 and representative outdoor air levels discussed in Section 5.5.1, reasonable maximum exposure (RME) levels for chloroform were calculated for different exposure groups and are presented in Table 5-10.

Table 5-10. Reasonable Maximum Exposure Daily Inhalation Dose in µg/kg/day and Administered Dermal Dose of Chloroform for the Target Person

Exposure group	Inhalation (µg/m^3)	Dermal (µg/kg/day)
Birth–<1 year	2.5	0.013
1–<2 years	2.5	0.012
2–<6 years	2.5	0.010
6–<11 years	2.5	0.0083
11–<16 years	2.5	0.0067
16–<21 years	2.5	0.0062
Adult	2.5	0.0061
Pregnant and breastfeeding women	2.5	0.0061

Source: ATSDR 2022b

An exposure study of 50 mothers was conducted in two areas with relatively high chloroform concentrations in municipal water compared to national averages, Corpus Christi, Texas, and Cobb County, Georgia, to determine effects to blood chloroform concentrations after showering. Median pre-showering levels were 25 and 70 pg/mL, respectively, and post-showering levels were significantly increased at 57 and 280 pg/mL, respectively (Lynberg et al. 2001). Monitoring of return to baseline was not included as part of the study.

Individuals may also be exposed to chloroform while swimming or lounging in chlorinated pools or spas through inhalation of volatilized chloroform in the air and dermal contact with the water. Monitoring studies have reported mean air concentrations of chloroform ranging from 2.66 to 105.73 ppbv at indoor recreational swimming pool facilities (Font-Ribera et al. 2010; Kanan et al. 2015; Nitter and Svendsen 2019; Sa et al. 2011). Pool water chloroform levels ranged from 3.04 to 114.5 µg/L (Aggazzotti et al. 1995; Cammann and Hübner 1995; Font-Ribera et al. 2010).

The Centers for Disease Control and Prevention (CDC) creates ongoing assessments on human exposure to environmental chemicals derived from data obtained from NHANES. The biomonitoring data reports levels of chloroform from a sample of people who represent the noninstitutionalized, civilian U.S. population during 2-year study periods conducted between 2011 and 2018. The data are presented in Table 5-11. Detectable levels of chloroform in blood are expected to reflect recent exposure; blood levels of chloroform can raise 2–4 times over baseline levels and return to normal rapidly after 1–2 hours (CDC 2022a, 2022b).

Table 5-11. Geometric Mean and Selected Percentiles of Chloroform Blood Concentrations (pg/mL) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) (2011–2018)

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)[a]				Sample Size
			50th	75th	90th	95th	
Total	2011–2012	6.32 (5.24–7.62)	6.06 (4.73–8.00)	12.6 (10.1–16.2)	22.5 (19.8–27.0)	33.5 (27.7–40.2)	2,589
	2013–2014	*	8.00 (<LOD–10.0)	16.0 (14.0–19.0)	27.0 (24.0–32.0)	40.0 (33.0–50.0)	3,136
	2015–2016	*	9.00 (8.00–11.0)	17.0 (15.0–20.0)	30.0 (28.0–33.0)	47.0 (42.0–54.0)	2,989
	2017-2018	*	8.00 (<LOD–9.00)	14.0 (11.0–17.0)	24.0 (20.0–30.0)	34.0 (30.0–42.0)	2,858
Age 12–19 years	2011–2012	5.08 (4.10–6.29)	4.76 (3.42–6.21)	9.22 (7.76–12.4)	20.8 (15.5–25.8)	28.7 (21.5–34.6)	487
	2013–2014	*	<LOD	14.0 (12.0–18.0)	28.0 (21.0–33.0)	35.0 (28.0–53.0)	588
	2015–2016	*	<LOD	14.0 (11.0–17.0)	26.0 (19.0–35.0)	37.0 (26.0–53.0)	537
	2017-2018	*	<LOD	11.0 (9.00–15.0)	23.0 (18.0–30.0)	32.0 (26.0–44.0)	471
Age 20+ years	2011–2012	6.54 (5.41–7.89)	6.26 (4.91–8.40)	12.9 (10.5–16.7)	23.2 (19.9–27.3)	34.1 (28.0–43.1)	2,102
	2013–2014	*	8.00 (<LOD–10.0)	16.0 (14.0–19.0)	27.0 (25.0–32.0)	41.0 (34.0–50.0)	2,548
	2015–2016	*	9.00 (8.00–11.0)	17.0 (16.0–20.0)	30.0 (28.0–35.0)	47.0 (40.0–57.0)	2,452
	2017-2018	*	8.00 (<LOD–9.00)	14.0 (11.0–17.0)	24.0 (20.0–30.0)	34.0 (30.0–43.0)	2,387
Males	2011–2012	6.27 (5.14–7.64)	6.18 (4.74–7.98)	12.3 (10.1–15.1)	22.3 (18.5–28.1)	35.6 (27.3–47.2)	1,307
	2013–2014	*	<LOD	16.0 (14.0–19.0)	26.0 (22.0–35.0)	38.0 (31.0–51.0)	1,512
	2015–2016	*	9.00 (<LOD–11.0)	17.0 (16.0–19.0)	31.0 (27.0–37.0)	51.0 (43.0–58.0)	1,485
	2017-2018	*	8.00 (<LOD–9.00)	14.0 (11.0–17.0)	26.0 (20.0–32.0)	35.0 (30.0–44.0)	1,390
Females	2011–2012	6.38 (5.25–7.75)	5.85 (4.62–8.16)	12.9 (9.94–17.9)	22.9 (19.4–28.0)	32.1 (26.6–41.0)	1,282
	2013–2014	*	9.00 (<LOD–10.0)	16.0 (14.0–19.0)	28.0 (25.0–34.0)	41.0 (33.0–52.0)	1,624
	2015–2016	*	9.00 (8.00–10.0)	17.0 (15.0–20.0)	29.0 (26.0–33.0)	42.0 (35.0–55.0)	1,504
	2017-2018	*	8.00 (<LOD–9.00)	14.0 (11.0–17.0)	23.0 (20.0–29.0)	32.0 (29.0–39.0)	1,468
Mexican Americans	2011–2012	6.14 (4.94–7.62)	5.91 (4.76–7.80)	11.7 (9.94–15.0)	23.1 (15.0–33.1)	32.5 (24.9–46.4)	269
	2013–2014	*	<LOD	9.00 (<LOD–14.0)	16.0 (12.0–22.0)	22.0 (16.0–33.0)	503
	2015–2016	*	8.00 (<LOD–10.0)	16.0 (12.0–21.0)	28.0 (23.0–35.0)	41.0 (32.0–46.0)	542
	2017-2018	*	<LOD	10.0 (8.00–16.0)	19.0 (14.0–27.0)	27.0 (19.0–36.0)	418
Non-Hispanic blacks	2011–2012	8.64 (6.08–12.3)	8.28 (5.22–13.6)	17.0 (10.8–27.0)	30.8 (20.0–45.2)	42.6 (32.3–56.5)	697
	2013–2014	*	10.0 (<LOD–14.0)	18.0 (14.0–24.0)	30.0 (23.0–40.0)	40.0 (30.0–65.0)	595
	2015–2016	11.6 (10.2–13.2)	10.0 (9.00–13.0)	18.0 (15.0–21.0)	28.0 (23.0–34.0)	38.0 (33.0–46.0)	619
	2017-2018	10.8 (9.40–12.5)	9.00 (<LOD–11.0)	16.0 (13.0–20.0)	27.0 (24.0–32.0)	39.0 (27.0–64.0)	635

Table 5-11. Geometric Mean and Selected Percentiles of Chloroform Blood Concentrations (pg/mL) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) (2011–2018)

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)[a]				Sample Size
			50th	75th	90th	95th	
Non-Hispanic whites	2011–2012	5.58 (4.50–6.90)	5.35 (3.85–7.41)	11.2 (7.93–14.6)	21.1 (16.7–25.4)	28.0 (24.0–37.0)	901
	2013–2014	*	9.00 (<LOD–11.0)	17.0 (14.0–20.0)	28.0 (23.0–36.0)	41.0 (32.0–57.0)	1,266
	2015–2016	*	9.00 (<LOD–11.0)	17.0 (15.0–19.0)	30.0 (27.0–33.0)	49.0 (37.0–58.0)	989
	2017-2018	*	<LOD	14.0 (11.0–16.0)	24.0 (20.0–29.0)	34.0 (30.0–38.0)	976
All Hispanics	2011–2012	7.85 (6.08–10.1)	8.03 (5.44–10.9)	15.3 (10.6–22.3)	26.3 (21.2–35.6)	37.2 (31.6–47.0)	546
	2013–2014	*	<LOD	12.0 (<LOD–17.0)	23.0 (17.0–29.0)	30.0 (23.0–45.0)	806
	2015–2016	*	9.00 (<LOD–11.0)	18.0 (14.0–22.0)	32.0 (27.0–36.0)	46.0 (36.0–52.0)	909
	2017-2018	*	<LOD	12.0 (9.00–17.0)	22.0 (16.0–30.0)	31.0 (21.0–42.0)	678
Asians	2011–2012	7.71 (6.25–9.51)	7.99 (6.25–10.0)	14.2 (11.1–18.1)	22.9 (18.8–30.2)	31.3 (24.2–43.1)	371
	2013–2014	*	<LOD	14.0 (10.0–20.0)	25.0 (17.0–39.0)	39.0 (27.0–47.0)	361
	2015–2016	*	9.00 (<LOD–11.0)	16.0 (11.0–23.0)	32.0 (22.0–46.0)	47.0 (34.0–62.0)	346
	2017-2018	*	8.00 (<LOD–18.0)	18.0 (9.00–29.0)	29.0 (18.0–52.0)	41.0 (26.0–71.0)	405

[a]LODs for survey years 2011–2012, 2013–2014, 2015–2016, and 2017–2018 are 2.1, 8, 8, and 8 pg/mL, respectively.

* = not calculated; proportion of results below limit of detection was too high to provide a valid result; CI = confidence interval; LOD = limit of detection

Source: CDC 2022a, 2022b

Limited other biomonitoring data are available. One study identified the VOCs present in amniotic fluid of 76 pregnant French women during the second trimester of their healthy pregnancy (Minet-Quinard et al. 2023). Chloroform was detected with a 44.7% frequency in the women's amniotic fluid.

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Limited current data were located regarding occupational exposure to chloroform. Although some of the exposure levels encountered in workplaces may be comparable to exposure that workers receive in their own homes, there are probably many specific jobs that expose workers to significantly higher levels of chloroform. These occupations likely include work at or near source-dominated areas such as chemical plants and other facilities that manufacture or use chloroform, operation of chlorination processes in drinking-water plants, work at or near wastewater-treatment plants and paper and pulp plants, and other facilities where large amounts of chloroform are released (e.g., hazardous and municipal-waste incinerators). Additionally, workers who also live in communities near production/use facilities (e.g., fence line communities) have an even greater potential for exposure. A maximum level of 3.8 ppbv was found in the air at an activated sludge wastewater treatment plant (Lurker et al. 1983). Maintenance workers, attendants, and lifeguards at indoor pools and spas may encounter concentrations between 32 and 207 ppb in water or 2.66–105.73 ppbv in air (Ahmadpour et al. 2022; Kanan et al. 2015; Nitter and Svendsen 2019; Sekar et al. 2022). Air may be a significant route of exposure for these workers, as one study found no significant difference between chloroform concentrations collected in close proximity to the pool surface (0.5 m) compared to approximate worker height (1.5 m) (Ahamdpour et al. 2022). For sewage/effluent treatment operators working in pulp, paper, and paper product mills, exposures to 15–1,670 ppb chloroform in water have been reported in the United States (Teschke et al. 1999). While occupational exposures are expected to be predominantly inhalation, there is potential for exposure via dermal contact with vapor or liquid.

Persons who use tap water often, especially if it is heated and/or sprayed (e.g., water used for cleaning, washing clothes and dishes, showering, and cooking), may also be exposed to higher than background levels. Levels in personal air samples as high as 22 and 11 ppb have been measured during household cleaning activities and showering (Wallace et al. 1987b). Persons using certain cleaning agents and pesticides in enclosed spaces with poor ventilation or persons working where these materials are used may be exposed to relatively high levels of chloroform. While the use of activated carbon filters may provide some reduction in the tap water levels for cold water, such filters are not effective with hot water where the elevated temperatures will induce volatilization from the filter media.

Individuals employed as cleaners (e.g., janitors, hotel housekeeping, domestic staff) form an occupational group that may have increased risk of exposure to chloroform (Lin et al. 2022; Wolfe et al. 2020). The potential for chloroform exposure in these occupations is elevated not only due to increased usage of disinfected water but also from chloroform generated during use of chlorine-containing disinfectants, such as bleach (Bruchard et al. 2023; Odabasi 2008; Lin et al. 2022). A particularly vulnerable group may be Hispanic women, who make up 58.9% of domestic house cleaners in the United States (Wolfe et al. 2020). Switching from traditional cleaning products to products labelled as "green" cleaning products has been shown to reduce chloroform exposure during domestic cleaning (Harley et al. 2021). Geometric mean personal air concentrations of chloroform while cleaning were 0.5 and 0.066 ppb while using traditional and "green" cleaning products, respectively.

Increased non-occupational exposure to chloroform is likely to occur from increased contact with chlorinated water. People who regularly swim in chlorinated pools, such as competitive swimmers, may have increased exposure to chloroform through inhalation and dermal contact with the water. Exposure levels are higher in heated, indoor pools, compared to outdoor training facilities (Aiking et al. 1994). In addition to increased frequency of exposure, kinetic studies show that increased physical exercise while in a chlorinated pool increases absorption of chloroform during the exposure period (Cammann and Hübner 1995). Several factors likely impact the chloroform levels reported in air from indoor swimming facilities, which ranged from 3.99 to 205.6 ppbv (Ahmadpour et al. 2022; Font-Ribera et al. 2010; Nitter and Svendsen 2019; Sa et al. 2011). These may include pool temperature, concentration of chlorine used, how crowded the pool is, and ventilation systems used.

Persons living in certain source-dominated areas may be at risk for higher than background exposures to chloroform. These may include persons living near industries and facilities that manufacture and use chloroform, municipal and industrial wastewater-treatment plants and incinerators, landfills and other waste sites, and paper and pulp plants. Likewise, persons who derive their drinking water from groundwater sources contaminated with chloroform from hazardous waste sites may have higher exposures. Additionally, exposure to chloroform in air may be increased in homes with vapor intrusion problems, and sewer lines below residences should be investigated as a potential source of chloroform emissions (ATSDR 2016; McHugh et al. 2017).

Individuals with inhalant substance use disorder that repeatedly and intentionally self-administer inhalants to achieve intoxication may have increased risk of exposure to chloroform depending upon the products abused. Chloroform is among the many chemicals in commonly abused products (Howard et al. 2011).

CHAPTER 6. ADEQUACY OF THE DATABASE

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

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

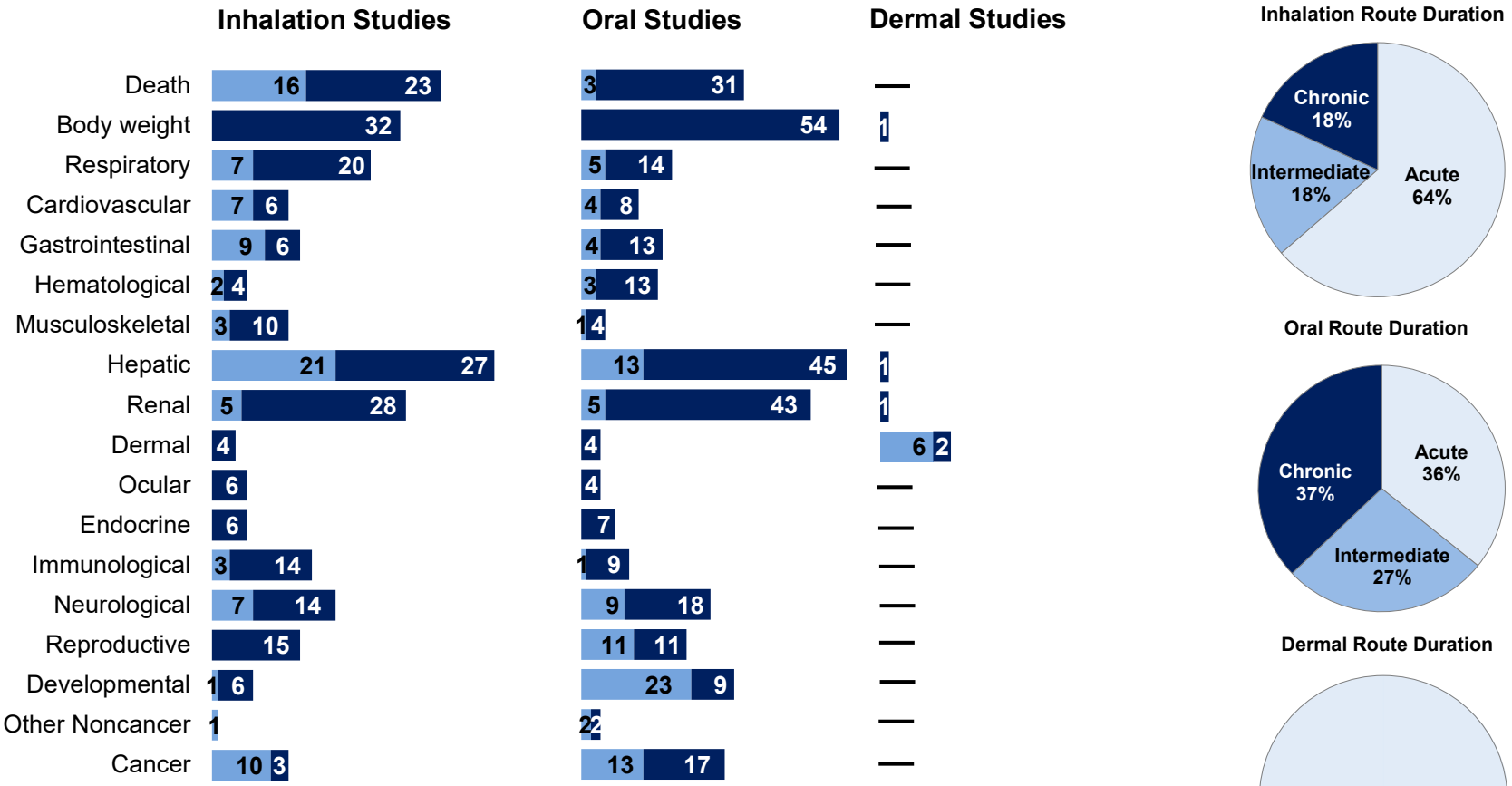
6.1 INFORMATION ON HEALTH EFFECTS

Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to chloroform that are discussed in Chapter 2 are summarized in Figure 6-1. The purpose of this figure is to illustrate the information concerning the health effects of chloroform. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

As shown in Figure 6-1, information on the health effects in humans are available from inhalation, oral, and dermal exposure. For the purposes of Figure 6-1, 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 6-1 due to unknown route(s) of exposure. The organs or systems adversely affected in humans after exposure to chloroform include the respiratory, liver, kidney, and neurological system. Death as well as multisystem damage may occur at sufficiently high exposure levels. Findings pertaining to reproductive, developmental, and carcinogenic effects of chloroform exposure in humans are mixed.

Figure 6-1. Summary of Existing Health Effects Studies on Chloroform by Route and Endpoint*

Potential liver, body weight, and kidney effects were the most studied endpoints
The majority of the studies examined oral or inhalation exposure in **animals** (versus **humans**)



*Includes studies discussed in Chapter 2. The number of studies include those finding no effect; 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 (e.g., exposure assessed via biomarker of exposure only) are not included in this figure or the study count reported above.

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 studies. The organs or systems adversely affected in animals were body weight, respiratory, hepatic, and renal effects. Death as well as multisystem damage may occur at sufficiently high exposure levels. Findings pertaining to cardiovascular, immunological, reproductive, and developmental effects of chloroform exposure in animals are mixed. Chloroform caused hepatic and renal cancer in animals following chronic-duration inhalation or oral exposure.

6.2 IDENTIFICATION OF DATA NEEDS

Missing information in Figure 6-1 should not be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Acute-Duration MRLs. The inhalation database was adequate to derive acute-duration inhalation and oral MRLs. While there are numerous acute-duration oral studies, most of the studies administered chloroform via gavage, which is considered less relevant to human exposure (due to saturation of detoxification pathways following bolus gavage exposure). Only two acute-duration drinking water studies were available. Additional, multi-dose, acute-duration drinking water studies could decrease uncertainty in the acute-duration oral MRL.

Intermediate-Duration MRLs. The inhalation database was adequate to derive an intermediate-duration inhalation MRL. Additional low-dose studies designed to identify a NOAEL for the critical effect (nasal lesions) could decrease uncertainty in the intermediate-duration inhalation MRL. The oral database is adequate to derive an intermediate-duration oral MRL.

Chronic-Duration MRLs. The inhalation database was adequate to derive a chronic-duration inhalation MRL. The oral database is adequate to derive a chronic-duration oral MRL. Additional low-dose studies designed to identify a NOAEL for the critical effects (nasal lesions via inhalation, hepatotoxicity via oral exposure) could decrease uncertainty in the chronic-duration MRLs.

Health Effects. Identification of data needs for health effects in animal studies is limited to targets included in the systematic review.

Respiratory. Respiratory effects noted in humans include respiratory depression and/or arrest at exposure levels associated with CNS depression; lung damage has been observed in fatal exposure cases (Ago et al. 2011; Featherstone 1947; Giusti and Chiarotti 1981; Harada et al. 1997; Piersol et al. 1933; Royston 1924; Schroeder 1965; Whitaker and Jones 1965). In animals, the nasal epithelium is a sensitive target of toxicity following inhalation and oral exposure (Constan et al. 1999; Dorman et al. 1997; Kasai et al. 2002; Larson et al. 1994c, 1995b, 1996; Mery et al. 1994; Templin et al. 1996a, 1996b; Yamamoto et al. 2002). Damage to the lower respiratory tract in animals was generally only observed at lethal exposure levels (Bowman et al. 1978; Kasai et al. 2002; NCI 1976). However, there is limited evidence of inflammatory responses in the lung at low inhalation exposure levels in mice (de Oliveira et al. 2015). Studies evaluating the potential for nasal effects in humans would be useful. Research on the potential respiratory effects of multi-route exposure to chloroform in residential water supplies estimating exposure levels via respiratory, oral, and dermal exposure routes would also be useful. Of specific interest is inflammatory respiratory responses, if analyses adequately control for confounders, particularly exposures to other known water disinfection byproducts. Additional low-dose animal studies evaluating inflammatory lung effects are needed to confirm findings by de Oliveira et al. (2015).

Hepatic. Numerous studies establish that the hepatic system is a target of chloroform toxicity in humans and animals via inhalation and oral exposure (Section 2.9). However, additional drinking water studies in animals could help better define the dose-response relationship, and how it compares to findings observed in gavage studies. Research on the potential hepatic effects of multi-route exposure to chloroform in residential water supplies would also be useful if analyses adequately control for confounders, particularly exposures to other known water disinfection byproducts.

Renal. Numerous studies establish that the renal system is a target of chloroform toxicity in humans and animals via inhalation and oral exposure (Section 2.10). However, additional drinking water studies in animals could help better define the dose-response relationship, and how it compares to findings observed in gavage studies. Research on the potential renal effects of multi-route exposure to chloroform in residential water supplies would also be useful if analyses

adequately control for confounders, particularly exposures to other known water disinfection byproducts.

Neurological. The CNS is a target organ for chloroform toxicity in humans after inhalation and oral exposure. The neurotoxic effect is well documented in studies of patients exposed to chloroform via anesthesia (Featherstone 1947; Smith et al. 1973; Whitaker and Jones 1965) or of individuals who intentionally and accidentally ingested the chemical (Piersol et al. 1933; Schroeder 1965; Storms 1973). CNS depression has also been clearly demonstrated in animals exposed via inhalation (Constan et al. 1999; EPA 1978; Gehring 1968; Lehmann and Flury 1943) or oral exposure (Bowman et al. 1978; NTP 1988a; Jones et al. 1958). Data pertaining to neurological effects at exposure levels below those associated with frank CNS depression are limited but suggest neurobehavioral changes in humans following occupational exposure (Challen et al. 1958; Li et al. 1993) and in animals following oral exposure (Balster and Borzelleca 1982; Landauer et al. 1982; Wada et al. 2015). Additional studies in humans and/or animals evaluating comprehensive neurological endpoints at low exposure levels via inhalation or oral exposure would be useful to establish dose-response relationships for mild neurological effects. Research on the potential neurological effects of multi-route exposure to chloroform in residential water supplies would also be useful if analyses adequately control for confounders, particularly exposures to other known water disinfection byproducts. More information regarding the mechanism of chloroform-induced neurotoxicity would be helpful.

Developmental. Numerous studies have evaluated potential associations between developmental effects and exposure to disinfection byproducts in chlorinated water, including chloroform. Some of these found associations between impaired growth and measured chloroform level in water, estimated total residential chloroform intake, or chloroform blood levels (Botton et al. 2015; Grazuleviciene et al. 2011; Kramer et al. 1992; Summerhayes et al. 2012; Wright et al. 2004), while others did not (Bonou et al. 2017; Cao et al. 2016; Hinckley et al. 2005; Liu et al. 2021; Porter et al. 2005; Villanueva et al. 2011). A main limitation of these studies is lack of control for other known water disinfection byproducts. Therefore, additional studies evaluating potential associations between residential chloroform exposure and developmental effects with adequate control for exposure to other known water disinfection byproducts could be useful.

Developmental studies in animals provide some evidence of fetal malformation after inhalation exposure (Murray et al. 1979; Schwetz et al. 1974). However, these defects were not observed in additional developmental studies in rats exposed via inhalation (Baeder and Hofmann 1988; EPA 1978) or rats or rabbits exposed orally (Ruddick et al. 1983; Thompson et al. 1974). Evidence for delayed ossification and impaired growth after inhalation or oral exposure indicate these findings are generally only observed at maternally toxic doses (Baeder and Hofmann 1988; Murray et al. 1979; Ruddick et al. 1983; Schwetz et al. 1974; Thompson et al. 1974). Additional low-dose studies are needed to confirm findings by Murray et al. (1979) and Schwetz et al. (1974) and/or establish dose-response data for developmental effects below those associated with maternal toxicity. One developmental study reported altered glucose homeostasis in offspring following developmental exposure to very low doses in drinking water (Lim et al. 2004). However, due to reporting deficiencies, there is uncertainty in the exposure estimate. Additional developmental studies evaluating low-dose drinking water exposures with adequate monitoring of body weight and intake are needed to corroborate findings from this study.

Epidemiology and Human Dosimetry Studies. Populations may be exposed to chloroform in the workplace, near hazardous waste sites containing chloroform, from chlorinated water, and from various consumer products that contain chloroform. Limited information was obtained from occupational studies reporting CNS and liver effects in exposed workers (Bomski et al. 1967; Challen et al. 1958; Phoon et al. 1983). However, exposure measurements in these studies were not rigorous. Occupational studies with quality external exposure assessments and/or reliable dosimetry data correlating occupational exposure with signs of toxic effects would be useful. Epidemiology studies suggest an association between elevated chloroform levels in drinking water and certain types of cancer in humans (Bove et al. 2007; Doyle et al. 1997; Font-Ribera et al. 2018; Gao et al. 2014) or impaired growth during development (Botton et al. 2015; Grazuleviciene et al. 2011; Kramer et al. 1992; Summerhayes et al. 2012; Wright et al. 2004). However, all of these studies were limited by a lack of control for important confounders, namely co-exposure to other known disinfection byproducts (e.g., trihalomethanes). Epidemiological studies with adequate control for other disinfection byproducts would be helpful. Since toxicokinetics of lipophilic compounds are expected to be different in lean versus obese individuals (La Merrill and Birnbaum 2011), epidemiological studies stratifying analyses by body mass index (BMI) may be useful in determining if there is increased risk of chloroform-related toxicity in obese individuals.

Biomarkers of Exposure and Effect. Methods for detecting chloroform in exhaled breath, blood, breast milk, urine, and tissues are available. Nevertheless, it is difficult to correlate chloroform levels in

biological samples with exposure, because of the volatility and short half-life of chloroform in biological tissues. Several studies monitored chloroform levels in environmentally exposed populations (Antoine et al. 1986; Hajimiragha et al. 1986; Peoples et al. 1979); however, the measured levels probably reflect both inhalation and oral exposure. Moreover, increased tissue levels of chloroform or its metabolites may reflect exposure to other chlorinated hydrocarbons. Studies designed to determine and validate more reliable biomarkers of exposure to allow for better quantitation of chloroform exposure would enhance the database.

No biomarkers were identified that are particularly useful in characterizing the effects induced by exposure to chloroform. The target organs of chloroform toxicity are the CNS, liver, and kidneys; however, damage to these organs may result from exposure to other chemicals. More effort to identify subtle biochemical changes to serve as biomarkers of effects of chloroform exposure would be useful in detecting early, subtle signs of chloroform-induced damage.

Absorption, Distribution, Metabolism, and Excretion. Human data indicate that chloroform absorption from the lungs is rapid and fairly complete after inhalation exposure (Smith et al. 1973). The data also indicate that absorption after oral exposure is close to 100% for both animals and humans (Brown et al. 1974a; Fry et al. 1972; Taylor et al. 1974). Dermal absorption in humans and animals also occurs to some extent, and it is governed by both ambient temperature and its rate of diffusion through the skin (Cammann and Hübner 1995; Fan et al. 2007; Gordon et al. 1998; Islam et al. 1995, 1996, 1999a, 1999b; Lévesque et al. 1994; Tsuruta 1975; Xu and Weisel 2005). Although there are no experimental data regarding dermal absorption in humans, some data have been extrapolated from mouse studies (Tsuruta 1975). The rate of absorption following oral or inhalation exposure is rapid (within 1–2 hours).

Data are available regarding the distribution of chloroform in animals after inhalation, oral, and dermal exposure to chloroform (Brown et al. 1974a; Chenoweth et al. 1962; Cohen and Hood 1969; Corley et al. 1990; Danielsson et al. 1986; Islam et al. 1995; Taylor et al. 1974); however, data regarding the distribution of chloroform in humans are very limited (Feingold and Holaday 1977) and warrant further investigation. Animal studies indicate that distribution following oral exposure is similar to that following inhalation exposure (Brown et al. 1974a; Pfaffenberger et al. 1980; Take et al. 2010); another well-conducted animal study focusing on distribution and excretion after dermal exposure would be useful to assess exposure via this route.

The metabolic pathways of chloroform metabolites are well understood (Ade et al. 1994; Constan et al. 1999; Lipscomb et al. 2004; Liu et al. 2013; Testai et al. 1996). Based on clear differences in toxicity between gavage and drinking water studies in rodents (Larson et al. 1993, 1994b, 1995a, 1995b), it appears that the mode of oral administration affects metabolism. Increased toxicity in rodents following acute-duration gavage exposure, compared to drinking water, is likely due to saturation of detoxification pathways following bolus gavage exposure, which exacerbates toxicity due to accumulation of toxic metabolites in hepatic and renal tissues. Specifically, it is proposed that the reaction of chloroform metabolites with GSH acts as a detoxifying mechanism. This is supported by observations that chloroform doses that caused liver GSH depletion produced liver necrosis (Docks and Krishna 1976). However, increased toxicity is not observed in gavage studies (Dunnick and Melnick 1993; NCI 1976; Roe et al. 1979) following chronic-duration exposure in rodents when compared to drinking water studies (Hard et al. 2000; Jorgenson et al. 1985; Nagano et al. 2006). Several factors may contribute to this differential finding in longer-duration studies, including: (1) adaptive metabolic changes with chronic-duration exposure leading to blunting or attenuation of bolus effects; (2) lack of evaluation at low gavage doses in some studies (which may have potentially identified lower LOAELs); and/or (3) evaluation of different strains in chronic versus shorter-duration studies that may have differential susceptibility. Additional data investigating the impact of mode of oral exposure, duration of administration, and impact of strain would be useful in order to understand the role of these factors in the mechanism of chloroform's toxicity.

The excretion of chloroform and its metabolites is understood, based on human and animal data derived from oral and inhalation studies (Brown et al. 1974a; Corley et al. 1990; Fry et al. 1972; Taylor et al. 1974) and in animals following dermal exposure (Islam et al. 1996, 1999a). The major route of chloroform elimination is pulmonary, but minor pathways are through enterohepatic circulation, urine, and feces as parent compound or metabolites (Corley et al. 1990).

Comparative Toxicokinetics. Target organs for chloroform distribution appear to be similar in humans and animals. Nonetheless, human and animal studies indicate that there are large interspecies differences in chloroform metabolism and tissue partition coefficients (Brown et al. 1974a; Corley et al. 1990). Since hepatic, renal, and nasal toxicity is attributed to reactive metabolites (e.g., phosgene), differential activity of CYP2E1 across species and sexes will confer a difference in susceptibility to toxic effects (Constan et al. 1999). Data on CYP2E1 activity in human olfactory epithelial tissue are limited and conflicting (Green et al. 2001; Longo and Ingelman-Sundberg 1993); studies designed to evaluate the ability of human olfactory mucosa to metabolize chloroform would be useful to decrease the uncertainty

in the dosimetry extrapolation in the inhalation MRLs. Marked sex-related differences in tissue distribution and covalent binding to tissue macromolecules in mice also have been observed (Taylor et al. 1974). Excretion data indicate that humans and nonhuman primates excrete chloroform in the breath primarily as unchanged chloroform; mice eliminate almost 80% of an oral chloroform dose as CO_2 (Brown et al. 1974a). Thus, toxicokinetic data indicate that it may be difficult to compare the toxicokinetics of chloroform in animals with that in humans. There are many oral studies, relatively few inhalation studies, and a limited number of dermal studies regarding the toxicokinetics of chloroform. Quantitative toxicokinetic studies in several animal species involving exposure to chloroform via all three routes, especially inhalation and dermal, would help complete the database. In addition, further refining of the existing PBPK/PD models and/or additional PBPK/PD model development would further advance our understanding of chloroform tissue dosimetry in humans and animals. For example, current nasal dosimetry models are limited to rats (Sarangapani et al. 2002); a PBPK/PD model for nasal dosimetry in humans would reduce uncertainty in dosimetry extrapolation in the inhalation MRLs. For oral MRLs, current PBPK/PD models extrapolate the internal doses from delivery of a large bolus dose. PBPK/PD models based on more environmentally-relevant exposure scenarios (drinking water) may be useful and could reduce uncertainty in dosimetry extrapolation for the acute-duration oral MRL. PBPK/PD models in additional species (e.g., dogs) would reduce uncertainty in dosimetry extrapolation for the intermediate- and chronic-duration oral MRLs.

Children's Susceptibility. It is unknown if developing fetuses, infants, or children are uniquely susceptible to chloroform toxicity. There may be age-related susceptibility to chloroform, as observed in rodent lethality studies (Deringer et al. 1953; Kimura et al. 1971). As discussed above (under Developmental Toxicity), developmental findings in human studies are mixed. In mice, it has been shown that chloroform passes the placenta (Danielsson et al. 1986). However, available evidence suggests that developmental effects in rodents are most likely to occur only at exposure levels associated with maternal toxicity. Serious effects (cleft palate, imperforate anus) were only observed in one study each, and both were at or above concentrations associated with maternal toxicity (Murray et al. 1979; Schwetz et al. 1974). These defects were not observed in additional developmental studies in rats exposed via inhalation (Baeder and Hofmann 1988; EPA 1978) or rats or rabbits exposed orally (Ruddick et al. 1983; Thompson et al. 1974), even at maternally toxic exposure levels. Similarly, many studies reported delayed ossification and/or impaired growth at maternally toxic inhalation or oral doses (Baeder and Hofmann 1988; Murray et al. 1979; Ruddick et al. 1983; Schwetz et al. 1974; Thompson et al. 1974). Additional studies at low, non-maternally toxic doses, are needed to fully evaluate children's susceptibility.

Physical and Chemical Properties. As reported in Table 4-2, the physical and chemical properties of chloroform have been characterized sufficiently to permit estimation of its environmental fate.

Production, Import/Export, Use, Release, and Disposal. Data regarding the production methods, production capacity volumes (current, past, projected future), and current import and export volumes are available (EPA 2023a; Holbrook 2003; Ohligschläger et al. 2019; USITC 2023). However, these statistics will generally not include all instances where chloroform is generated as a chemical intermediate or waste product. Except for the partial coverage provided in the TRI, comprehensive information regarding current release and disposal patterns, are lacking. General disposal information is adequately detailed in the literature, and information regarding disposal regulations of chloroform is available (EPA 1988a, 1988b). Production, release, and disposal data are useful to determine where environmental exposure to chloroform may be high.

Environmental Fate. Chloroform partitions mainly into the atmosphere and into groundwater. Experimental data are available regarding the transport and partitioning properties of chloroform in surface waters (Bean et al. 1985; Clark et al. 1982; Class and Ballschmidter 1986; Dilling 1977; Ferrario et al. 1985; Piwoni et al. 1986; Sawhney 1989). Chloroform can be transported long distances in air.

Data are available regarding the degradation of chloroform in the atmosphere, but less is known about degradation rates in water and soil (Anderson et al. 1991; Bouwer et al. 1981a, 1981b; Dilling et al. 1975; DOT 1980; Henson et al. 1988; Jeffers et al. 1989; Park et al. 1988; Singh et al. 1981; Tabak et al. 1981; Wilson et al. 1981). Hydrolysis and direct photodegradation are not significant removal processes. Although data regarding biodegradation rates in natural media are lacking, volatilization is expected to dominate over biodegradation as a removal process from surface water and near-surface soil. Chloroform seems relatively persistent in the atmosphere and groundwater. The environmental fate of chloroform is sufficiently determined by the available data. Considering the documented occurrence (Class and Ballschmidter 1986) of chloroform in remote, often pristine areas, further study is warranted to help quantify the relative role of long-range transport processes. These more localized processes could include the reaction of naturally generated chlorinated oxidants with organic materials to yield chloroform. More data would be useful on the half-lives of chloroform in media.

Bioavailability from Environmental Media. Chloroform is absorbed following inhalation, oral, and dermal contact. Toxicity studies of exposure to chloroform in air, water, and food demonstrated the

bioavailability of chloroform by these routes. Data are lacking on the bioavailability of chloroform following ingestion of contaminated soils. However, near-surface soil concentrations can be expected to be low due to volatilization (Piwoni et al. 1986; Wilson et al. 1981), suggesting that soil ingestion is not a likely route of exposure.

Food Chain Bioaccumulation. Data are available that indicate that chloroform does not bioconcentrate in aquatic organisms (Barrows et al. 1980; Veith et al. 1980). Bioconcentration studies are lacking for plants and other animals (e.g., plants, macroinvertebrates). Similarly, no studies were located regarding the biomagnification potential of chloroform in terrestrial and aquatic food chains. Additional information on bioconcentration and biomagnification could be useful in establishing the significance of food chain bioaccumulation as a route of human exposure.

Exposure Levels in Environmental Media. All humans are exposed to at least low levels of chloroform via inhalation of contaminated air, and most humans are exposed by drinking contaminated water. Estimates from intake via inhalation and ingestion of drinking water, based on limited data, are available (EPA 2023d; USGS 2015a, 2015b; WQP 2024). The quantitation of chloroform levels in food has been studied (Cao et al. 2024; Fleming-Jones and Smith 2003; Huang and Batterman 2009, 2010). Current information on exposure to chloroform for workers or people who live near manufacturing and use facilities, water and wastewater-treatment plants, municipal and industrial incinerators, hazardous waste sites, and other sources of significant release would be useful. Likewise, current indoor air exposure levels would be valuable.

Exposure Levels in Humans. Data regarding occupational exposure levels in humans are incomplete and are usually the result of limited, special studies. Studies designed to obtain better, current estimates of expected chloroform exposures in various workplace settings would be useful, including industrial settings (facilities that manufacture or use chloroform, drinking-water plants, wastewater-treatment plants, paper and pulp plants), indoor pools and spas, and industrial and domestic cleaning scenarios. Chloroform has been found in human blood of adults in the U.S. population (CDC 2022a, 2022b). A detailed database of exposure would be helpful in determining the current exposure levels, thus allowing an estimation of the average daily dose associated with various scenarios, such as living near a point source of release, drinking contaminated water, or working in a contaminated place. This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. Although NHANES data does include information on ages 12–19, more studies are needed on exposure levels to children at all stages of development. This information would be useful in determining how exposure of children differs from adults.

6.3 ONGOING STUDIES

No ongoing studies were identified in the National Institute of Health (NIH) RePORTER (2024) database.

CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding chloroform in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency.

ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the MRLs for chloroform.

Table 7-1. Regulations and Guidelines Applicable to Chloroform

Agency	Description	Information	Reference
	Air		
EPA	RfC	Not evaluated	IRIS 2001
WHO	Air quality guidelines	Not listed	WHO 2010
	Water & Food		
EPA	Drinking water standards and health advisories		EPA 2018b
	1-Day health advisory (10-kg child)	4 mg/L	
	10-Day health advisory (10-kg child)	4 mg/L	
	DWEL	0.35 mg/L	
	Lifetime health advisory	0.07 mg/L	
	National primary drinking water regulations		EPA 2023e
	Total trihalomethanes—MCL	0.080 mg/L	
	Chloroform—MCLG	0.07 mg/L	
	RfD	0.01 mg/kg/day[a]	IRIS 2001
WHO	Drinking water quality guidelines		WHO 2022
	Guideline value	0.3 mg/L	
	TDI	15 µg/kg body weight	
FDA	Substances added to food[b]	Approved for some indirect additives uses	FDA 2024a
	Allowable level in bottled water		FDA 2023
	Total trihalomethanes	0.080 mg/L	

Table 7-1. Regulations and Guidelines Applicable to Chloroform

Agency	Description	Information	Reference
	Cancer		
HHS	Carcinogenicity classification	Reasonably anticipated to be a human carcinogen	NTP 2021
EPA	Carcinogenicity classification	Likely to be carcinogenic to humans[c,d]	IRIS 2001
	IUR	2.3×10^{-5} per µg/m^3 [e]	
IARC	Carcinogenicity classification	Group 2B[f]	IARC 1999
	Occupational		
OSHA	PEL (ceiling limit[g]) for general industry and construction	50 ppm (240 mg/m^3)	OSHA 2022a, 2022c
	PEL (8-hour TWA) for shipyards	50 ppm (240 mg/m^3)	OSHA 2022b
NIOSH	STEL (60-minute)	2 ppm (9.78 mg/m^3)[h]	NIOSH 2019
	IDLH	500 ppm[h]	
	Emergency Criteria		
EPA	AEGLs-air		EPA 2018c
	AEGL 1[i]	Not recommended	
	AEGL 2[i]		
	10-minute	120 ppm	
	30-minute	80 ppm	
	60-minute	64 ppm	
	4-hour	40 ppm	
	8-hour	29 ppm	
	AEGL 3[i]		
	10-minute	4,000 ppm	
	30-minute	4,000 ppm	
	60-minute	3,200 ppm	
	4-hour	2,000 ppm	
	8-hour	1,600 ppm	

Table 7-1. Regulations and Guidelines Applicable to Chloroform

Agency	Description	Information	Reference
DOE	PACs-air		DOE 2018a
	PAC-1[j]	2 ppm	
	PAC-2[j]	64 ppm	
	PAC-3[j]	3,200 ppm	

[a]RfD for noncancer effect also considered to be protective against cancer risk (IRIS 2001).
[b]The Substances Added to Food inventory replaces EAFUS and contains the following types of ingredients: food and color additives listed in FDA regulations, flavoring substances evaluated by FEMA or JECFA, GRAS substances listed in FDA regulations, substances approved for specific uses in food prior to September 6, 1958, substances that are listed in FDA regulations as prohibited from use in food, delisted color additives, and some substances "no longer FEMA GRAS."
[c]Under the EPA's 1986 guidelines for carcinogen risk assessment (EPA 1986), Group B2 contains agents classified as probable human carcinogens.
[d]Using draft revised guidelines for carcinogen risk assessment (EPA 1996), which were finalized later (EPA 2005), chloroform was classified as: (1) 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, and (2) not likely to be carcinogenic to humans by any route of exposure under exposure conditions that do not cause cytotoxicity and cell regeneration.
[e]EPA reported that this IUR was developed in 1987 and does not incorporate newer data or the updated EPA cancer assessment guidelines. Per EPA, the methodology used to derive the IUR has two shortcomings: (1) it utilized a route-to-route extrapolation approach that did not employ a PBPK model, and (2) it incorporated a linear extrapolation approach for dose-response that implicitly assumes a risk of cancer at all nonzero exposures to chloroform (i.e., no threshold). EPA's mode-of-action analysis added in 2001, however, concluded that for cancer, chloroform exhibits a "threshold" by all routes of exposure. Thus, a chloroform dose exists that does not elicit cytotoxicity and presents no cancer risk. Therefore, the assumption underlying EPA's IUR dose-response approach (linear extrapolation with no threshold) is inconsistent with EPA's subsequent mode-of-action analysis.
[f]Group 2B: possibly carcinogenic to humans.
[g]Value not to be exceeded at any time.
[h]NIOSH considers the compound to be a potential occupational carcinogen.
[i]Definitions of AEGL terminology are available from EPA (2018d).
[j]Definitions of PAC terminology are available from DOE (2018b).

AEGL = acute exposure guideline levels; DOE = Department of Energy; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; FAO = Food and Agriculture Organization; FDA = Food and Drug Administration; FEMA = Flavor and Extract Manufacturers Association of the United States; GRAS = generally recognized as safe; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; IUR = inhalation unit risk; JECFA = Joint FAO/WHO Expert Committee on Food Additives; MCL = maximum contaminant level; MCLG = maximum contaminant level goal; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = protective action criteria; PBPK = physiologically based pharmacokinetic; PEL = permissible exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; STEL = short-term exposure limit; TDI = tolerable daily intake; TWA = time-weighted average; WHO = World Health Organization

CHAPTER 8. REFERENCES

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APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. LOAELs for serious health effects (such as irreparable damage to the liver or kidneys, or serious birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

APPENDIX A

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Office of Innovation and Analytics, Toxicology Section, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Office of Innovation and Analytics, Toxicology Section, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S106-5, Atlanta, Georgia 30329-4027.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Inhalation
Duration: Acute
MRL: 0.001 ppm (0.005 mg/m^3)
Critical Effect: Nasal lesions
Reference: Larson et al. 1996; Templin et al. 1996b
Point of Departure: NOAEL of 2 ppm ($NOAEL_{HEC}$ of 0.04 ppm)
Uncertainty Factor: 30
LSE Graph Key: 9, 24
Species: Rat, Mouse

MRL Summary: An acute-duration inhalation MRL of 0.001 ppm was derived for chloroform based on nasal lesions in rats and mice exposed to concentrations ≥10 ppm for 4 days (6 hours/day); a NOAEL of 2 ppm was identified (Larson et al. 1996; Templin et al. 1996b). The MRL is based on the NOAEL of 2 ppm, which was adjusted to continuous duration exposure and converted to a $NOAEL_{HEC}$ of 0.04 ppm and divided by a total uncertainty factor of 30 (3 for extrapolation from animals to humans after dosimetric adjustment and 10 for human variability). The $LOAEL_{HEC}$ value was 0.19 ppm.

Selection of the Critical Effect: No acute-duration human studies with reliable exposure estimates were identified. The most sensitive effects following acute-duration inhalation exposure were hepatic and respiratory effects (Table A-1). Changes to nasal bones and olfactory neuron loss were also observed at similar concentrations as nasal epithelial changes.

Table A-1. Selected NOAEL and LOAEL Values in Animals Following Acute-Duration Inhalation Exposure to Chloroform

		Effect level (ppm)			
Species	Duration	NOAEL	LOAEL	Effect	Reference
Hepatic effects					
B6C3F1 mouse	7 days 6 hours/day	1.2	3	18% increase in relative liver weight	Larson et al. 1994c
B6C3F1 mouse	4 days 6 hours/day	2	10	Diffuse lipid hepatocytic vacuolation, scattered hepatocyte necrosis	Larson et al. 1996
Respiratory effects					
C57BL/6 mouse	5 days 1 hour/day (20 minutes 3 times/day)	ND	7	Increased white blood cells in BALF, increased alveolar area, and decreased density of alveolar septa; decreased relative lung weight in females	de Oliveira et al. 2015
Fischer 344 rat	4 days 6 hours/day	2	10	Loss of olfactory glands; periosteal hypercellularity and proliferation; mineralization of the basal lamina; new nasal bone growth	Templin et al. 1996b

Table A-1. Selected NOAEL and LOAEL Values in Animals Following Acute-Duration Inhalation Exposure to Chloroform

		Effect level (ppm)			
Species	Duration	NOAEL	LOAEL	Effect	Reference
B6C3F1 mouse	4 days 6 hours/day	2	10	Connective tissue proliferation in the nasal lamina propria; periosteal cell proliferation in nasal cavity	Larson et al. 1996
B6C3F1 mouse	7 days 6 hours/day	3	10	Nasal periosteal cell proliferation	Mery et al. 1994
Fischer 344 rat	7 days 6 hours/day	3.1	10.4	Goblet cell hyperplasia in nasal respiratory epithelium; olfactory gland degeneration in lamina propria; periosteal proliferation and new bone formation	Larson et al. 1994c; Mery et al. 1994
Nervous system effects					
Fischer 344 rat	7 days 6 hours/day	3.1	10.4	Olfactory neuron loss	Larson et al. 1994c; Mery et al. 1994

BALF = bronchoalveolar lavage fluid; LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level

In order to identify the most sensitive POD, benchmark dose (BMD) modeling was attempted for endpoints in Table A-1 when data were amenable to modeling. The data were fit to all available continuous models in EPA's Benchmark Dose Software (BMDS; version 3.3) using a benchmark response (BMR) of 1 SD for liver weight, nasal lesion severity score, periosteal labeling index (proliferation), and nasal turbinate width. Adequate model fit was judged by four criteria: goodness-of-fit statistics (p-value >0.1), visual inspection of the dose-response curve, a 95% lower confidence limit on the BMD (BMCL) that is not 10 times lower than the lowest non-zero dose, and scaled residual within ±2 units at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest 95% lower confidence limit concentration (BMCL) was selected as the POD when the difference between the BMCLs estimated from these models was >3-fold; otherwise, the BMCL from the model with the lowest Akaike information criterion (AIC) was chosen.

The datasets used for BMD modeling are presented in Table A-2 for elevated liver weight in mice reported by Larson et al. (1994c), Table A-3 for nasal lesion severity score and periosteal proliferation in mice reported by Larson et al. (1996), Table A-4 for periosteal proliferation and width of central nasal turbinate in rats reported by Mery et al. (1994), Table A-5 for periosteal proliferation in mice reported by Mery et al. (1994), and Table A-6 for periosteal proliferation in rats reported by Templin et al. (1996b). Data for increased severity of hepatic lesions in mice were not suitable for modeling because mean severity scores were reported without a measure of variance (Larson et al. 1996). Data for pulmonary effects reported by de Oliveira et al. (2015) were not suitable for modeling because only one exposure group was included. Data for nasal epithelial lesions in rats were not suitable for modeling because incidence data were not provided and/or mean severity scores were reported without a measure of variance (Larson et al. 1994c; Mery et al. 1994; Templin et al. 1996b). ATSDR used the NOAEL/LOAEL approach for endpoints with data unsuitable for BMD modeling.

Table A-2. Relative Liver Weights in Female Mice Following Inhalation Exposure to Chloroform for 7 Days (6 Hours/Day)

	Analytical concentration (ppm)						
Endpoint[a]	0	1.2	3	10	29.5	101	288
Relative liver weight (% body weight)	5.7±0.6 (5)	6.3±0.5 (5)	6.7±0.7[b] (5)	7.0±1.1[b] (5)	7.3±0.6[b] (5)	9.5±1.7[b] (5)	10.1±1.1[b] (5)

[a]Mean±SD (number of animals).
[b]p<0.05.

BW = body weight; SD = standard deviation

Source: Larson et al. 1994c

Table A-3. Nasal Lesions and Periosteal Proliferation in Female Mice Following Inhalation Exposure to Chloroform for 4 Days (6 Hours/Day)

	Analytical concentration (ppm)					
Endpoint[a]	0	0.3	2	10	30	88
Severity score[b]	0±0 (5)	0±0 (5)	0.5±0.5 (5)	1.6±0.5[c] (5)	1.8±1.0[c] (5)	2.4±0.5[c] (5)
Nasal turbinate lamina propria labeling index	15±8 (5)	9±3 (5)	16±5 (5)	164±49[d] (5)	281±158[d] (5)	397±27[d] (5)

[a]Mean±SD (number of animals).
[b]Nasal lesions were scored according to a 1–4 score: 0 = within normal limits; 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
[c]p<0.05, as calculated for this review.
[d]p<0.05, as reported by the study authors.

SD = standard deviation

Source: Larson et al. 1996

Table A-4. Periosteal Proliferation and Endoturbinate Width in Male Rats Following Inhalation Exposure to Chloroform for 7 Days (6 Hours/Day)

	Analytical concentration (ppm)						
Endpoint[a]	0	1.5	3.1	10.4	29.3	100	271
Labelled cells in nasal turbinate							
Proximal	55±30 (5)	52±41 (5)	140±130 (5)	270±54[b] (5)	330±100[b] (5)	250±95[b] (5)	450±110[b] (5)
Central	26±15 (5)	19±13 (5)	90±13 (5)	220±80[b] (5)	200±60[b] (5)	230±110[b] (5)	340±140[b] (5)
Distal	36±19 (5)	34±19 (5)	96±19 (5)	150±69[b] (5)	120±52[b] (5)	130±47[b] (5)	220±93[b] (5)

Table A-4. Periosteal Proliferation and Endoturbinate Width in Male Rats Following Inhalation Exposure to Chloroform for 7 Days (6 Hours/Day)

	Analytical concentration (ppm)						
Endpoint[a]	0	1.5	3.1	10.4	29.3	100	271
Width of central turbinate (µm)	41±12 (5)	45±17 (5)	40±9 (5)	61±17[b] (5)	51±16[b] (5)	66±8[b] (5)	68±10[b] (5)

[a]Mean±SD (number of animals).
[b]p<0.05, as reported by the study authors.

SD = standard deviation

Source: Mery et al. 1994

Table A-5. Periosteal Proliferation in Female Mice Following Inhalation Exposure to Chloroform for 7 Days (6 Hours/Day)

	Analytical concentration (ppm)						
Endpoint[a]	0	1.2	3	10	29.5	101	288
Labelled cells in nasal turbinate							
Proximal	19±11 (5)	31±32 (5)	63±34 (5)	360±94[b] (5)	190±130[b] (5)	190±100[b] (5)	330±70[b] (5)
Distal	14±11 (5)	21±12 (5)	15±10 (5)	82±42[b] (5)	54±48[b] (5)	77±24[b] (5)	100±30[b] (5)
Ventral	31±23 (5)	95±130 (5)	110±140 (5)	310±49[b] (5)	230±140[b] (5)	260±160[b] (5)	370±130[b] (5)
Dorsal	21±13 (5)	36±69 (5)	27±14 (5)	200±11[b] (5)	120±74[b] (5)	110±140[b] (5)	220±140[b] (5)

[a]Mean±SD (number of animals).
[b]p<0.05, as reported by the study authors.

SD = standard deviation

Source: Mery et al. 1994

Table A-6. Periosteal Proliferation in Male Rats Following Inhalation Exposure to Chloroform for 4 Days (6 Hours/Day)

	Concentration (ppm)					
Endpoint[a]	0	2	10	30	90	300
Proximal turbinate labeling index	30±15 (5)	24±11 (5)	490±99[b] (5)	566±155[b] (5)	752±74[b] (5)	809±48[b] (5)

[a]Mean±SD, estimated from graphically presented data using *GrabIt!* software (number of animals).
[b]p<0.05, as reported by the study authors.

SD = standard deviation

Source: Templin et al. 1996b

Details of the modeling results for the model predictions for relative liver weight in female mice reported by Larson et al. (1994c) are in Table A-7. The frequentist, restricted, Exponential 5 model was selected based on the selection criteria outlined above. No adequate models were identified for connective tissue or periosteal cell proliferation in mice following exposure for 4 days (Larson et al. 1996) or periosteal cell proliferation in rats following exposure for 4 days (Templin et al. 1996b) or 7 days (Mery et al. 1994) because they failed to meet conventional goodness-of-fit criteria using constant or nonconstant variance. While statistical model fits were identified for increased width of the central turbinate in rats exposed for 7 days and distal turbinate labeling index in mice exposed for 7 days, inspection of the recommended and alternate models showed poor visual fit, particularly in the low-exposure region of the curves.

Table A-7. Results from Benchmark Dose (BMD) Analysis (Constant Variance) of Relative Liver Weight in Female Mice Following Inhalation Exposure to Chloroform for 7 Days (6 Hours/Day) (Larson et al. 1994c)

					Scaled residuals[c]	
Model	BMC_{1SD}[a] (ppm)	$BMCL_{1SD}$[a] (ppm)	p-Value[b]	AIC	Dose below BMC	Dose above BMC
Exponential (model 3)[d]			0.001	116.36	3.07	-0.84
Exponential (model 5)[d,e]	**16.72**	**9.89**	**0.49**	**101.74**	0.64	-0.82
Hill[f]	13.97	6.94	0.43	102.16	0.46	-1.03
Polynomial (3-degree)[f]			0.004	113.93	0.46	2.86
Polynomial (2-degree)[f]			0.004	113.93	0.46	2.86
Power			0.004	113.93	0.46	2.86
Linear			0.004	113.93	0.46	2.86

[a]BMC and BMCLs values for models that do not provide adequate fit are not included in this table.
[b]Values <0.1 fail to meet conventional χ^2 goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMC.
[d]Power restricted to ≥1.
[e]Selected model. The constant variance model provided an adequate fit. Only the Exponential 5 and Hill models provided an adequate fit to the means. BMCLs were sufficiently close (differed by <3-fold). Therefore, the model with the lowest AIC was selected (Exponential 5).
[f]Coefficients restricted to be positive.

AIC = Akaike Information Criterion; BMC = benchmark concentration (maximum likelihood estimate of the concentration associated with the selected benchmark response); BMCL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., $_{1SD}$ = exposure dose associated with a change of 1 standard deviation from the control); NA = not applicable

In order to accurately compare candidate PODs across different species and target tissues, POD values were converted into human equivalent concentrations (HECs). For systemic (hepatic) effects, exposure was not adjusted for continuous exposure because data provided by the PBPK model by Corley et al. (1990) demonstrate that the arterial blood concentration (CA) of chloroform in the mouse exposed to chloroform for 6 hours reached "periodicity" (the pattern of repeated increases and decreases in arterial blood concentration that occurs when steady state is achieved during repeated intermittent exposures) within 15 minutes following exposure (Table A-8). Therefore, adjustment from 6 hours to 24 hours is not required.

Table A-8. Corley PBPK Model for Chloroform to Simulate 6-Hour Inhalation Exposure in Mice

Time (hours)	Blood concentration (CA) (mg/L)
0.00	0.014
0.25	0.040
0.50	0.041
0.75	0.041
1.25	0.042
1.50	0.042
1.75	0.042
2.00	0.042
2.25	0.042
2.50	0.042
3.375	0.042
4.5	0.042
5.625	0.042
6.75 (post-exposure)	0.0006

Source: Corley et al. (1990) in the Scop version (courtesy of Dr. Nancy Chiu, EPA)

The NOAELs for hepatic effects in mice reported by Larson et al. (1994c, 1996) were converted into $NOAEL_{HEC}$ values using guidance from EPA (1994) on dosimetric adjustments for systemic effects using the ratio of animal:human blood gas partition coefficients. In the case of chloroform, using reported blood:air partition coefficients of 21.3 for the mouse and 7.34 for the human (Corley et al. 1990) provides a ratio of mouse: human partition coefficients >1; therefore, a default value of 1 is used to derive the $NOAEL_{HEC}$.

Larson et al. (1994c), 7-day mouse study (increased relative liver weight):

$$BMCL_{HEC} = BMCL \times \frac{mouse\ partition\ coefficient}{human\ partition\ coefficent} = 9.9\ ppm \times 1 = 9.9\ ppm$$

Larson et al. (1996), 4-day mouse study (hepatic lesions):

$$NOAEL_{HEC} = NOAEL \times \frac{mouse\ partition\ coefficient}{human\ partition\ coefficent} = 2\ ppm \times 1 = 2\ ppm$$

The candidate POD values for nasal effects were adjusted to continuous exposure because kinetic data reported by Sarangapani et al. (2002) indicate that the periodicity reported by Corley et al. (1990) is not applicable to nasal tissue exposures. Using a PBPK model, Sarangapani et al. (2002) showed a steeper external exposure-internal dose relationship for the nasal compartment compared to the hepatic compartment. This steeper dose relationship is driven by the tissue:air partition coefficient and is relatively insensitive to the blood perfusion rate or other systemic parameters. Additionally, longer-duration studies indicate increased severity of nasal lesions with increased duration of exposure, which further supports duration-adjustment for nasal effects. Since the nasal bone effects and olfactory neuron loss are presumably due to portal-of-entry effects, extrathoracic HEC calculations were applied for these endpoints as well.

For each study evaluating nasal endpoints, POD_{ADJ} values were converted to POD_{HEC} values using guidance from EPA (1994) on dosimetric adjustments for respiratory effects using the regional gas dose ratio (RGDR) for extrathoracic effects ($RGDR_{ET}$). This $RGDR_{ET}$ is calculated using the following equation as defined by EPA (1994):

$$RGDR_{ET} = \frac{V_{E\,a}}{SA_a} \div \frac{V_{E\,h}}{SA_h}$$

where:
$V_{E\,a}$ = ventilation rate for animals: male F344 rats = 0.137 L/minute; female B6C3F1 mice = 0.028 L/minute
SA_a = surface area of the extrathoracic region in rats = 15 cm^2
$V_{E\,h}$ = ventilation rate for humans = 13.8 L/minute
SA_h = surface area of the extrathoracic region in humans = 200 cm^2

Note, below, that rat and mouse have different extrathoracic RGDR values and these will be critical in calculating $NOAEL_{HEC}$ values for each endpoint.

$$Rat\text{:}\ RGDR_{ET} = \frac{V_{E\,a}}{SA_a} \div \frac{V_{E\,h}}{SA_h} = \frac{0.137}{15} \div \frac{13.8}{200} = 0.132$$

$$Mouse\text{:}\ RGDR_{ET} = \frac{V_{E\,a}}{SA_a} \div \frac{V_{E\,h}}{SA_h} = \frac{0.028}{3} \div \frac{13.8}{200} = 0.136$$

Templin et al. (1996b); 4-day study in rats (nasal lesions and bone growth, periosteal proliferation):

$$NOAEL_{ADj} = NOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 2\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{4\ days}{7\ days} = 0.3\ ppm$$

$$NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR = 0.3\ ppm \times 0.132 = 0.04\ ppm$$

Larson et al. (1996); 4-day study in mice (nasal lesions, periosteal proliferation):

$$NOAEL_{ADj} = NOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 2\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{4\ days}{7\ days} = 0.3\ ppm$$

$$NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR = 0.3\ ppm \times 0.136 = 0.04\ ppm$$

Larson et al. (1994c) and Mery et al. (1994); 7-day study in rats (nasal lesions, bone growth, periosteal proliferation; olfactory neuron loss):

$$NOAEL_{ADJ} = NOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 3.1\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{7\ days}{7\ days} = 0.78\ ppm$$

$$NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR = 0.78\ ppm \times 0.132 = 0.10\ ppm$$

Mery et al. (1994); 7-day study in mice (nasal periosteal proliferation):

$$NOAEL_{ADJ} = NOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 3\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{7\ days}{7\ days} = 0.8\ ppm$$

$$NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR = 0.8\ ppm \times 0.136 = 0.1\ ppm$$

The LOAEL value for pulmonary effects in mice reported by de Oliveira et al. (2015) was adjusted to continuous exposure because it is unknown if the periodicity reported by Corley et al. (1990) for systemic effects is applicable to pulmonary effects. The $LOAEL_{ADJ}$ was then converted to a $LOAEL_{HEC}$ using guidance from EPA (1994) on dosimetric adjustments for respiratory effects using the RGDR for pulmonary effects ($RGDR_{PU}$). The $RGDR_{PU}$ is calculated using the following equation as defined by EPA (1994):

$$RGDR_{PU} = \frac{Q_{alv_a}}{SA_a} \div \frac{Q_{alv_h}}{SA_h} = \frac{0.028}{0.05} \div \frac{13.8}{54} = 2.19$$

where:
Q_{alv_a} = alveolar ventilation rate for B6C3F1 mice = 0.028 L/minute
SA_a = surface area of the pulmonary region in mice = 0.05 m^2
Q_{alv_h} = alveolar ventilation rate for humans = 13.8 L/minute
SA_h = surface area of the pulmonary region in humans = 54 m^2

Applying this equation results in an RGDR of 2.191304 for pulmonary effects in mice, and the HEC is calculated as shown below.

de Oliveira et al. (2015); 5-day study in mice (pulmonary effects):

$$LOAEL_{ADj} = LOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 7\ ppm \times \frac{1\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 0.2\ ppm$$

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 0.2\ ppm \times 2.19 = 0.4\ ppm$$

All candidate POD_{HEC} values are summarized in Table A-9. Based on POD_{HEC} values, the lowest POD identified was for nasal effects in rats and mice, with a $NOAEL_{HEC}$ value of 0.04 ppm. Therefore, nasal effects were selected as the critical effect.

Table A-9. Summary of Candidate Effects and POD Values Considered for Derivation of an Acute-Duration Inhalation MRL for Chloroform

Species	Duration	Effect	Candidate POD (ppm)	POD type	Reference
Hepatic effects					
B6C3F1 mouse	7 days 6 hours/day	Increased relative liver weight	9.9	$BMCL_{HEC}$	Larson et al. 1994c
B6C3F1 mouse	4 days 6 hours/day	Increased severity of hepatic lesions	2	$NOAEL_{HEC}$	Larson et al. 1996

Table A-9. Summary of Candidate Effects and POD Values Considered for Derivation of an Acute-Duration Inhalation MRL for Chloroform

Species	Duration	Effect	Candidate POD (ppm)	POD type	Reference
Respiratory effects					
C57BL/6 mouse	5 days 1 hour/day (20 minutes 3 times/day)	Increased white blood cells in BALF, increased alveolar area, and decreased density of alveolar septa; decreased relative lung weight in females	0.4	$LOAEL_{HEC}$	de Oliveira et al. 2015
Fischer 344 rat	4 days 6 hours/day	Nasal epithelial lesions, periosteal proliferation, new bone growth	0.04	$NOAEL_{HEC}$	Templin et al. 1996b
B6C3F1 mouse	4 days 6 hours/day	Nasal epithelial lesions, periosteal proliferation	0.04	$NOAEL_{HEC}$	Larson et al. 1996
B6C3F1 mouse	7 days 6 hours/day	Nasal periosteal proliferation	0.1	$NOAEL_{HEC}$	Mery et al. 1994
Fischer 344 rat	7 days 6 hours/day	Nasal epithelial lesions, periosteal proliferation, new bone growth	0.10	$NOAEL_{HEC}$	Larson et al. 1994c; Mery et al. 1994
Nervous system effects					
Fischer 344 rat	7 days 6 hours/day	Olfactory neuron loss	0.10	$NOAEL_{HEC}$	Larson et al. 1994c; Mery et al. 1994

BALF = bronchoalveolar lavage fluid; BMC = benchmark concentration (maximum likelihood estimate of the concentration associated with the selected benchmark response); BMCL = 95% lower confidence limit on the BMC; HEC = human equivalent concentration; LOAEL = lowest observed adverse effect level; MRL = Minimal Risk Level; NOAEL = no-observed-adverse-effect level; POD = point of departure

Selection of the Principal Study: Templin et al. (1996b) and Larson et al. (1996) were selected as co-principal studies because they provided the lowest candidate POD (0.04 ppm) for the critical effect (nasal lesions).

Summary of the Principal Study:

Templin MV, Larson JL, Butterworth BE, et al. 1996b. A 90-day chloroform inhalation study in F-344 rats: profile of toxicity and relevance to cancer studies. Fundam Appl Toxicol 32(1):109-125.

Larson JL, Templin MV, Wolf DC, et al. 1996. A 90-day chloroform inhalation study in female and male B6C3F1 mice: implications for cancer risk assessment. Fundam Appl Toxicol 30(1):118-137. https://doi.org/10.1006/faat.1996.0049.

Templin et al. (1996b) exposed 9-week-old male F344 rats (5/sex/group) to target concentrations of 0, 2, 10, 30, 90, or 300 ppm chloroform for 4 days (6 hours/day). In all animals, bromodeoxyuridine (BrdU) was administered 3.5 days prior to sacrifice. Endpoints evaluated included clinical signs, body weight, gross necropsy, histopathology (liver, kidney, nasal cavity, non-nasal bones (sternum, rib, vertebrae, tibia, femur), and cellular proliferation (BrdU labeling index) in liver, kidney, and bone.

Average analytical exposure concentrations were always within 4.5% of the target (quantitative values not reported). No deaths were reported. Body weight gains were significantly decreased compared to control in all exposure groups. Controls gained approximately 3% during the exposure period, while rats exposed to 2, 10, 30, 90, or 300 ppm lost approximately 2, 3, 3, 5, and 14% of their initial body weight (estimated based on graphically presented data). No histopathological lesions were observed in the liver, but the BrdU labelling index showed significantly increased hepatocellular proliferation at 300 ppm. Minimal vacuolation of proximal convoluted tubules was observed in 5/5 mice at 300 ppm; no renal cell proliferation was noted. In the nasal cavity, lesions were noted in at ≥10 ppm. The lesions were primarily observed in the ethmoid portion lined with olfactory epithelium (ventral and lateral regions of turbinates), including lesions within the lamina propria characterized by edema, loss of deep Bowman's glands, periosteal hypercellularity, and new bone growth in the proximal portions of the ethmoturbinates. The severity and relative distribution of the lesions were concentration-dependent, ranging from minimal involvement in rats exposed to 10 ppm to moderate to severe effects in rats exposed to 300 ppm. Focal atrophy of the olfactory epithelium was noted in rats exposed to 90 or 300 ppm.

Larson et al. (1996) investigated the ability of chloroform vapors to produce toxicity and regenerative cell proliferation in the liver, kidneys, and nasal passages of female B6C3F1 mice. Groups of five animals were exposed to target concentrations of 0, 0.3, 2, 10, 30, or 90 ppm chloroform (via inhalation for 6 hours/day for 4 consecutive days). At necropsy, livers and kidneys were removed, weighed, examined macroscopically, and prepared for microscopic evaluation. The nasal cavities and non-nasal bones (sternum with rib, vertebrae, tibia, femur) were also removed and prepared for microscopic evaluation. Animals were administered BrdU via an implanted osmotic pump for the last 3.5 days. Cell proliferation was quantitated as the percentage of cells in S-phase (labeling index [LI]) measured by immuno-histochemical detection of BrdU-labeled nuclei.

Analytical concentrations were 0, 0.3, 1.99, 10.0, 29.6, and 88 ppm. No clinical signs of toxicity were noted in females exposed to chloroform for 4 days. Relative kidney weights were similar to controls at all chloroform exposure levels; however, exposure to 90 ppm chloroform resulted in increased relative liver weights. Female mice exposed to chloroform for 4 days experienced a dose-dependent mild response of uniform hepatocyte lipid vacuolization. Scattered hepatocyte necrosis also occurred in a dose-dependent manner. Hepatic LI was significantly elevated in female mice in the 90-ppm dose group after 4 days exposure (9-fold; $p<0.05$). Kidneys of female mice exposed to chloroform were not different from those of controls at any dose. Exposure to chloroform did not significantly affect the kidney cortex LI in females at any dose. Mild, transient changes occurred in the posterior ventral areas of nasal tissue in female mice exposed to 10, 30, and 90 ppm chloroform. The lesions were characterized by mild proliferative responses in the periosteum consisting of a thickening of this bone. The adjacent lamina also exhibited loss of acini of Bowman's glands and vascular congestion. No microscopic changes were noted in non-nasal bones, nor were non-nasal bone LIs different from those of controls.

Selection of the Point of Departure for the MRL: The NOAEL of 2 ppm based on nasal lesions in rats and mice was selected as the POD as it provides the lowest POD for the critical effect. As mentioned above, data were either unavailable for BMD modeling (Templin et al. 1996b) or failed to produce any model fits (Larson et al. 1996).

Adjustment for Intermittent Exposure and Human Equivalent Concentration: As shown above in equations after Table A-8, the NOAEL of 2 ppm was adjusted for continuous exposure and converted into a $NOAEL_{HEC}$ of 0.04 ppm. The associated LOAEL of 10 ppm was adjusted for continuous exposure and converted into a $LOAEL_{HEC}$ of 0.19 ppm as shown below.

Templin et al. (1996b); rats:

$$LOAEL_{ADj} = LOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 10\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{4\ days}{7\ days} = 1.4\ ppm$$

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 1.4\ ppm \times 0.132 = 0.19\ ppm$$

Larson et al. (1996); mice:

$$LOAEL_{ADj} = LOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 10\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{4\ days}{7\ days} = 1.4\ ppm$$

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 1.4\ ppm \times 0.136 = 0.19\ ppm$$

Uncertainty Factors: The following uncertainty factors were applied to the $NOAEL_{HEC}$ to derive the MRL:

- Uncertainty factor of 3 for extrapolation from animals to humans with dosimetric adjustments
- Uncertainty factor of 10 for human variability

Subsequently, the MRL for acute-duration exposure to chloroform via inhalation is:

$$MRL = \frac{NOAEL_{HEC}}{(UF)} = \frac{0.04\ ppm}{30} = 0.001\ ppm$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that respiratory effects are a presumed health effect following exposure to chloroform based on inadequate evidence from human epidemiology studies and a high level of evidence from animal studies (Appendix C).

Lung damage has been reported in several fatal cases of inhalation exposure (Ago et al. 2011; Featherstone 1947; Giusti and Chiarotti 1981; Harada et al. 1997; Piersol et al. 1933; Royston 1924; Schroeder 1965). Case reports have also documented changes in respiratory rate and/or respiratory arrest at high exposure levels (Jayaweera et al. 2017; Storms 1973; Whitaker and Jones 1965), but these effects are likely secondary to CNS depression. No data pertaining to potential nasal effects in humans following exposure to chloroform were identified. In animals, the nasal epithelium is a consistent target of toxicity in rodents following acute-, intermediate-, and chronic-duration inhalation exposure (Constan et al. 1999; Kasai et al. 2002; Larson et al. 1994c, 1996; Mery et al. 1994; Templin et al. 1996b; Yamamoto et al. 2002) and acute- and intermediate-duration gavage exposure (Dorman et al. 1997; Larson et al. 1995b; Templin et al. 1996a). Findings show a dose- and duration-dependent increase in the incidence and severity of lesions. The MRL based on nasal lesions is expected to be protective of lower respiratory effects, as damage to the lower respiratory tract in animals was generally only observed at lethal exposure levels (Bowman et al. 1978; Kasai et al. 2002; NCI 1976). Only minimal evidence of inflammatory responses has been reported in the lung at low inhalation exposure levels in mice (de Oliveira et al. 2015).

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MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Inhalation
Duration: Intermediate
MRL: 0.0008 ppm (0.004 mg/m^3)
Critical Effect: Nasal lesions
Reference: Templin et al. 1996b
Point of Departure: LOAEL of 2 ppm ($LOAEL_{HEC}$ of 0.07 ppm)
Uncertainty Factor: 90
LSE Graph Key: 37
Species: Rat

MRL Summary: An intermediate-duration inhalation MRL of 0.0008 ppm was derived for chloroform based on nasal lesions in rats exposed to concentrations ≥2 ppm for 13 weeks (7 days/week; 6 hours/day); a NOAEL was not identified (Templin et al. 1996b). The MRL is based on the LOAEL of 2 ppm, which was adjusted to continuous duration exposure and converted to a $LOAEL_{HEC}$ of 0.07 ppm and divided by a total uncertainty factor of 90 (3 for use of a minimal LOAEL, 3 for extrapolation from animals to humans after dosimetric adjustment, and 10 for human variability).

Selection of the Critical Effect: No intermediate-duration human studies with reliable exposure estimates were identified. The most sensitive effects following intermediate-duration inhalation exposure were respiratory effects, specifically damage to the nasal turbinates and overlying epithelial tissues (Table A-10).

Table A-10. Selected NOAEL and LOAEL Values in Animals Following Intermediate-Duration Inhalation Exposure to Chloroform

		Effect level (ppm)			
Species	Duration	NOAEL	LOAEL	Effect	Reference
Respiratory					
Fischer 344 rats	6 or 13 weeks 7 days/week 6 hours/day	ND	2	Loss of olfactory glands and edema in the nasal lamina propria; atrophy of ethmoid turbinate	Templin et al. 1996b
Fischer 344 rats	3 weeks 7 days/week 6 hours/day	2	10	Loss of olfactory glands; edema, and cellular proliferation in the nasal lamina propria	Templin et al. 1996b
BDF1 mouse	13 weeks 5 days/week 6 hours/day	ND	12	Eosinophilic change of olfactory and respiratory epithelia in females; thickening of nasal bones in both sexes	Kasai et al. 2002
Body weight					
BDF1 mouse	13 weeks 5 days/week 6 hours/day	ND	5	17% decrease in percent body weight gain	Templin et al. 1998

Table A-10. Selected NOAEL and LOAEL Values in Animals Following Intermediate-Duration Inhalation Exposure to Chloroform

Species	Duration	Effect level (ppm) NOAEL	LOAEL	Effect	Reference
Renal					
BDF1 mouse	13 weeks 5 days/week 6 hours/day	ND	12	Necrosis and cytoplasmic basophilia in the proximal tubules and proteinuria in males	Kasai et al. 2002

LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level

Selection of the Principal Study: The 13-week study in rats by Templin et al. (1996b) was selected as the principal study because it provided the lowest candidate POD for the critical effect (nasal lesions).

Summary of the Principal Study:

Templin MV, Larson JL, Butterworth BE, et al. 1996b. A 90-day chloroform inhalation study in F-344 rats: profile of toxicity and relevance to cancer studies. Fundam Appl Toxicol 32(1):109-125.

Templin et al. (1996b) exposed nine-week-old male and female F344 rats (10/sex/group) to target concentrations of 0, 2, 10, 30, 90, or 300 ppm chloroform for 13 weeks via whole-body inhalation (7 days/week; 6 hours/day). BrdU was administered 3.5 days prior to sacrifice in 8/group in control and 30-, 90-, and 300-ppm groups. Endpoints evaluated included clinical signs, body weight, organ weights (liver and kidney), gross necropsy, histopathology on a complete set of tissues, and cell proliferation in the liver, kidney, and nasal tissues.

Average analytical exposure concentrations were always within 4.5% of the target (quantitative data not reported). No deaths were reported. Rats receiving the higher concentrations of chloroform exhibited signs of mild dehydration in the second week and, at the later time points, slight hair loss, discharge from the eyes and anogenital staining (data not shown). Body weight gains were significantly decreased in males at 90 ppm (40%) and 300 ppm (9%), compared to control (54%); estimated based on graphically presented data. In females, body weight gain was significantly decreased at 10 ppm (29%), 30 ppm (30%), 90 ppm (20%), and 300 ppm (5%), compared to control (36%); estimated based on graphically presented data. Relative liver weights were increased in males at 300 ppm (~30%) and in females increased at 90 ppm (~10%) and 300 ppm (~50%). Relative kidney weights were increased at 90 ppm in males (~10%) and females (~25%) and at 300 ppm in males (~30%) and females (~50%). Organ weight findings may be secondary to body weight effects (absolute organ weights were not reported); however, the study authors noted that increased female liver weight was associated with periductal fibrosis. Hepatocellular vacuolation and hepatocyte degeneration and/or necrosis was observed in both sexes at ≥90 ppm. Foci of adenofibrosis (intestinal-crypt-like ducts with periductular fibrosis) were observed in both sexes at 300 ppm (more severe in females). Hepatocellular proliferation was observed in both sexes at 300 ppm. In the kidney, vacuolation in the proximal convoluted tubule and scattered focal necrosis were observed in males at ≥90 ppm. Females showed scattered regenerating proximal convoluted tubules with anisokaryosis and megalokaryosis. Renal cell proliferation was observed in both sexes at ≥30 ppm. Nasal lesions were observed in 100% of exposed male rats; no nasal lesions were observed in control males. The most prevalent effects were atrophy of the ethmoid turbinates, loss of Bowman's glands, and mild-to-moderate edema in the lamina propria. Mineralization of the basal lamina was observed at 300 ppm and the olfactory epithelium showed focal edema and conversion to respiratory epithelium. Lesions were minimal at 2 ppm, mild at 10 and 30 ppm, mild-to-moderate at 90 ppm, and moderate-to-severe at

300 ppm. The study authors noted that nasal lesions in female rats were "similar to those found in the male;" however, quantitative data were not provided. Nasal cellular proliferation was noted at ≥10 ppm. No other tissue was affected by chloroform exposure.

Selection of the Point of Departure for the MRL: The LOAEL of 2 ppm based on nasal lesions in male rats was selected as the POD as it provides the lowest POD for the critical effect. The data are not amenable to BMD modeling because the response in male rats goes from 0% incidence in the control group to 100% incidence in all exposure groups.

Adjustment for Intermittent Exposure: The LOAEL of 2 ppm was adjusted for continuous exposure.

$$LOAEL_{ADJ} = LOAEL \times \frac{hours/day}{24\ hours} \times \frac{days/week}{7\ days} = 2\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{7\ days}{7\ days} = 0.5\ ppm$$

Human Equivalent Concentration: Sarangapani et al. (2002) is the only published chloroform model that simulates doses to the nasal cavity tissues. The model has been validated against observations made in rats, but not other laboratory animal models or humans. ATSDR typically requires that models used for dosimetry extrapolation in derivation of MRLs be validated in the species to which they are applied. As the principal study uses mice, the Sarangapani et al. (2002) model was not used for dosimetry extrapolation in deriving the intermediate-duration inhalation MRL. Instead, the $LOAEL_{ADJ}$ was converted to a $LOAEL_{HEC}$ using guidance from EPA (1994) on dosimetric adjustments for respiratory effects using the $RGDR_{ET}$. This $RGDR_{ET}$ is calculated using the following equation as defined by EPA (1994):

$$RGDR_{ET} = \frac{V_{E_a}}{SA_a} \div \frac{V_{E_h}}{SA_h} = \frac{0.137}{15} \div \frac{13.8}{200} = 0.132$$

where:
V_{E_a} = ventilation rate for male F344 rats = 0.137 L/minute
SA_a = surface area of the extrathoracic region in rats = 15 cm^2
V_{E_h} = ventilation rate for humans = 13.8 L/minute
SA_h = surface area of the extrathoracic region in humans = 200 cm^2

Applying this equation results in an RGDR of 0.13 for extrathoracic effects in F344 rats, and the HEC is calculated as:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 0.5\ ppm \times 0.132 = 0.07\ ppm$$

Uncertainty Factors: The following uncertainty factors were applied to the $LOAEL_{HEC}$ to derive the MRL:

- Uncertainty factor of 3 for use of a minimal LOAEL (nasal lesions of minimal severity)
- Uncertainty factor of 3 for animal to human extrapolation with applying dosimetric adjustment
- Uncertainty factor of 10 for human variability

Subsequently, the inhalation MRL for intermediate-duration exposure to chloroform is:

$$MRL = \frac{LOAEL_{HEC}}{(UF)} = \frac{0.07\ ppm}{90} = 0.0008\ ppm$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that respiratory effects are a presumed health effect following exposure to chloroform based on inadequate evidence from human epidemiology studies and a high level of evidence from animal studies (Appendix C).

Lung damage has been reported in several fatal cases of inhalation exposure (Ago et al. 2011; Featherstone 1947; Giusti and Chiarotti 1981; Harada et al. 1997; Piersol et al. 1933; Royston 1924; Schroeder 1965). Case reports have also documented changes in respiratory rate and/or respiratory arrest at high exposure levels (Jayaweera et al. 2017; Storms 1973; Whitaker and Jones 1965), but these effects are likely secondary to CNS depression. No data pertaining to potential nasal effects in humans following exposure to chloroform were identified. In animals, the nasal epithelium is a consistent target of toxicity in rodents following acute-, intermediate-, and chronic-duration inhalation exposure (Constan et al. 1999; Kasai et al. 2002; Larson et al. 1994c, 1996; Mery et al. 1994; Templin et al. 1996b; Yamamoto et al. 2002) and acute- and intermediate-duration gavage exposure (Dorman et al. 1997; Larson et al. 1995b; Templin et al. 1996a). Findings show a dose- and duration-dependent increase in the incidence and severity of lesions.

Agency Contact (Chemical Manager): Rae T. Benedict, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Inhalation
Duration: Chronic
MRL: 0.0004 ppm (0.002 mg/m^3)
Critical Effect: Nasal lesions
Reference: Yamamoto et al. 2002
Point of Departure: LOAEL of 5.0 ppm ($LOAEL_{HEC}$ of 0.11 ppm)
Uncertainty Factor: 300
LSE Graph Key: 52
Species: Mouse

MRL Summary: A chronic-duration inhalation MRL of 0.0004 ppm was derived for chloroform based on nasal lesions in female mice exposed to concentrations ≥5 ppm for 104 weeks (5 days/week; 6 hours/day); a NOAEL was not identified (Yamamoto et al. 2002). The MRL is based on the LOAEL of 5.0 ppm, which was adjusted to continuous duration exposure and converted to a $LOAEL_{HEC}$ of 0.11 ppm and divided by a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans after dosimetric adjustment, and 10 for human variability).

Selection of the Critical Effect: Both human and animal data were considered while determining the critical effects (Table A-11). The only chronic-duration human study with dose-response data is an occupational study by Li et al. (1993). A LOAEL of 2.76 ppm was identified for workers occupationally exposed to chloroform for 1–15 years based on impaired performance on the pursuit aiming task, indicating impaired hand-eye coordination. In animal studies, the most sensitive target of toxicity was nasal effects in mice at ≥5 ppm.

Table A-11. Selected NOAEL and LOAEL Values in Humans and Animals Following Chronic-Duration Inhalation Exposure to Chloroform

Species	Duration	Effect level (ppm) NOAEL	LOAEL	Effect	Reference
Neurological					
Human	1–15 years 5 days/week[a] 8 hours/day[a]	ND	2.76	Impaired hand-eye coordination	Li et al. 1993
Respiratory					
BDF1 mouse	104 weeks 5 days/week 6 hours/day	ND	5.0	Atrophy and respiratory metaplasia of the olfactory epithelium; thickening of nasal bones	Yamamoto et al. 2002
Fischer 344 rat	104 weeks 5 days/week 6 hours/day	ND	10.1	Atrophy and respiratory metaplasia of the olfactory epithelium; thickening of nasal bones	Yamamoto et al. 2002

Table A-11. Selected NOAEL and LOAEL Values in Humans and Animals Following Chronic-Duration Inhalation Exposure to Chloroform

Species	Duration	Effect level (ppm) NOAEL	LOAEL	Effect	Reference
Renal					
Fischer 344 rat	104 weeks 5 days/week 6 hours/day	10.1	30.0	Nuclear enlargement of the proximal tubules and dilation of the tubular lumen	Yamamoto et al. 2002

[a]Assuming a 40-day work week.

LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level

In order to accurately compare PODs across study designs, species, and target tissues, candidate PODs were adjusted for continuous exposure in both studies, and a HEC value was calculated for the nasal effects in mice.

Li et al. (1993); human (neurological effects):

$$LOAEL_{ADj} = LOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 2.76\ ppm \times \frac{8\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 0.657\ ppm$$

Yamamoto et al. (2002); mouse (nasal effects):

The nasal LOAEL was adjusted for continuous exposure.

$$LOAEL_{ADj} = LOAEL \times \frac{\text{hours/day}}{24\ hours} \times \frac{\text{days/week}}{7\ days} = 5.0\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 0.89\ ppm$$

The $LOAEL_{ADJ}$ was converted to a $LOAEL_{HEC}$ using guidance from EPA (1994) on dosimetric adjustments for respiratory effects using the $RGDR_{ET}$. This $RGDR_{ET}$ is calculated using the following equation as defined by EPA (1994):

$$RGDR_{ET} = \frac{V_{Ea}}{SA_a} \div \frac{V_{Eh}}{SA_h} = \frac{0.0245}{3} \div \frac{13.8}{200} = 0.118$$

where:
V_{E_a} = ventilation rate for female BDF1 mice = 0.0245 L/minute (Yamamoto et al. 2002)
SA_a = surface area of the extrathoracic region in mice = 3 cm^2 (EPA 1994)
V_{E_h} = ventilation rate for humans = 13.8 L/minute (EPA 1994)
SA_h = surface area of the extrathoracic region in humans = 200 cm^2 (EPA 1994)

Applying this equation results in an RGDR of 0.118 for extrathoracic effects in female BDF1 mice, and the HEC is calculated as shown below.

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 0.89\ ppm \times 0.118 = 0.11\ ppm$$

The candidate human and animal chronic-duration inhalation PODs are summarized in Table A-12.

Table A-12. Summary of Candidate Effects and POD Values Considered for Derivation of a Chronic-Duration Inhalation MRL for Chloroform

Species	Duration	Effect	Candidate POD (ppm)	POD type	Reference
Neurological effects					
Human	1–15 years 5 days/week[a] 8 hours/day[a]	Impaired hand-eye coordination	0.657	$LOAEL_{ADJ}$	Li et al. 1993
Respiratory effects					
BDF1 mouse	104 weeks 5 days/week 6 hours/day	Atrophy and respiratory metaplasia of the olfactory epithelium; thickening of nasal bones	0.11	$LOAEL_{HEC}$	Yamamoto et al. 2002

[a]Assuming a 40-hour work week.

ADJ = adjusted; HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; MRL = Minimal Risk Level; POD = point of departure

Based on values in Table A-12, nasal lesions were selected as the critical effect because they provide the lowest candidate POD. Additionally, there is clear evidence of concentration- and duration-dependent increases in incidence and/or severity of nasal lesions in acute-, intermediate-, and chronic-duration animal studies. While selecting neurological effects from the human study would decrease uncertainty with regard to animal to human extrapolation, there are considerable uncertainties associated with the study by Li et al. (1993), including: 1) limited information regarding methods and timing of exposure assessment; 2) limited information regarding controls (identified only as individuals "without obvious exposure to occupational hazards"; 3) no information on potential concurrent exposures to other solvents or potentially neurotoxic compounds; and 4) relatively small group numbers, especially at the LOAEL (60 control, 14 low-exposure [2.76 ppm], 46 high-exposure [6.04 ppm]). Based on these limitations, systematic review determined that the Li et al. (1993) is a second-tier risk of bias study of low confidence (Appendix C).

Selection of the Principal Study: The 104-week study in mice by Yamamoto et al. (2002) was selected as the principal study because it provided the lowest candidate POD (0.11 ppm) for the critical effect (nasal lesions).

Summary of the Principal Study:

Yamamoto S, Kasai T, Matsumoto, et al. 2002. Carcinogenicity and chronic toxicity in rats and mice exposed to chloroform by inhalation. J Occup Health 44(5):283-293. https://doi.org/10.1539/joh.44.283.

Six-week-old male and female Crj:BDF1 mice (50/sex/group) were exposed to 0, 5, 30, or 90 ppm chloroform via whole-body inhalation for 6 hours/day, 5 days/week, for 104 weeks. Analytical concentrations were reported as 5.0, 10.1, 30.0, and 90.1 ppm. To avoid lethality, the 30- and 90-ppm exposure groups underwent stepwise exposure paradigms over the first 4–6 weeks. Time weighted averages (TWAs) were calculated from the analytical concentrations and exposure duration (2 weeks at 5.0 ppm, 2 weeks at 10.1 ppm, and 100 weeks at 30.0 ppm for the 30-ppm group; 2 weeks at 5.0 ppm,

2 weeks at 10.1 ppm, 2 weeks at 30.0 ppm, and 98 weeks at 90.1 ppm for the 90-ppm group), resulting in final TWA exposure concentrations of 0, 5.0, 29.1, and 85.8 ppm. Endpoints evaluated included lethality, clinical signs, body weight, food and water intake, hematology, blood chemistry, urinalysis, organ weights, gross necropsy, and histopathology. A complete set of tissues were examined.

Chloroform exposure did not affect survival rate (50–76%) or lead to any overt clinical signs of toxicity compared to control (using the stepwise protocol). Body weight was significantly decreased in males and females at all doses throughout the first year of the study, but subsequently recovered to control levels in the two lower dose female groups. The magnitude of decrease is unknown (data not reported). Food consumption was similar between exposed and control mice. No significant changes in hematological parameters were observed (data not shown). Serum chemistry changes included significant increases in serum AST, ALT, and BUN in males and females at 85.8 ppm. Serum ALP was also increased in males. No difference was seen in the urinalysis. Absolute, but not relative, kidney weight was significantly increased in males at 85.8 ppm (data not shown; attributed to tumors). No other organ weight data were reported. Gross examination showed increased incidences of renal nodules in males at 29.1 and 85.8 ppm, but not in the females (data not shown). Microscopic changes included significant increases in fatty change in the liver of males and females at 85.8 ppm and lesions in the renal proximal tubule (nuclear enlargement, cytoplasmic basophilia, hyperplasia) in males at ≥29.1 ppm. Kidney damage in females was markedly lower than in males, with the only change being a slight significant increase in cytoplasmic basophilia at 85.8 ppm. In the nasal cavity, thickening of bone was noted in both sexes at ≥5.0 ppm exposure with atrophy and respiratory metaplasia of the olfactory epithelium occurring in males at 85.8 ppm and in females at ≥5.0 ppm. In males, significant increases were seen in the incidence of renal adenoma or carcinoma (combined) at 29.1 ppm (7/50) and 85.8 ppm (12/48) and renal carcinoma at 85.8 ppm (11/48) compared to control (a significant positive trend for these tumors was noted). No renal tumors occurred in control males or female mice of any group. Incidence of liver tumors was not increased in any exposure group, although significant positive trends were found for hepatocellular adenoma or carcinoma (combined) and carcinoma in both males and females. No nonneoplastic or neoplastic lesions were increased in other organs.

Selection of the Point of Departure for the MRL: The LOAEL of 5.0 ppm for nasal lesions in mice was selected as it provided the lowest POD for the critical effect. The principal study only provided incidence data for neoplastic lesions; however, incidence data were available in unpublished Japanese-language reports with English tables (MHLW 1994b). Data for nasal lesions could not be BMD modeled because incidence went from 0% in controls to 100% at the lowest concentration.

Adjustment for Intermittent Exposure and Human Equivalent Concentration: Sarangapani et al. (2002) is the only published chloroform model that simulates doses to the nasal cavity tissues. The model has been validated against observations made in rats, but not other laboratory animal models or humans. ATSDR typically requires that models used for dosimetry extrapolation in derivation of MRLs be validated in the species to which they are applied. As the principal study uses mice, the Sarangapani et al. (2002) model was not used for dosimetry extrapolation in deriving the chronic-duration inhalation MRL. Therefore, the LOAEL was converted to a $LOAEL_{HEC}$ using guidance from EPA (1994) on dosimetric adjustments for respiratory effects using the $RGDR_{ET}$, as shown in the equations after Table A-11. The LOAEL of 5.0 ppm was adjusted for continuous exposure and converted into a $LOAEL_{HEC}$ of 0.11 ppm.

Uncertainty Factors: The following uncertainty factors were then applied to the $LOAEL_{HEC}$ to derive the MRL.

- 10 for use of a LOAEL
- 3 for extrapolation from animals to humans with dosimetric adjustments

- 10 for human variability

Subsequently, the inhalation MRL for chronic-duration exposure to chloroform is:

$$MRL = \frac{LOAEL_{HEC}}{UFs} = \frac{0.11\ ppm}{300} = 0.00037\ ppm\ \approx 0.0004\ ppm$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that respiratory effects are a presumed health effect following exposure to chloroform based on inadequate evidence from human epidemiology studies and a high level of evidence from animal studies (Appendix C).

Lung damage has been reported in several fatal cases of inhalation exposure (Ago et al. 2011; Featherstone 1947; Giusti and Chiarotti 1981; Harada et al. 1997; Piersol et al. 1933; Royston 1924; Schroeder 1965). Case reports have also documented changes in respiratory rate and/or respiratory arrest at high exposure levels (Jayaweera et al. 2017; Storms 1973; Whitaker and Jones 1965), but these effects are likely secondary to CNS depression. In animals, the nasal epithelium is a consistent target of toxicity in rodents following acute-, intermediate-, and chronic-duration inhalation exposure (Constan et al. 1999; Kasai et al. 2002; Larson et al. 1994c, 1996; Mery et al. 1994; Templin et al. 1996b; Yamamoto et al. 2002) and acute- and intermediate-duration gavage exposure (Dorman et al. 1997; Larson et al. 1995b; Templin et al. 1996a). Findings show a dose- and duration-dependent increase in the incidence and severity of lesions.

Agency Contact (Chemical Manager): Rae T. Benedict, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Oral
Duration: Acute
MRL: 0.3 mg/kg/day
Critical Effect: Hepatotoxicity (hepatic lesions)
Reference: Larson et al. 1994b
Point of Departure: NOAEL of 26 mg/kg/day
Uncertainty Factor: 100
LSE Graph Key: 33
Species: Mouse

MRL Summary: An acute-duration oral MRL of 0.3 mg/kg/day was derived for chloroform based on hepatic effects (centrilobular hepatocyte eosinophilic cytoplasm) in B6C3F1 mice following exposure to chloroform in drinking water for 4 days (Larson et al. 1994b). The MRL is based on a NOAEL of 26 mg/kg/day, which was divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Selection of the Critical Effect: No adequate acute-duration human data were available. Experimental acute-duration oral data in animals clearly show that rodents are more susceptible to chloroform toxicity via gavage exposure than drinking water exposure. The lowest acute-duration LOAELs identified in rats and mice via gavage exposure range from 10 to 34 mg/kg/day for respiratory, hepatic, renal, neurological, and developmental effects (Table A-13). In contrast, the lowest acute-duration LOAELs identified in rats and mice exposed via drinking water range from 53 to 81 mg/kg/day for hepatic effects and decreased body weights (Table A-14).

Table A-13. Selected NOAEL and LOAEL Values in Animals Following Acute-Duration Gavage Exposure to Chloroform

Species	Duration	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Renal					
Osborne-Mendel rat	Once	ND	10	Regenerative cell proliferation in the epithelial cells of the proximal tubules of the renal cortex	Templin et al. 1996a
Fischer 344 rat	4 days	10	34	Mild-to-moderate degeneration of renal proximal tubules and tubule epithelial cell proliferation	Larson et al. 1993
Fischer 344 rat	Once	ND	34	Scattered necrosis of the renal proximal tubule	Larson et al. 1993
B6C3F1 mouse	4 days	ND	34	Extensive acute necrosis of the proximal convoluted tubule, regenerative cell proliferation in the renal cortex and medulla	Larson et al. 1994d

Table A-13. Selected NOAEL and LOAEL Values in Animals Following Acute-Duration Gavage Exposure to Chloroform

Species	Duration	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Developmental					
Dutch belted rabbit	13 days GDs 6–18	ND	20	8% decrease in fetal body weight, delayed ossification	Thompson et al. 1974
Neurological					
CD-1 mouse	10 days	10	30	Conditioned taste aversion to saccharin	Landauer et al. 1982
Hepatic					
Fischer 344 rat	4 days	10	34	Increased relative liver weight	Larson et al. 1993
Respiratory					
Fischer 344 rat	4 days	ND	34	Degeneration of the olfactory epithelium and superficial Bowman's glands; periosteal hypercellularity	Larson et al. 1995b

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level

Table A-14. Selected NOAEL and LOAEL Values in Animals Following Acute-Duration Drinking Water Exposure to Chloroform

Species	Duration	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Renal					
Fischer 344 rat	Drinking water	68.1	ND		Larson et al. 1995a
B6C3F1 mouse	Drinking water	105	ND		Larson et al. 1994b
Hepatic					
B6C3F1 mouse	Drinking water	26	53	Centrilobular hepatocyte eosinophilic cytoplasm	Larson et al. 1994b
Fischer 344 rat	Drinking water	68.1	ND		Larson et al. 1995a
Body weight					
Fischer 344 rat	Drinking water	33.2	57.5	17% decrease in body weight gain	Larson et al. 1995a
B6C3F1 mouse	Drinking water	53	81	20% body weight loss	Larson et al. 1994b

LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level

Increased toxicity in rodents following acute-duration gavage exposure, compared to drinking water, is likely due to saturation of detoxification pathways following bolus gavage exposure, which exacerbates toxicity due to accumulation of toxic metabolites in hepatic and renal tissues. Specifically, it is proposed that the reaction of chloroform metabolites with GSH acts as a detoxifying mechanism. This is supported by observations that chloroform doses that caused liver GSH depletion produced liver necrosis (Docks and Krishna 1976). Additionally, exposure to chloroform via drinking water over the course of the day, rather than in a single bolus dose, may result in adaptive mechanisms. 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). No literature was identified indicating similar adaptive changes regarding detoxification capacity following gavage exposure. Considering these chloroform-specific data regarding differential toxicity and toxicokinetics via gavage versus drinking water exposure, basing an oral MRL on the most sensitive endpoint following gavage exposure (renal toxicity) may be overly conservative and not applicable to lower, environmentally relevant exposure levels. Based on this rationale, findings from drinking water studies are considered more relevant to environmental exposure levels and scenarios. The most sensitive effects following drinking water exposure are hepatic effects in mice at 53 mg/kg/day (Table A-14). Therefore, hepatic effects are selected as the critical effect for derivation of the acute-duration oral MRL.

Selection of the Principal Study: The 4-day study in mice by Larson et al. (1994b) is selected as the principal study because it provides the highest NOAEL below the lowest LOAEL for the critical effect (hepatotoxicity).

Summary of the Principal Study:

Larson JL, Wolf DC, Butterworth BE. 1994b. Induced cytotoxicity and cell proliferation in the hepatocarcinogenicity of chloroform in female B6C3F1 mice: comparison of administration by gavage in corn oil vs ad libitum in drinking water. Fund Appl Toxicol 22:90-102.

Groups of female B6C3F1 mice (14/group) were exposed to chloroform at drinking water concentrations of 0, 60, 200, 400, 900, or 1,800 ppm for 4 days. Mice were housed individually so accurate dose calculations could be made based on individual water consumption data. Body weights were recorded. After the 4-day exposure period, mice were sacrificed, and all animals were examined for macroscopic changes in the liver and kidney prior to being divided into three groups for analysis. Group 1 (five animals per group) was evaluated for serum clinical chemistry (ALT, SDH), liver and kidney weight, and liver and kidney histology. Group 2 (four animals per group) was evaluated for kidney histology. Group 3 (five animals per group) was evaluated for cellular proliferation (via BrdU labelling) in the liver and kidney.

Based on measured water intake and body weights, the study authors calculated average chloroform intakes of 0, 16.0, 26.4, 53.5, 80.9, and 105 mg/kg/day at 0, 60, 200, 400, 900, and 1,800 ppm, respectively. Dose-related decreases in body weights and water intake were observed, with an approximate 20% body weight loss during the exposure period at ≥900 ppm (data presented graphically). At necropsy, no exposure-related changes in serum ALT or SDH, gross pathology, or liver or kidney weights were observed. The study authors reported tinctorial changes, characterized by pale cytoplasmic eosinophilic staining of centrilobular hepatocytes, in 2/5, 8/10, and 4/5 mice, respectively; it is noted that the methods section indicates that only five per group were evaluated for liver histology. Liver histology at ≤200 ppm was reportedly not different from control (incidence data not reported). No exposure-related histopathological changes were noted in the kidney. Chloroform exposure via drinking water did not induce cell proliferation in either the liver or kidney.

Selection of the Point of Departure for the MRL: The NOAEL of 26 mg/kg/day for hepatic effects in the study by Larson et al. (1994b) was selected as the POD. While the study authors reported incidence data at ≥400 ppm (53.5 mg/kg/day), incidence data for ≤200 ppm (≤26 mg/kg/day) were not provided; therefore, BMD modeling was not used to derive this MRL.

Calculations: None. Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al. (1990) and Reitz et al. (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Both studies relied on data from studies of a single gavage dose (or in the case of humans, gelatin capsule dosing) of chloroform in oil-based vehicles. The models have not been validated for simulating dosimetry of repeated continuous exposures, such as daily ingestion of chloroform in drinking water. Application of either model to dosimetry extrapolation in the derivation of the acute MRL would be highly uncertain. The major uncertainty would be in extrapolating the internal doses from delivery of a large bolus dose to the liver from an oil gavage dose to the internal dose expected for repeated ingestion of chloroform in water. This extrapolation has not been validated. Therefore, the models were not used for dosimetry extrapolation in deriving the acute-duration MRL.

Uncertainty Factors: The following uncertainty factors were applied to the NOAEL to derive the MRL:

- Uncertainty factor of 10 for extrapolation from animals to humans
- Uncertainty factor of 10 for human variability

Subsequently, the oral MRL for acute-duration exposure to chloroform is:

$$Provisional\ MRL = \frac{NOAEL}{(UF)} = \frac{26\ mg/kg/day}{100} = 0.26\ mg/kg/day \approx 0.3\ mg/kg/day$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that hepatic effects are a known health effect following exposure 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 (Appendix C).

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 (Section 2.9). In fatal ingestion cases, acute liver failure and/or severe liver damage have been found at autopsy (Dettling et al. 2016; Piersol et al. 1933). In nonfatal cases of oral ingestion, clinical signs of hepatotoxicity manifested within 1–7 days of exposure (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). Impaired liver function was observed in a man who ingested 21 mg/kg/day chloroform in a cough medicine for 10 years (Wallace 1950).

In animal studies, the liver is also a clear target of toxicity 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 (Section 2.9). Findings in rodents following gavage exposure range from changes in clinical chemistry and mild histopathological damage after lower, shorter exposures (e.g., lipid accumulation, cellular swelling and vacuolation, scattered necrosis, hepatocellular proliferation), with widespread and severe necrosis and degeneration with higher and/or longer durations. As discussed above, the rodent liver is less susceptible to toxicity following drinking water exposure, presumably due to saturation of metabolic detoxification pathways with bolus gavage exposure.

Agency Contact (Chemical Manager): Rae T. Benedict, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Oral
Duration: Intermediate
MRL: 0.1 mg/kg/day
Critical Effect: Hepatotoxicity (increased serum ALT)
Reference: Heywood et al. 1979
Point of Departure: NOAEL of 15 mg/kg/day ($NOAEL_{ADJ}$ of 13 mg/kg/day)
Uncertainty Factor: 100
LSE Graph Key: 74
Species: Dog

MRL Summary: An intermediate-duration oral MRL of 0.1 mg/kg/day was derived for chloroform based on hepatic effects (~2-fold increase in serum ALT) in Beagle dogs following exposure to chloroform for 26–52 weeks (6 days/week) via toothpaste capsule (Heywood et al. 1979). The MRL is based on a NOAEL of 15 mg/kg/day, which was adjusted to a continuous duration dose ($NOAEL_{ADJ}$) of 13 mg/kg/day and divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Selection of the Critical Effect: No adequate intermediate-duration oral studies in humans were identified. As discussed in the acute-duration oral MRL worksheet above, rodents are more susceptible to chloroform toxicity via gavage exposure than drinking water exposure following intermediate-duration oral exposure. This pattern is clearly shown in a series of 21-day studies in rats and mice by Larson et al. (1994b, 1995a; Table A-15).

Table A-15. Comparison of Toxicity in Rodents Following a 21-Day Exposure to Chloroform via Gavage versus Drinking Water Exposure

Species	Route	Effect level (mg/kg/day) NOAEL	Effect level (mg/kg/day) LOAEL	Effect	Reference
Hepatic					
Fischer 344 rat	Gavage in oil	34	100	Increased hepatocellular proliferation	Larson et al. 1995a
Fischer 344 rat	Drinking water	106	ND		
B6C3F1 mouse	Gavage in oil	10	34	Mild vacuolation of hepatocytes, increased serum ALT and SDH	Larson et al. 1994b
B6C3F1 mouse	Drinking water	43	82	Increased relative liver weight	

Table A-15. Comparison of Toxicity in Rodents Following a 21-Day Exposure to Chloroform via Gavage versus Drinking Water Exposure

Species	Route	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Renal					
Fischer 344 rat	Gavage in oil	34	100	Increased proliferation of proximal tubule epithelial cells in renal cortex	Larson et al. 1995a
Fischer 344 rat	Drinking water	106	ND		

ALT = alanine aminotransferase; LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level; SDH = sorbitol dehydrogenase

Based on the rationale discussed in the acute-duration oral MRL worksheet above, gavage studies in rodents were not considered for intermediate-duration oral MRL derivation. The most sensitive effects in drinking water studies in rodents and oral exposure studies in other species are hepatic effects in dogs at ≥30 mg/kg/day and renal and gastrointestinal effects in Eker rats at ≥27 mg/kg/day (Table A-16). Findings in Eker rats is not considered an appropriate basis for the MRL since it is an animal model of hereditary renal cancer (McDorman et al. 2003a). Additionally, no additional drinking water studies in rats or mice report adverse renal or gastrointestinal effects (Table A-16). Therefore, hepatic effects are selected as the critical effect for derivation of the intermediate-duration oral MRL.

Table A-16. Selected NOAEL and LOAEL Values in Animals Following Intermediate-Duration Oral Exposure to Chloroform

Species	Duration (route)	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Hepatic effects					
Beagle dog	26–52 weeks (C) 6 days/week	15	30	~2-fold increase in serum ALT	Heywood et al. 1979
B6C3F1 mouse	3 weeks (W)	43	82	Increased relative liver weight	Larson et al. 1994b
Fischer 344 rat	3 weeks (W)	106	ND		Larson et al. 1995a
B6C3F1 mouse	90 days (W)	145	290	Increased fat content of the liver; centrilobular fatty changes	EPA 1980
Osborne-Mendel rat	90 days (W)	160	ND		EPA 1980
Fischer 344 rat	28 or 90 days (W)	200	ND		Chu et al. 1982a, 1982b

Table A-16. Selected NOAEL and LOAEL Values in Animals Following Intermediate-Duration Oral Exposure to Chloroform

Species	Duration (route)	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Renal effects					
Eker[a] rat	10 months (W)	ND	27	Increased incidence of atypical tubules and hyperplasia	McDorman et al. 2003a
Fischer 344 rat	3 weeks (W)	106	ND		Larson et al. 1995a
Osborne-Mendel rat	90 days (W)	160	ND		EPA 1980
Fischer 344 rat	28 or 90 days (W)	200	ND		Chu et al. 1982a, 1982b
B6C3F1 mouse	3 weeks (W)	329	ND		Larson et al. 1994b
B6C3F1 mouse	90 days (W)	435	ND		EPA 1980
Gastrointestinal effects					
Eker[a] rat	10 months (W)	ND	27	Increased incidence of aberrant crypt foci in the colon	McDorman et al. 2003a
Fischer 344 rat	13 weeks (W)	34	ND		DeAngelo et al. 2002
Fischer 344 rat	26 weeks (W)	35	ND		Geter et al. 2004b

[a]Animal model of hereditary renal cancer.

ALT = alanine aminotransaminase; (C) = capsule; LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level; (W) = drinking water

Selection of the Principal Study: The 26–52-week study in dogs by Heywood et al. (1979) is selected as the principal study because it provides the highest NOAEL below the lowest LOAEL for the critical effect (hepatotoxicity). In the study by Heywood et al. (1979), dose delivery was via toothpaste-containing gelatin capsule. This route of exposure is not expected to mimic the bolus dose conditions of gavage administration. The capsule will disintegrate over time, resulting in a slower release of contents compared to bolus administration; thus, this mode of administration was considered to be relevant to human exposure conditions.

Summary of the Principal Study:

Heywood R, Sortwell RJ, Noel PRB, et al. 1979. Safety evaluation of toothpaste containing chloroform. III. Long-term study in beagle dogs. J Environ Pathol Toxicol 2:835-851.

In order to assess safety of toothpaste containing chloroform, groups of male and female Beagle dogs (8/sex/group) were exposed to chloroform in toothpaste-containing capsules at doses of 15 or 30 mg/kg/day for 6 days/week for up to 7.5 years. Control groups included untreated controls (8/sex),

vehicle (capsule) controls (16/sex), and an alternative non-chloroform toothpaste control (8/sex). During the intermediate-phase of the study (<1 year), blood was collected to measure hematology and clinical chemistry parameters at 6 and 13 weeks of exposure and at intervals of 8–32 weeks thereafter. Body weight, food intake, water intake, and clinical signs were monitored throughout the exposure period.

No dogs died during the first year of the study. No clinical signs of toxicity or body weight effects were observed. Serum ALT was significantly increased in males and females exposed to 30 mg/kg/day beginning at 6 weeks and at every interval thereafter. The observed increase was approximately 2-fold starting on week 26. ALT activity was not increased in dogs exposed to 15 mg/kg/day group until week 130. Therefore, 15 mg/kg/day is considered a NOAEL for intermediate-duration exposure. No additional changes in serum clinical chemistry or hematology were noted.

Selection of the Point of Departure for the MRL: The NOAEL of 15 mg/kg/day for hepatic effects in the study by Heywood et al. (1979) was selected as the POD. While study authors reported mean ALT activity values and results of the statistical analysis, a measure of variance was not provided; therefore, BMD modeling could not be used to derive this MRL.

Calculations: The NOAEL of 15 mg/kg/day was adjusted for a daily exposure scenario:

$$NOAEL_{ADJ} = NOAEL \times \frac{days\ exposed}{7\ days} = 15\ mg/kg/day \times \frac{6\ days}{7\ days} = 13\ mg/kg/day$$

Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al. (1990) and Reitz et al. (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Neither of these studies evaluated dogs and are therefore not suitable for dose extrapolation.

Uncertainty Factors: The following uncertainty factors were applied to the $NOAEL_{ADJ}$ to derive the MRL:

- Uncertainty factor of 10 for extrapolation from animals to humans
- Uncertainty factor of 10 for human variability

Subsequently, the oral MRL for intermediate-duration exposure to chloroform is:

$$MRL = \frac{NOAEL_{ADJ}}{(UF)} = \frac{13\ mg/kg/day}{100} = 0.13\ mg/kg/day \approx 0.1\ mg/kg/day$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that hepatic effects are a known health effect following exposure 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 (Appendix C).

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 (Section 2.9). In fatal ingestion cases, acute liver failure and/or severe liver damage have been found at autopsy (Piersol et al. 1933; Dettling et al. 2016). In nonfatal cases of oral ingestion, clinical signs of hepatotoxicity manifest within 1–7 days of exposure (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). Impaired liver function was observed in a man who ingested 21 mg/kg/day chloroform in a cough medicine for 10 years (Wallace 1950).

In animal studies, the liver is also a clear target of toxicity 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 (Section 2.9). Findings in rodents following oral gavage exposure range from changes in clinical chemistry and mild histopathological damage after lower, shorter exposures (e.g., lipid accumulation, cellular swelling and vacuolation, scattered necrosis, hepatocellular proliferation), with widespread and severe necrosis and degeneration with higher and/or longer exposure durations. As discussed above, the rodent liver is less susceptible to toxicity following drinking water exposure, presumably due to saturation of metabolic detoxification pathways with bolus gavage exposure.

Agency Contact (Chemical Manager): Rae T. Benedict, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Chloroform
CAS Numbers: 67-66-3
Date: October 2024
Profile Status: Final
Route: Oral
Duration: Chronic
MRL: 0.02 mg/kg/day
Critical Effect: Hepatotoxicity (moderate-to-marked fatty cysts)
Reference: Heywood et al. 1979
Point of Departure: $BMDL_{10}$ of 2.15 mg/kg/day ($BMDL_{ADJ}$ of 1.84 mg/kg/day)
Uncertainty Factor: 100
LSE Graph Key: 84
Species: Dog

MRL Summary: A chronic-duration oral MRL of 0.02 mg/kg/day was derived for chloroform based on hepatic effects (moderate-to-marked fatty cysts) in Beagle dogs following exposure to chloroform for up to 7.5 years (6 days/week) via toothpaste capsule (Heywood et al. 1979). The MRL is based on a $BMDL_{10}$ of 2.15 mg/kg/day in male dogs, which was adjusted to a continuous duration dose ($BMDL_{ADJ}$) of 1.84 mg/kg/day and divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Selection of the Critical Effect: No adequate chronic-duration oral studies in humans were identified. The most sensitive chronic-duration oral LOAELs are shown in Table A-17. In contrast to findings in acute- and intermediate-duration studies, a clear increase in susceptibility was not observed in rodents exposed via gavage, compared to those exposed via drinking water (i.e., comparable lowest LOAEL values following chronic exposure). Several factors may contribute to this finding, including: (1) adaptive metabolic changes with chronic-duration exposure leading to blunting or attenuation of bolus effects; (2) lack of evaluation at low gavage doses in some studies (which may have potentially identified lower LOAELs); and/or (3) evaluation of different strains in chronic versus shorter-duration studies that may have differential susceptibility. However, dogs are more sensitive than rodents, regardless of oral exposure methodology. Therefore, the most sensitive endpoint in dogs (hepatotoxicity) is selected as the critical effect for derivation of the intermediate-duration oral MRL.

Table A-17. Selected NOAEL and LOAEL Values in Animals Following Chronic-Duration Oral Exposure to Chloroform

Species	Duration (route)	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
Hepatic					
Beagle dog	7.5 years (C)	ND	15	Moderate-to-marked fatty cysts; ~2-fold increase in serum ALT	Heywood et al. 1979
ICI mouse	80 weeks (GO)	60	ND		Roe et al. 1979
Osborne-Mendel rat	78 weeks (GO)	100	200	Necrosis of hepatic parenchyma	NCI 1976

Table A-17. Selected NOAEL and LOAEL Values in Animals Following Chronic-Duration Oral Exposure to Chloroform

Species	Duration (route)	Effect level (mg/kg/day) NOAEL	LOAEL	Effect	Reference
B6C3F1 mouse	78 weeks (GO)	ND	M: 138 F: 238	Nodular hyperplasia	NCI 1976
Renal					
Beagle dog	7.5 years (C)	15	30	Fat deposition in glomeruli	Heywood et al. 1979
Fischer 344 rat	104 weeks (W)	ND	45	Increased incidences of cytoplasmic basophilia and tubular lumen dilation in the proximal tubule	Nagano et al. 2006
ICI mouse	80 weeks (GO)	F: 60	M: 60	Moderate-to-severe kidney disease	Roe et al. 1979
Osborne-Mendel rat	104 weeks (W)	38	81	Renal tubule cell alterations	Hard et al. 2000; Jorgenson et al. 1985
Osborne-Mendel rat	78 weeks (GO)	200	ND		NCI 1976
B6C3F1 mouse	78 weeks (GO)	M: 277 F: 477	ND		NCI 1976

ALT = alanine aminotransaminase; (C) = capsule; F = females; (GO) = gavage in oil; LOAEL = lowest-observed-adverse-effect level; M = males; ND = not determined; NOAEL = no-observed-adverse-effect level; (W) = drinking water

Selection of the Principal Study: The 7.5-year study in dogs by Heywood et al. (1979) is selected as the principal study because it provides the highest NOAEL below the lowest LOAEL for the critical effect (hepatotoxicity). In the study by Heywood et al. (1979), dose delivery was via toothpaste-containing gelatin capsule. This route of exposure is not expected to mimic the bolus dose conditions of gavage administration. The capsule will disintegrate over time, resulting in a slower release of contents compared to bolus administration; thus, this mode of administration was considered to be relevant to human exposure conditions.

Summary of the Principal Study:

Heywood R, Sortwell RJ, Noel PRB, et al. 1979. Safety evaluation of toothpaste containing chloroform. III. Long-term study in beagle dogs. J Environ Pathol Toxicol 2:835-851.

In order to assess safety of toothpaste containing chloroform, groups of male and female Beagle dogs (8/sex/group) were exposed to chloroform in toothpaste orally via gelatin capsules at doses of 15 or 30 mg/kg/day for 6 days/week for up to 7.5 years followed by a 20–24-week observation period. Control groups included untreated controls (8/sex), vehicle (capsule) controls (16/sex), and an alternative non-chloroform toothpaste control (8/sex). Survival, clinical signs, food intake, and water intake were monitored throughout the exposure period. Blood was collected to measure hematology and clinical chemistry parameters at 6 and 13 weeks of exposure and at intervals of 8–32 weeks thereafter.

Ophthalmoscopy was performed prior to exposure and at 3-month intervals thereafter. During the 6th year of the study, bromosulfalein retention tests were conducted to assess liver function. At natural death or scheduled sacrifice, main organs (brain, pituitary, spinal cord, heart, lungs, liver, spleen, pancreas, thymus, prostate, uterus, kidneys, thyroids, adrenals, testes, ovaries) were removed and weighed, and a full microscopic examination was conducted on these tissues and all abnormalities. Electron microscopy was performed on liver and kidney sections from two untreated controls and three high-dose dogs (sex unspecified).

Several dogs died prior to scheduled sacrifice between week 87 and 328; however, mortalities were not exposure related. In male dogs, observed deaths included one from each of the following groups: untreated control, vehicle control, low-exposure, and high-exposure groups. In female dogs, three untreated controls and four vehicle controls died; all exposed animals survived until scheduled sacrifice. The study authors noted that about 20% of the dogs were hyperexcitable, mainly during the first 2–3 years. Some had convulsions, and 10 of the 11 reported fatalities occurred after such an attack. While study authors did not indicate which animal groups showed excitability, based on a lack of dose-related mortality it is assumed that neurological signs were not dose-related. No exposure-related changes were observed for body weight, food intake, or water intake. No exposure-related ophthalmological or hematological changes were noted. Serum ALT levels were significantly increased at 15 and 30 mg/kg/day starting on week 130 and 6, respectively. Elevations of ~2-fold were observed at week 260 and 26, respectively. Approximate 2-fold elevations in serum AST and serum ALP were also observed at the end of the exposure period (no statistical analysis provided). Serum enzyme levels recovered somewhat during the recovery period. The bromosulfalein retention test during the 6th year did not reveal any liver impairment. No organ weight changes were found in the exposed groups. Exposure-related nonneoplastic histopathological changes were observed in the liver and kidney. Fatty cysts were observed in the liver in all groups; however, incidence and severity increased in a dose-related manner, with moderate-to-marked fatty cysts significantly elevated in treated groups, compared to control. In males, moderate-to-marked fatty cysts were observed in 1/15, 6/7, and 6/7 dogs at 0 (vehicle control), 15, and 30 mg/kg/day, respectively. In females, moderate-to-marked fatty cysts were observed in 0/12, 3/8, and 7/8 dogs at 0 (vehicle control), 15, and 30 mg/kg/day, respectively. No moderate-to-marked fatty cysts were observed in untreated or non-chloroform toothpaste controls. Fat deposition in renal glomeruli was reportedly higher in the 30 mg/kg/day chloroform group (incidence data were not provided). No remarkable nonneoplastic histopathological differences were observed in other evaluated tissues. No exposure-related tumors were observed.

Selection of the Point of Departure for the MRL: In order to identify the most sensitive POD, BMD modeling was attempted for the incidence data for fatty cysts in male and female dogs (Heywood et al. 1979). BMD modeling was not conducted for serum ALT data because the study authors did not report a measure of variance for the means. The incidence data were fit to all available dichotomous models in EPA's BMDS (version 3.3) using a BMR of 10% extra risk. Adequate model fit was judged by four criteria: goodness-of-fit statistics (p-value >0.1), visual inspection of the dose-response curve, a 95% lower confidence limit on the BMD (BMDL) that is not 10 times lower than the lowest non-zero dose, and a scaled residual within ±2 units at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest BMDL was selected as the POD when the difference between the BMDLs estimated from these models was >3-fold; otherwise, the BMDL from the model with the lowest AIC was chosen.

The datasets used for BMD modeling are presented in Table A-18. Details of the modeling results for the model predictions for hepatic lesions in male and female dogs are in Tables A-19 and A-20, respectively. In accordance with the selection criteria mentioned above, the Logistic model, a frequentist, unrestricted model, was selected for males and the Probit model, a frequentist, unrestricted model, was selected for females.

Table A-18. Moderate-to-Marked Hepatic Fatty Cysts in Dogs Following Oral Exposure to Chloroform for up to 7.5 years

	Dose (mg/kg/day)		
	0 (vehicle)	15	30
Males Incidence (percent incidence)	1/15 (7%)	6/7[a] (86%)	6/7[a] (86%)
Females Incidence (percent incidence)	0/12 (0%)	3/8[a] (38%)	7/8[a] (88%)

[a]p<0.05 (2-tailed Fisher's Exact Probability Test, conducted for this review).

Source: Heywood et al. 1979

Table A-19. Model Predictions for Increased Incidence of Moderate-to-Marked Hepatic Fatty Cysts in Male Dogs Following Oral Exposure to Chloroform for up to 7.5 Years (Heywood et al. 1979)

					Scaled residuals[c]	
Model	BMD_{10}[a] (mg/kg/day)	$BMDL_{10}$[a] (mg/kg/day)	p-Value[b]	AIC	Dose below BMD	Dose above BMD
Dichotomous Hill			NA	26.83	-5.25×10^{-9}	1.25×10^{-8}
Gamma[d]			0.69	23.81	-0.04	0.35
Log-Logistic[e]			0.87	23.03	-0.002	0.12
Multistage Degree 2[f]			0.69	23.81	-0.04	0.35
Multistage Degree 1[f]			0.69	23.81	-0.04	0.35
Weibull[d]			0.69	23.81	-0.04	0.35
Logistic[g]	**3.83**	**2.15**	**0.35**	**26.09**	**-0.56**	**0.69**
Log-Probit			NA	NA	NA	NA
Probit	3.83	2.36	0.27	26.50	-0.58	0.89
Quantal Linear			0.69	23.81	-0.04	0.35

[a]BMD and BMDLs values for models that do not provide adequate fit are not included in this table.
[b]Values <0.1 fail to meet conventional χ^2 goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD.
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.
[g]Selected model. Only Logistic and Probit modes provided an adequate fit to the data. BMDLs were sufficiently close (differed by <3-fold). Therefore, the model with the lowest AIC was selected (Logistic).

AIC = Akaike Information Criterion; BMD = benchmark dose (maximum likelihood estimate of the dose associated with the selected benchmark response); $BMDL_{10}$ = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., 10 = dose associated with 10% extra risk; NA = computation failed

Table A-20. Model Predictions for Increased Incidence of Moderate-to-Marked Hepatic Fatty Cysts in Female Dogs Following Oral Exposure to Chloroform for up to 7.5 Years (Heywood et al. 1979)

Model	BMD_{10}[a] (mg/kg/day)	$BMDL_{10}$[a] (mg/kg/day)	p-Value[b]	AIC	Scaled residuals[c]	
					Dose below BMD	Dose above BMD
Dichotomous Hill			NA	22.61	-4.28×10^{-4}	3.05×10^{-9}
Gamma[d]			1.00	20.61	-4.28×10^{-4}	5.54×10^{-10}
Log-Logistic[e]			1.00	20.61	-4.28×10^{-4}	3.49×10^{-9}
Multistage Degree 2[f]			1.00	18.63	-4.28×10^{-4}	-0.08
Multistage Degree 1[f]			0.80	19.87	-4.28×10^{-4}	-0.56
Weibull[d]			1.00	20.61	-4.28×10^{-4}	-4.68×10^{-9}
Logistic	9.04	4.86	0.55	21.35	-0.50	0.32
Log-Probit			1.00	20.61	-4.28×10^{-4}	3.20×10^{-10}
Probit[g]	**8.70**	**4.63**	**0.62**	**21.09**	**-0.39**	**0.30**
Quantal Linear			0.80	19.87	-4.28×10^{-4}	-0.56

[a]BMD and BMDLs values for models that do not provide adequate fit are not included in this table.
[b]Values <0.1 fail to meet conventional χ^2 goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD.
[d]Power restricted to ≥1.
[e]Slope restricted to ≥1.
[f]Betas restricted to ≥0.
[g]Selected model. Only Logistic and Probit modes provided an adequate fit to the data. BMDLs were sufficiently close (differed by <3-fold). Therefore, the model with the lowest AIC was selected (Probit).

AIC = Akaike Information Criterion; BMD = benchmark dose (maximum likelihood estimate of the dose associated with the selected benchmark response); $BMDL_{10}$ = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., 10 = dose associated with 10% extra risk; NA = computation failed

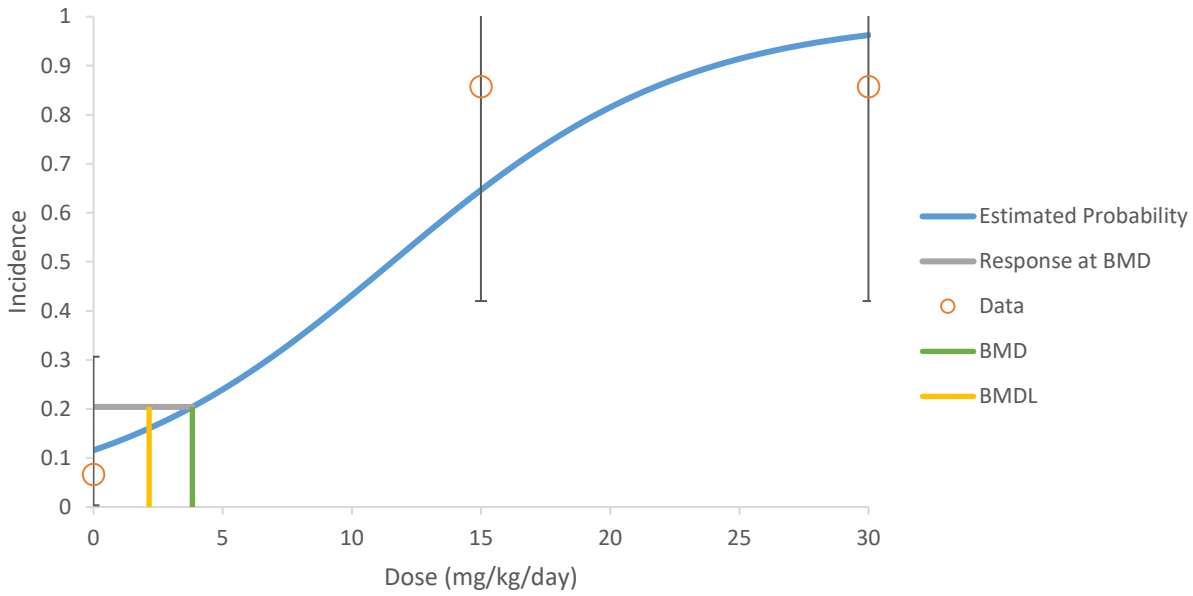
The candidate PODs for hepatic effects in dogs are summarized in Table A-21. Confidence is higher in the PODs based on BMD modeling; from these, the lowest POD identified was 2.15 mg/kg/day. Therefore, the $BMDL_{10}$ of 2.15 mg/kg/day for increased incidence of moderate-to-marked fatty cysts in the liver of male dogs was selected as the POD for the chronic-duration oral MRL. Model fit for the hepatic lesions in male dogs is shown in Figure A-1 (Logistic model).

Table A-21. Summary of Candidate POD Values Considered for Derivation of a Chronic-Duration Oral MRL for Chloroform

Species (sex)	Duration	Effect	Candidate POD (mg/kg/day)	POD type	Reference
Dog (male and female)	7.5 years (6 days/week)	>2-fold increase in serum ALT	15	LOAEL	Heywood et al. 1979
Dog (male)	7.5 years (6 days/week)	Increased incidence of moderate-to-marked hepatic fatty cysts	2.15	BMDL	Heywood et al. 1979
Dog (female)	7.5 years (6 days/week)	Increased incidence of moderate-to-marked hepatic fatty cysts	4.63	BMDL	Heywood et al. 1979

ALT = alanine aminotransferase; BMD = maximum likelihood estimate of the exposure dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD; LOAEL = lowest observed adverse effect level; MRL = Minimal Risk Level; POD = point of departure

Figure A-1. Fit of Logistic Model to Incidence Data for Moderate-to-Marked Hepatic Fatty Cysts in Male Dogs Following Oral Exposure to Chloroform for up to 7.5 Years (Heywood et al. 1979)



Calculations: The $BMDL_{10}$ of 2.15 mg/kg/day was adjusted for a daily exposure scenario:

$$BMDL_{ADJ} = BMDL_{10} \times \frac{days\ exposed}{7\ days} = 2.15\ mg/kg/day \times \frac{6\ days}{7\ days} = 1.84\ mg/kg/day$$

Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al. (1990) and Reitz et al. (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Neither of these studies evaluated dogs and are therefore not suitable for dose extrapolation.

Uncertainty Factors: The following uncertainty factors were applied to the $BMDL_{ADJ}$ to derive the MRL:

- Uncertainty factor of 10 for extrapolation from animals to humans
- Uncertainty factor of 10 for human variability

Subsequently, the oral MRL for chronic-duration exposure to chloroform is:

$$MRL = \frac{BMDL_{ADJ}}{(UF)} = \frac{1.84\ mg/kg/day}{100} = 0.0184\ mg/kg/day \approx 0.02\ mg/kg/day$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Systematic review concluded that hepatic effects are a known health effect following exposure 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 (Appendix C).

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 (Section 2.9). In fatal ingestions cases, acute liver failure and/or severe liver damage have been found at autopsy (Dettling et al. 2016; Piersol et al. 1933). In nonfatal cases of oral ingestion, clinical signs of hepatotoxicity manifest within 1–7 days of exposure (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). Impaired liver function was observed in a man who ingested 21 mg/kg/day chloroform in a cough medicine for 10 years (Wallace 1950).

In animal studies, the liver is also a clear target of toxicity 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 (Section 2.9). Findings in rodents following gavage exposure range from changes in clinical chemistry and mild histopathological damage after lower, shorter exposures (e.g., lipid accumulation, cellular swelling and vacuolation, scattered necrosis, hepatocellular proliferation), with widespread and severe necrosis and degeneration with higher and/or longer durations. As discussed above, the rodent liver is less susceptible to toxicity following drinking water exposure, presumably due to saturation of metabolic detoxification pathways with bolus gavage exposure.

Agency Contact (Chemical Manager): Rae T. Benedict, Ph.D.

APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR CHLOROFORM

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to chloroform.

B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen were conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for chloroform. ATSDR primarily focused on peer-reviewed articles without language restrictions. Foreign language studies are reviewed based on available English-language abstracts and/or tables (or summaries in regulatory assessments, such as International Agency for Research on Cancer [IARC] documents). If the study appears critical for hazard identification or MRL derivation, translation into English is requested. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of chloroform have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of chloroform are presented in Table B-1.

Table B-1. Inclusion Criteria for the Literature Search and Screen[a]

Health Effects
Species
Human
Laboratory mammals
Route of exposure
Inhalation
Oral
Dermal (or ocular)
Parenteral (these studies will be considered supporting data)
In vitro (these studies will be considered supporting data)
Health outcome
Death
Systemic effects
Body weight effects
Respiratory effects
Cardiovascular effects
Gastrointestinal effects
Hematological effects
Musculoskeletal effects
Hepatic effects
Renal effects
Dermal effects
Ocular effects
Endocrine effects
Immunological effects
Neurological effects

Table B-1. Inclusion Criteria for the Literature Search and Screen[a]

Reproductive effects
Developmental effects
Other noncancer effects
Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models
Biomarkers
Biomarkers of exposure
Biomarkers of effect
Interactions with other chemicals
Potential for human exposure
Releases to the environment
Air
Water
Soil
Environmental fate
Transport and partitioning
Transformation and degradation
Environmental monitoring
Air
Water
Sediment and soil
Other media
Biomonitoring
General populations
Occupation populations

[a]Physical-chemical properties are not generally obtained from literature searches, but rather from curated governmental databases such as PubChem.

Prioritization of Human Data. Numerous epidemiological studies evaluate potential associations between exposure to chlorinated drinking water and adverse health outcomes, particularly developmental endpoints and cancer. Epidemiological studies evaluating associations with consumption of chlorinated water or total trihalomethane exposure only were not included in the profile due to availability of studies with chloroform-specific exposure estimates and analyses. Additionally, human epidemiological studies without monitoring data, such as ecological studies based on proximity to emission sources or cohort studies with only self-reported ever/never exposed classifications, were not included in the profile. These studies have limited usefulness due to high risk of exposure misclassification and no information on intensity of potential exposure.

Prioritization of Animal Data. The acute- and intermediate-duration databases for hepatic and renal endpoints in animals following inhalation or oral exposure are extensive. Therefore, animal studies evaluating hepatic and renal endpoints were prioritized for efficient review. Inclusion of hepatic and renal animal studies in Chapter 2 (and the systematic review) was based on the following criteria:

- Acute- and intermediate-duration, single-dose studies that focused only on hepatic and renal endpoints were excluded. All chronic-duration studies and studies evaluating multiple systems were retained regardless of number of dose groups. Lethality data were retained from all studies.
- Only acute- and intermediate-duration studies that evaluated at least one dose within the same order of magnitude (e.g., 0–9, 10–99, 100–999, etc.) of the lowest identified LOAEL for hepatic or renal effects in the 1997 toxicological profile were included. Route- and duration-specific lowest LOAELs are shown in Table B-2. Based on these LOAEL values, only acute-duration inhalation studies evaluating at least one concentration <10 or <100 ppm were included for hepatic and renal endpoints, respectively. For intermediate-duration inhalation studies, only studies evaluating at least one concentration <100 ppm were included for hepatic and renal endpoints. For acute- and intermediate-duration oral studies, only studies evaluating at least one concentration <100 mg/kg/day were included for hepatic and renal endpoints. All chronic-duration studies and studies that evaluated multiple systems were retained regardless of the lowest dose level. Lethality data were retained from all studies.

Table B-2. Lowest LOAELs for Hepatic and Renal Endpoints Reported in 1997 Toxicological Profile

System	Inhalation (ppm)	Oral (mg/kg/day)
Hepatic		
Acute	3	34
Intermediate	25	30
Renal		
Acute	29	34
Intermediate	10	17.4

B.1.1 Literature Search

The literature search was conducted to update the Toxicological Profile for Chloroform released in 1997. All literature cited in the previous (1997) toxicological profile were considered for inclusion in the updated profile. The initial literature search, which was performed in September 2020, was restricted to studies added to databases since January 1995. An updated literature search was performed after the Toxicological Profile for Chloroform Draft for Public Comment was released in January 2024 to identify any additional studies added to databases between September 2020 and February 2024. The following main databases were searched in September 2020 and February 2024:

- PubMed
- National Technical Reports Library (NTRL)
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, Medical Subject Headings (MeSH) headings, and keywords for chloroform. The query strings used for the literature search are presented in Table B-3.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures

and Results (NIH RePORTER) databases using the queries presented in Table B-4. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance Priority List (SPL) resource page, and other items as needed. Regulations applicable to chloroform were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

Table B-3. Database Query Strings

Database search date	Query string
PubMed	
02/2024	(("Chloroform"[mh] OR 67-66-3[rn]) AND (2020/09/01:3000[mhda])) OR ((("1,1,1-Trichloromethane"[tw] OR "chloroform"[tw] OR "methane trichloro"[tw] OR "Trichloromethane"[tw] OR "Formyl trichloride"[tw] OR "carbon trichloride"[tw] OR "Freon 20"[tw] OR "HCC 20"[tw] OR "Methane trichloride"[tw] OR "Methenyl chloride"[tw] OR "Methenyl trichloride"[tw] OR "Methyl trichloride"[tw] OR "Methylidyne trichloride"[tw] OR "Trichloroform"[tw] OR (("F 20"[tw] OR "F20"[tw]) AND "freon*") OR (("R 20"[tw] OR "R20"[tw]) AND "refrigerant*")) NOT medline[sb]) AND (2020/09/01:3000[dp] OR 2020/09/01:3000[crdt] OR 2020/09/01:3000[edat]))
09/2020	((((("Chloroform/toxicity"[mh] OR "Chloroform/adverse effects"[mh] OR "Chloroform/poisoning"[mh] OR "Chloroform/pharmacokinetics"[mh]) OR ("Chloroform"[mh] AND ("environmental exposure"[mh] OR ci[sh])) OR ("Chloroform"[mh] AND toxicokinetics[mh:noexp]) OR ("Chloroform/blood"[mh] OR "Chloroform/cerebrospinal fluid"[mh] OR "Chloroform/urine"[mh]) OR ("Chloroform"[mh] AND ("endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh])) OR ("Chloroform"[mh] AND ("computational biology"[mh] OR "medical informatics"[mh] OR genomics[mh] OR genome[mh] OR proteomics[mh] OR proteome[mh] OR metabolomics[mh] OR metabolome[mh] OR genes[mh] OR "gene expression"[mh] OR phenotype[mh] OR genetics[mh] OR genotype[mh] OR transcriptome[mh] OR ("systems biology"[mh] AND ("environmental exposure"[mh] OR "epidemiological monitoring"[mh] OR analysis[sh])) OR "transcription, genetic "[mh] OR "reverse transcription"[mh] OR "transcriptional activation"[mh] OR "transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, messenger"[mh] OR "RNA, transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "reverse transcriptase polymerase chain reaction"[mh] OR "base sequence"[mh] OR "trans-activators"[mh] OR "gene expression profiling"[mh])) OR ("Chloroform/antagonists and inhibitors"[mh]) OR ("Chloroform/metabolism"[mh] AND ("humans"[mh] OR "animals"[mh])) OR ("Chloroform"[mh] AND cancer[sb]) OR ("Chloroform/pharmacology"[majr])) OR (("1,1,1-Trichloromethane"[tw] OR "CARBON TRICHLORIDE"[tw] OR "chloroform"[tw] OR "Formyl trichloride"[tw] OR "Freon 20"[tw] OR "HCC 20"[tw] OR "Methane trichloride"[tw] OR "Methane, trichloro-"[tw] OR "Methenyl chloride"[tw] OR "Methenyl trichloride"[tw] OR "Methyl trichloride"[tw] OR "Methylidyne trichloride"[tw] OR "Trichloroform"[tw] OR "Trichloromethane"[tw] OR (("F 20"[tw] OR "F20"[tw]) AND freon*[tw]) OR (("R 20"[tw] OR "R20"[tw]) AND refrigerant*[tw])) NOT medline[sb]))) AND (1995:3000[dp] OR 1995:3000[mhda] OR 1995:3000[crdt] OR 1995:3000[edat])

Table B-3. Database Query Strings

Database search date	Query string
NTRL	
02/2024	Terms in Title or Keyword; limited to 2020-present "1,1,1-Trichloromethane" OR "chloroform" OR "methane trichloro" OR "Trichloromethane" OR "Formyl trichloride" OR "carbon trichloride" OR "Freon 20" OR "HCC 20" OR "Methane trichloride" OR "Methenyl chloride" OR "Methenyl trichloride" OR "Methyl trichloride" OR "Methylidyne trichloride" OR "Trichloroform"
09/2020	Date Published 1995 to 2020 Fields: Title or Keyword "1,1,1-Trichloromethane" OR "CARBON TRICHLORIDE" OR "chloroform" OR "Formyl trichloride" OR "Freon 20" OR "HCC 20" OR "Methane trichloride" OR "Methane, trichloro-" OR "Methenyl chloride" OR "Methenyl trichloride" OR "Methyl trichloride" OR "Methylidyne trichloride" OR "Trichloroform" OR "Trichloromethane"
Toxcenter	
02/2024	FILE 'TOXCENTER' ENTERED AT 13:04:32 ON 14 FEB 2024 L1 38527 SEA FILE=TOXCENTER 67-66-3 L2 27919 SEA FILE=TOXCENTER L1 NOT PATENT/DT L3 4855 SEA FILE=TOXCENTER L2 AND ED>=20200901 ACT TOXQUERY/Q --------- L4 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L5 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L6 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L7 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L8 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L9 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L10 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) L11 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE)) L12 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L13 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L14 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L15 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L16 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) L17 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)

Table B-3. Database Query Strings

Database search date	Query string
	L18 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?) L19 QUE (ENDOCRIN? AND DISRUPT?) L20 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?) L21 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?) L22 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?) L23 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?) L24 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?) L25 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?) L26 QUE (NEPHROTOX? OR HEPATOTOX?) L27 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?) L28 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?) L29 QUE L4 OR L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 L30 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?) L31 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE) L32 QUE L29 OR L30 OR L31 L33 QUE (NONHUMAN MAMMALS)/ORGN L34 QUE L32 OR L33 L35 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?) L36 QUE L34 OR L35 --------- L37 2780 SEA FILE=TOXCENTER L3 AND L36 L38 104 SEA FILE=TOXCENTER L37 AND MEDLINE/FS L39 2676 SEA FILE=TOXCENTER L37 NOT MEDLINE/FS L40 2687 DUP REM L38 L39 (93 DUPLICATES REMOVED) L*** DEL 104 S L37 AND MEDLINE/FS L*** DEL 104 S L37 AND MEDLINE/FS L41 104 SEA FILE=TOXCENTER L40 L*** DEL 2676 S L37 NOT MEDLINE/FS L*** DEL 2676 S L37 NOT MEDLINE/FS L42 2583 SEA FILE=TOXCENTER L40 L43 2583 SEA FILE=TOXCENTER (L41 OR L42) NOT MEDLINE/FS D SET D SEL D CLUSTER

Table B-3. Database Query Strings

Database search date	Query string
	D SCAN L43
09/2020	FILE 'TOXCENTER' ENTERED AT 12:58:25 ON 25 SEP 2020 CHARGED TO COST=EH038.05.01.LB.03 L1 31178 SEA FILE=TOXCENTER 67-66-3 L2 30902 SEA FILE=TOXCENTER L1 NOT TSCATS/FS L3 21961 SEA FILE=TOXCENTER L2 NOT PATENT/DT L4 13834 SEA FILE=TOXCENTER L3 AND PY>1994 ACT TOXQUERY/Q --------- L5 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L6 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L7 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L8 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L9 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L10 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L11 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) L12 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE)) L13 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L14 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L15 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L16 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L17 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) L18 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?) L19 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?) L20 QUE (ENDOCRIN? AND DISRUPT?) L21 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?) L22 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?) L23 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?) L24 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?) L25 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)

Table B-3. Database Query Strings

Database search date	Query string
	L26 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L27 QUE (NEPHROTOX? OR HEPATOTOX?)
	L28 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L29 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L30 QUE L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29
	L31 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L32 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L33 QUE L30 OR L31 OR L32
	L34 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L35 QUE L33 OR L34

	L36 6707 SEA FILE=TOXCENTER L4 AND L35
	L37 732 SEA FILE=TOXCENTER L36 AND MEDLINE/FS
	L40 1002 SEA FILE=TOXCENTER L36 AND BIOSIS/FS
	L41 4927 SEA FILE=TOXCENTER L36 AND CAPLUS/FS
	L42 46 SEA FILE=TOXCENTER L36 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L43 5991 DUP REM L37 L40 L42 L41 (716 DUPLICATES REMOVED) ANSWERS '1-5991' FROM FILE TOXCENTER
	L*** DEL 732 S L36 AND MEDLINE/FS
	L*** DEL 732 S L36 AND MEDLINE/FS
	L44 732 SEA FILE=TOXCENTER L43
	L*** DEL 1002 S L36 AND BIOSIS/FS
	L*** DEL 1002 S L36 AND BIOSIS/FS
	L45 794 SEA FILE=TOXCENTER L43
	L*** DEL 4927 S L36 AND CAPLUS/FS
	L*** DEL 4927 S L36 AND CAPLUS/FS
	L46 4428 SEA FILE=TOXCENTER L43
	L*** DEL 46 S L36 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L*** DEL 46 S L36 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L47 37 SEA FILE=TOXCENTER L43
	L48 5259 SEA FILE=TOXCENTER (L44 OR L45 OR L46 OR L47) NOT MEDLINE/FS
	L53 732 SEA FILE=TOXCENTER (L49 OR L50 OR L51 OR L52) AND MEDLINE/FS
	L54 769 SEA FILE=TOXCENTER L48 AND PY<=2000
	L56 740 SEA FILE=TOXCENTER L48 AND PY>2000 AND PY<=2005
	L58 1008 SEA FILE=TOXCENTER L48 AND PY>2005 AND PY<=2010
	L60 1449 SEA FILE=TOXCENTER L48 AND PY>2010 AND PY<=2015
	L61 1293 SEA FILE=TOXCENTER L48 AND PY>2015
	L62 5259 SEA FILE=TOXCENTER L54 OR L56 OR L58 OR L60 OR L61

Table B-3. Database Query Strings

Database search date	Query string
	D SCAN L54 D SCAN L56 D SCAN L58 D SCAN L60 D SCAN L61

Table B-4. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS via ChemView	
02/2024; 09/2020	67-66-3
NTP	
02/2024	Date limited: 2020-2024; not dated 67-66-3 "chloroform" "Trichloromethane" "1,1,1-Trichloromethane" "methane trichloro" "Formyl trichloride" "carbon trichloride" "Freon 20" "HCC 20" "Methane trichloride" "Methenyl chloride" "Methenyl trichloride" "Methyl trichloride" "Methylidyne trichloride" "Trichloroform"
09/2020	Limited to content types: Reports & Publications; Systematic Reviews; ROC Profiles, Reviews, or Candidates; and Testing Status 67-66-3
NPIRS	
02/2024	EPA Registration #: 020701
Regulations.gov	
02/2024	Dockets and Documents (limited to 01/01/2020-02/12/2024 and Notices) 67-66-3 "chloroform" "Trichloromethane" "1,1,1-Trichloromethane" "methane trichloro" "Formyl trichloride" "carbon trichloride" "Freon 20" "HCC 20" "Methane trichloride" "Methenyl chloride" "Methenyl trichloride" "Methyl trichloride" "Methylidyne trichloride" "Trichloroform"

Table B-4. Strategies to Augment the Literature Search

Source	Query and number screened when available
09/2020	Limited to rules, proposed rules, notices, other 67-66-3
NIH RePORTER	
05/2024	"1,1,1-Trichloromethane" OR "chloroform" OR "methane trichloro" OR "Trichloromethane" OR "Formyl trichloride" OR "carbon trichloride" OR "Freon 20" OR "HCC 20" OR "Methane trichloride" OR "Methenyl chloride" OR "Methenyl trichloride" OR "Methyl trichloride" OR "Methylidyne trichloride" OR "Trichloroform"
02/2023	Text Search: "1,1,1-Trichloromethane" OR "CARBON TRICHLORIDE" OR "chloroform" OR "Formyl trichloride" OR "Freon 20" OR "HCC 20" OR "Methane trichloride" OR "Methane, trichloro-" OR "Methenyl chloride" OR "Methenyl trichloride" OR "Methyl trichloride" OR "Methylidyne trichloride" OR "Trichloroform" OR "Trichloromethane" (advanced) Limit to: Project Title, Project Terms, Project Abstracts
Other	Identified throughout the assessment process[a]

[a]References identified throughout the assessment process may include studies found by tree searching; recommended by intraagency, interagency, peer, or public reviewers; or published more recently than the date of the literature search (February 2024). Additional references include those for specific regulations or guidelines and publications found by targeted searches for specific information (e.g., searches for reviews of general [not chemical-specific] mechanisms of toxicity).

The 2020 pre-public comment search results were:

- Number of records identified from PubMed, NTRL, and TOXCENTER (after duplicate removal): 10,710
- Number of records identified from other strategies: 133
- Total number of records to undergo literature screening: 10,843

The 2024 post-public comment search results were:

- Number of records identified from PubMed, NTRL, and TOXCENTER (after duplicate removal): 4,578
- Number of records identified from other strategies: 166
- Total number of records to undergo literature screening: 4,744

B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on chloroform during the pre- and post-public comment drafts:

- Title and abstract screen
- Full text screen

Pre-Public Comment Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

- Number of titles and abstracts screened: 10,843

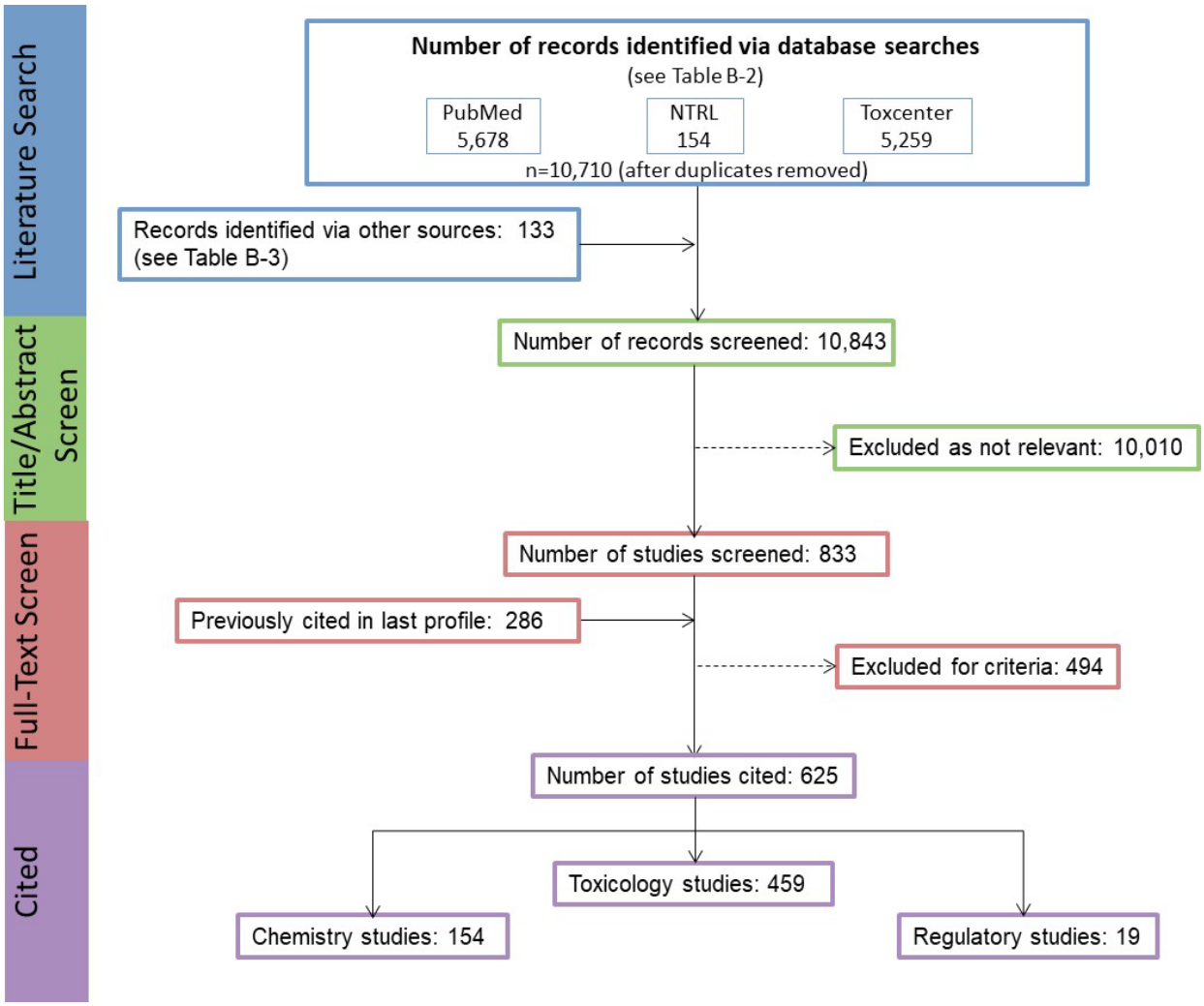
- Number of studies considered relevant and moved to the next step: 833

Pre-Public Comment Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 833
- Number of studies cited in the previous toxicological profile: 286
- Total number of studies cited in the profile: 625

A summary of the results of the pre-public literature search and screening is presented in Figure B-1.

Figure B-1. September 2020 Pre-Public Comment Literature Search Results and Screen for Chloroform



*The chemistry studies category includes studies pertaining to the potential for human exposure (Table B-1). The toxicology studies category includes human and animal studies of health effects as well as studies of toxicokinetics, biomarkers, and interactions with other chemicals (Table B-1). The regulatory studies category includes those studies cited in Chapter 7.

Post-Public Comment Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

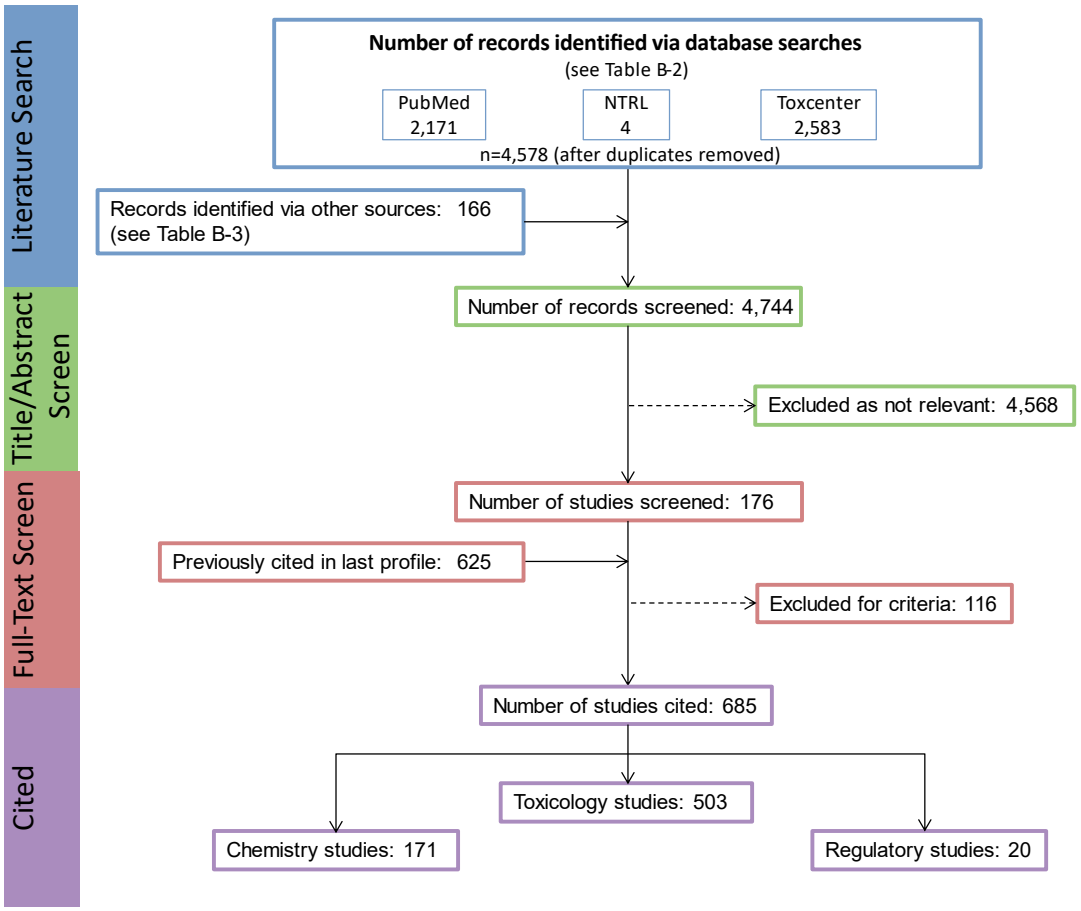
- Number of titles and abstracts screened: 4,744
- Number of studies considered relevant and moved to the next step: 176

Post-Public Comment Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 176
- Number of studies cited in the pre-public draft of the toxicological profile: 625
- Total number of studies cited in the profile: 685

A summary of the results of the post-public comment literature search and screening is presented in Figure B-2

Figure B-2. May 2024 Post-Public Comment Literature Search Results and Screen for Chloroform*



*The chemistry studies category includes studies pertaining to the potential for human exposure (Table B-1). The toxicology studies category includes human and animal studies of health effects as well as studies of toxicokinetics, biomarkers, and interactions with other chemicals (Table B-1). The regulatory studies category includes those studies cited in Chapter 7.

APPENDIX C. FRAMEWORK FOR ATSDR'S SYSTEMATIC REVIEW OF HEALTH EFFECTS DATA FOR CHLOROFORM

To increase the transparency of ATSDR's process of identifying, evaluating, synthesizing, and interpreting the scientific evidence on the health effects associated with exposure to chloroform, ATSDR utilized a slight modification of NTP's Office of Health Assessment and Translation (OHAT) systematic review methodology (NTP 2013, 2015; Rooney et al. 2014). ATSDR's framework is an eight-step process for systematic review with the goal of identifying the potential health hazards of exposure to chloroform:

- Step 1. Problem Formulation
- Step 2. Literature Search and Screen for Health Effects Studies
- Step 3. Extract Data from Health Effects Studies
- Step 4. Identify Potential Health Effect Outcomes of Concern
- Step 5. Assess the Risk of Bias for Individual Studies
- Step 6. Rate the Confidence in the Body of Evidence for Each Relevant Outcome
- Step 7. Translate Confidence Rating into Level of Evidence of Health Effects
- Step 8. Integrate Evidence to Develop Hazard Identification Conclusions

C.1 PROBLEM FORMULATION

The objective of the toxicological profile and this systematic review was to identify the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to chloroform. The inclusion criteria used to identify relevant studies examining the health effects of chloroform are presented in Table C-1.

Data from human and laboratory animal studies were considered relevant for addressing this objective. Human studies were divided into two broad categories: observational epidemiology studies and controlled exposure studies. The observational epidemiology studies were further divided: cohort studies (retrospective and prospective studies), population studies (with individual data or aggregate data), and case-control studies.

Table C-1. Inclusion Criteria for Identifying Health Effects Studies

Species
Human[a]
Laboratory mammals
Route of exposure
Inhalation
Oral
Dermal (or ocular)
Parenteral (these studies will be considered supporting data)
Health outcome
Death
Systemic effects
Body weight effects
Respiratory effects
Cardiovascular effects

Table C-1. Inclusion Criteria for Identifying Health Effects Studies

Gastrointestinal effects
Hematological effects
Musculoskeletal effects
Hepatic effects[b]
Renal effects[b]
Dermal effects
Ocular effects
Endocrine effects
Immunological effects
Neurological effects
Reproductive effects
Developmental effects
Other noncancer effects
Cancer

[a]Inclusion criteria were refined for human studies as described in Section B.1.1, *Prioritization of Human Data*.
[b]Inclusion criteria were refined for animal studies evaluating hepatic and renal effects as described in Section B.1.1, *Prioritization of Animal Data*.

C.2 LITERATURE SEARCH AND SCREEN FOR HEALTH EFFECTS STUDIES

A literature search and screen were conducted to identify studies examining the health effects of chloroform. The literature search framework for the toxicological profile is discussed in detail in Appendix B.

C.2.1 Literature Search

As noted in Appendix B, the current literature search was intended to update the Draft Toxicological Profile for Chloroform released for public comment in 2024; thus, the literature search was restricted to studies published between September 2020 and February 2024. See Appendix B for the databases searched and the search strategy.

A total of 10,843 and 4,744 records relevant to all sections of the toxicological profile were identified in the initial and update literature search, respectively (after duplicate removal).

C.2.2 Literature Screening

As described in Appendix B, a two-step process was used to screen the literature search to identify relevant studies examining the health effects of chloroform.

Title and Abstract Screen. In the Title and Abstract Screen step, 107 documents (inclusive of both literature searches) were considered to meet the health effects inclusion criteria in Table C-1 and were moved to the next step in the process.

Full Text Screen. In the second step in the literature screening process for the systematic review, a full text review of 191 health effect documents (documents identified in the update literature search and

documents cited in older versions of the profile) was performed. From those 191 documents (258 studies), 88 documents (137 studies) were included in the qualitative review.

C.3 EXTRACT DATA FROM HEALTH EFFECTS STUDIES

Relevant data extracted from the individual studies selected for inclusion in the systematic review were collected in customized data forms. A summary of the type of data extracted from each study is presented in Table C-2. For references that included more than one experiment or species, data extraction records were created for each experiment or species.

Table C-2. Data Extracted from Individual Studies

Citation
Chemical form
Route of exposure (e.g., inhalation, oral, dermal)
Specific route (e.g., gavage in oil, drinking water)
Species
Strain
Exposure duration category (e.g., acute, intermediate, chronic)
Exposure duration
Frequency of exposure (e.g., 6 hours/day, 5 days/week)
Exposure length
Number of animals or subjects per sex per group
Dose/exposure levels
Parameters monitored
Description of the study design and method
Summary of calculations used to estimate doses (if applicable)
Summary of the study results
Reviewer's comments on the study
Outcome summary (one entry for each examined outcome)
No-observed-adverse-effect level (NOAEL) value
Lowest-observed-adverse-effect level (LOAEL) value
Effect observed at the LOAEL value

A summary of the extracted data for each study is presented in the Supplemental Document for Chloroform and overviews of the results of the inhalation, oral, and dermal exposure studies are presented in Sections 2.2–2.19 of the profile and in the Levels Significant Exposures tables in Section 2.1 of the profile (Tables 2-1, 2-2, and 2-3, respectively).

C.4 IDENTIFY POTENTIAL HEALTH EFFECT OUTCOMES OF CONCERN

Overviews of the potential health effect outcomes for chloroform identified in human and animal studies are presented in Tables C-4 and C-5, respectively. Available human studies evaluating noncancer effects include numerous case studies and case-series reports, a limited number of occupational exposure studies, and general population exposure studies (primarily focusing on exposure to chloroform as a disinfection byproduct in residential water supplies). When evaluated together, these studies suggest that the respiratory, hepatic, renal, and neurological systems and the developing fetus may be susceptible to

chloroform toxicity. Animal studies evaluated a comprehensive set of endpoints following inhalation and oral exposure; dermal studies were limited to two acute-duration studies evaluating a limited number of endpoints. Respiratory and hepatic effects were considered sensitive outcomes following inhalation exposure in animals, and hepatic, renal, and developmental effects were considered sensitive outcomes following oral exposure in animals (i.e., effects were observed at low concentrations or doses; see Tables 2-1 and 2-2 and Figures 1-3, 1-4, 2-1, and 2-2 for further detail). Based on effects noted in human and animal studies, epidemiological and experimental studies examining these respiratory, hepatic, renal, neurological, and developmental outcomes were carried through to Steps 4–8 of the systematic review. Case studies and case-series reports were not included in the formal systematic review due to inherent high risk of bias and low confidence based on study design. However, consistent findings from numerous case studies were considered during the adjustment of the confidence rating (with regards to consistency and/or severity of observed effects). There were 136 studies (published in 88 documents) examining these potential outcomes carried through to Steps 4–8 of the systematic review.

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Table C-3. Overview of the Health Outcomes for Chloroform Evaluated in Human Studies

	Body weight	Respiratory	Cardiovascular	Gastrointestinal	Hematological	Musculoskeletal	Hepatic	Renal	Dermal	Ocular	Endocrine	Immunological	Neurological	Reproductive	Developmental	Other Noncancer	Caner
Inhalation studies																	
Cohort			1	2			3	1				1	2			1	
			1	2			1	0				1	2			1	
Case-control		6	4	4	1	3	13	3				1	3		1		10
		6	4	4	1	3	13	3				1	3		0		2
Population		1					1	1				1					
		0					0	0				0					
Case series			2	3	1		4						2				
			2	3	1		3						2				
Oral studies																	
Cohort														6	15		3
														2	3		3
Case-control		5	4	4	2	1	12	4				1	9	3	6	1	8
		5	4	4	2	1	12	4				1	9	0	3	1	3
Population					1		1	1						2	2	1	2
					1		1	1						0	1	1	0
Case series																	
Dermal studies																	
Cohort																	
Case-control				1			1		6				1				
				1			1		6				1				
Population																	
Case series																	

Number of studies examining endpoint	0	1	2	3	4	5–9	≥10
Number of studies reporting outcome	0	1	2	3	4	5–9	≥10

APPENDIX C

Table C-4. Overview of the Health Outcomes for Chloroform Evaluated in Experimental Animal Studies

	Body weight	Respiratory	Cardiovascular	Gastrointestinal	Hematological	Musculoskeletal	Hepatic	Renal	Dermal	Ocular	Endocrine	Immunological[a]	Neurological[a]	Reproductive[a]	Developmental	Other Noncancer	Caner
Inhalation studies																	
Acute-duration	14	9					11	11				6	8	7	6		
	9	8					10	9				3	8	5	6		
Intermediate-duration	15	10	4	4	2	8	15	15	2	4	4	6	4	6			
	7	6	0	0	0	0	14	12	0	0	0	0	0	0			
Chronic-duration	3	2	2	2	2	2	2	3	2	2	2	2	2	2			3
	2	2	0	0	0	0	2	3	0	0	0	0	0	0			1
Oral studies																	
Acute-duration	21	3	1	3	3		22	18	2	1		1	7	3	5		1
	12	3	1	1	2		19	14	2	1		1	7	3	4		
Intermediate-duration	22	8	4	8	6	2	19	16		2	5	6	7	5	4		7
	8	3	1	1	1	0	15	6		0	1	1	1	1	2		3
Chronic-duration	10	3	3	2	4	2	4	7	2	1	2	2	4	3			9
	4	1	1	0	0	0	3	3	0	0	0	0	0	0			7
Dermal studies																	
Acute-duration	1						1	1	2								
	1						0	1	2								
Intermediate-duration																	
Chronic-duration																	

Number of studies examining endpoint	0	1	2	3	4	5–9	≥10
Number of studies reporting outcome	0	1	2	3	4	5–9	≥10

[a]Number of studies examining endpoint includes study evaluating histopathology, but not evaluating function.

C.5 ASSESS THE RISK OF BIAS FOR INDIVIDUAL STUDIES

C.5.1 Risk of Bias Assessment

The risk of bias of individual studies was assessed by reviewers using the guidelines provided in OHAT's Risk of Bias Tool (NTP 2015). The risk of bias questions for observational epidemiology studies, human-controlled exposure studies, and animal experimental studies are presented in Tables C-6, C-7, and C-8, respectively. Each risk of bias question was answered on a four-point scale:

- **Definitely low risk of bias (++)**
- **Probably low risk of bias (+)**
- **Probably high risk of bias (-)**
- **Definitely high risk of bias (– –)**

In general, "definitely low risk of bias" or "definitely high risk of bias" were used if the question could be answered with information explicitly stated in the study report. If the response to the question could be inferred, then "probably low risk of bias" or "probably high risk of bias" responses were typically used.

Table C-5. Risk of Bias Questionnaire for Observational Epidemiology Studies

Selection bias
Were the comparison groups appropriate?
Confounding bias
Did the study design or analysis account for important confounding and modifying variables?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

Table C-6. Risk of Bias Questionnaire for Human-Controlled Exposure Studies

Selection bias
Was administered dose or exposure level adequately randomized?
Was the allocation to study groups adequately concealed?
Performance bias
Were the research personnel and human subjects blinded to the study group during the study?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

Table C-7. Risk of Bias Questionnaire for Experimental Animal Studies

Selection bias
Was administered dose or exposure level adequately randomized?
Was the allocation to study groups adequately concealed?
Performance bias
Were experimental conditions identical across study groups?
Were the research personnel blinded to the study group during the study?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

After the risk of bias questionnaires were completed for the health effects studies, the studies were assigned to one of three risk of bias tiers based on the responses to the key questions listed below and the responses to the remaining questions.

- Is there confidence in the exposure characterization? (only relevant for observational studies)
- Is there confidence in the outcome assessment?
- Does the study design or analysis account for important confounding and modifying variables? (only relevant for observational studies)

First Tier. Studies placed in the first tier received ratings of "definitely low" or "probably low" risk of bias on the key questions **AND** received a rating of "definitely low" or "probably low" risk of bias on the responses to at least 50% of the other applicable questions.

Second Tier. A study was placed in the second tier if it did not meet the criteria for the first or third tiers.

Third Tier. Studies placed in the third tier received ratings of "definitely high" or "probably high" risk of bias for the key questions **AND** received a rating of "definitely high" or "probably high" risk of bias on the response to at least 50% of the other applicable questions.

The results of the risk of bias assessment for the different types of chloroform health effects studies (observational epidemiology and animal experimental studies) are presented in Tables C-9 and C-10, respectively.

Table C-8. Summary of Risk of Bias Assessment for Chloroform—Observational Epidemiology Studies

Reference	Risk of bias criteria and ratings						Risk of bias tier
	Selection bias	Confounding bias	Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Were the comparison groups appropriate?	**Did the study design or analysis account for important confounding and modifying variables?***	Were outcome data complete without attrition or exclusion from analysis?	**Is there confidence in the exposure characterization?***	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Outcome: Respiratory effects							
Cross-sectional studies							
Font-Ribera et al. 2010	+	–	+	+	++	++	Second
Outcome: Hepatic effects							
Cohort studies							
Aiking et al. 1994	+	–	+	+	–	++	Second
Bomski et al. 1967	+	–	–	–	–	–	Third
Challen et al. 1958	+	+	+	+	+	+	First
Li et al. 1993	+	–	–	+	–	–	Second
Outcome: Renal effects							
Cohort studies							
Aiking et al. 1994	+	–	+	+	–	++	Second
Li et al. 1993	+	–	–	+	–	–	Second
Outcome: Neurological effects							
Cohort studies							
Challen et al. 1958	+	+	+	+	–	+	Second
Li et al. 1993	+	–	–	+	+	–	Second

Table C-8. Summary of Risk of Bias Assessment for Chloroform—Observational Epidemiology Studies

Reference	Risk of bias criteria and ratings						Risk of bias tier
	Selection bias	Confounding bias	Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Were the comparison groups appropriate?	**Did the study design or analysis account for important confounding and modifying variables?***	Were outcome data complete without attrition or exclusion from analysis?	**Is there confidence in the exposure characterization?***	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Outcome: Developmental effects							
Cohort studies							
Botton et al. 2015	+	–	++	+	+	++	Second
Cao et al. 2016	+	–	++	++	+	++	Second
Costet et al. 2011	+	–	++	+	+	++	Second
Dodds and King 2001	+	–	–	–	+	++	Second
Grazuleviciene et al. 2011	+	–	++	++	+	++	Second
Grazuleviciene et al. 2013	+	–	++	++	+	++	Second
Hinckley et al. 2005	+	–	++	–	+	++	Second
Hoffman et al. 2008	+	–	++	–	+	++	Second
Liu et al. 2021	+	–	++	+	+	+	Second
Rivera-Núñez and Wright 2013	+	+	+	–	+	++	Second
Sun et al. 2020	+	–	++	+	+	++	Second
Villanueva et al. 2018	+	–	++	++	++	++	Second
Villanueva et al. 2011	–	–	++	–	+	++	Second
Zhu et al. 2022	+	–	++	–	+	++	Second

Table C-8. Summary of Risk of Bias Assessment for Chloroform—Observational Epidemiology Studies

Reference	Risk of bias criteria and ratings						Risk of bias tier
	Selection bias	Confounding bias	Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Were the comparison groups appropriate?	**Did the study design or analysis account for important confounding and modifying variables?***	Were outcome data complete without attrition or exclusion from analysis?	**Is there confidence in the exposure characterization?***	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Population studies							
Porter et al. 2005	+	–	++	–	+	–	Second
Wright et al. 2004	+	–	++	–	+	++	Second
Case-control studies							
Bonou et al. 2017	++	–	++	+	+	++	Second
Kaufman et al. 2018	++	–	++	–	++	++	Second
Kaufman et al. 2020	++	–	++	–	++	++	Second
Kramer et al. 1992	–	–	++	–	–	–	Third
Levallois et al. 2012	+	–	++	+	+	++	Second
Summerhayes et al. 2012	+	–	++	–	+	++	Second
Swartz et al. 2015a, 2015b	+	++	++	–	+	++	Second
Zaganjor et al. 2020	+	–	++	+	++	++	Second

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias

*Key question

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Outcome: Respiratory effects									
Inhalation acute-duration exposure									
Constan et al. 1999 (Sv/129 mice)	+	+	++	++	++	++	++	++	First
Constan et al. 1999 (B6C3F1 mice)	+	+	++	++	++	++	++	++	First
de Oliveira et al. 2015	–	+	++	+	++	+	+	++	First
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	–	First
Larson et al. 1994c; Mery et al. 1994 (mouse)	–	+	++	+	++	+	+	++	First
Larson et al. 1994c; Mery et al. 1994 (rat)	–	+	++	+	++	+	+	++	First
Larson et al. 1996	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (4 days)	–	+	++	+	++	+	+	++	First
Inhalation intermediate-duration exposure									
Kasai et al. 2002 (mouse)	–	+	++	+	++	++	+	–	First
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	–	First
Larson et al. 1996 (13 weeks; 5 days/week)	–	+	++	+	++	–	+	++	First
Larson et al. 1996 (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Larson et al. 1996 (3 weeks)	–	+	++	+	++	+	+	++	First

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Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings: Selection bias: Was administered dose or exposure level adequately randomized?	Selection bias: Was the allocation to study groups adequately concealed?	Performance bias: Were the experimental conditions identical across study groups?	Performance bias: Were the research personnel blinded to the study group during the study?	Attrition / exclusion bias: Were outcome data complete without attrition or exclusion from analysis?	Detection bias: Is there confidence in the exposure characterization?	Detection bias: **Is there confidence in the outcome assessment?***	Selective reporting bias: Were all measured outcomes reported?	Risk of bias tier
Larson et al. 1996 (6 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 5 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (3 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (6 weeks)	–	+	++	+	++	+	+	++	First
Inhalation chronic-duration exposure									
Yamamoto et al. 2002 (mouse)	–	+	++	+	++	++	+	+	First
Yamamoto et al. 2002 (rat)	–	+	++	+	++	++	+	+	First
Oral acute-duration exposure									
Larson et al. 1995b	+	+	++	+	++	+	+	+	First
Templin et al. 1996a (Fischer 344)	+	+	++	+	++	+	+	++	First
Templin et al. 1996a (Osborne-Mendel)	+	+	++	+	++	+	+	++	First
Oral intermediate-duration exposure									
Chu et al. 1982a	–	+	+	+	–	+	+	–	First
Chu et al. 1982b	–	+	+	+	+	+	+	+	First
Dorman et al. 1997	– –	+	–	+	–	+	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
NTP 1988a	++	+	++	+	++	+	+	++	First
EPA 1980 (mouse)	++	+	–	+	+	+	+	++	First
EPA 1980 (rat)	++	+	–	+	+	+	+	++	First
Larson et al. 1995b	+	+	++	+	++	+	++	+	First
Sehata et al. 2002 (CB6F1)	–	+	++	+	+	+	++	++	First
Oral chronic-duration exposure									
Dunnick and Melnick 1993; NCI 1976 (mouse)	+	+	++	+	++	++	+	++	First
Dunnick and Melnick 1993; NCI 1976 (rat)	+	+	++	+	++	++	+	++	First
Roe et al. 1979 (Experiment 1)	+	+	–	+	–	++	–	+	Second
Outcome: Liver effects									
Inhalation acute-duration exposure									
Baeder and Hofmann 1988	–	+	++	+	++	+	–	++	Second
Constan et al. 1999 (Sv/129 mice)	+	+	++	+	++	+	+	++	First
Constan et al. 1999 (B6C3F1 mice)	+	+	++	+	++	+	+	++	First
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	++	First
Larson et al. 1994c; Mery et al. 1994 (mouse)	–	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Larson et al. 1994c; Mery et al. 1994 (rat)	–	+	++	+	++	+	+	++	First
Larson et al. 1996	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (4 days)	–	+	++	+	++	+	+	++	First
Templin et al. 1996c (2 weeks)	+	+	++	+	+	+	+	++	First
Templin et al. 1996c (4 days)	+	+	++	+	+	+	+	++	First
Inhalation intermediate-duration exposure									
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	++	First
Larson et al. 1996 (13 weeks; 5 days/week)	–	+	++	+	++	–	+	++	First
Larson et al. 1996 (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Larson et al. 1996 (3 weeks)	–	+	++	+	++	+	+	++	First
Larson et al. 1996 (6 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 5 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (3 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (6 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1998 (13 weeks)	+	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Templin et al. 1998 (3 weeks)	+	+	++	+	++	+	+	++	First
Templin et al. 1998 (7 weeks)	+	+	++	+	++	+	+	++	First
Torkelson et al. 1976 (rat 1–4 hours/day)	–	+	++	+	–	++	+	++	First
Torkelson et al. 1976 (rat 7 hours/day)	–	+	++	+	–	++	+	++	First
Inhalation chronic-duration exposure									
Yamamoto et al. 2002 (mouse)	–	+	++	+	++	++	++	+	First
Yamamoto et al. 2002 (rat)	–	+	++	+	++	++	++	+	First
Oral acute-duration exposure									
Chu et al. 1982b	–	+	++	+	–	+	+	+	First
Ewaid et al. 2020	+	+	–	+	–	+	+	+	First
Jones et al. 1958	– –	+	–	+	–	–	+	+	Second
Keegan et al. 1998	–	+	++	+	++	+	++	++	First
Larson et al. 1993 (mouse)	+	+	++	+	++	+	+	+	First
Larson et al. 1993 (rat)	+	+	++	+	++	+	+	+	First
Larson et al. 1994b (GO)	+	+	+	+	+	+	+	+	First
Larson et al. 1994b (W)	+	+	+	+	+	+	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Larson et al. 1994d	+	+	++	+	+	+	+	+	First
Larson et al. 1995a (DW)	+	+	++	+	+	+	+	+	First
Larson et al. 1995a (G)	+	+	++	+	+	+	+	+	First
Larson et al. 1995b	+	+	++	+	++	+	+	+	First
Lilly et al. 1997	–	+	++	+	++	+	++	+	First
Miyagawa et al. 1998	–	+	+	+	+	–	+	+	First
Moore et al. 1982 (G)	+	+	+	+	+	–	+	++	First
Moore et al. 1982 (GO)	+	+	+	+	+	–	+	++	First
Munson et al. 1982	–	+	+	+	–	–	++	+	First
Templin et al. 1996a (Fischer 344)	+	+	++	+	++	+	+	++	First
Templin et al. 1996a (Osborne-Mendel)	+	+	++	+	++	+	+	++	First
Thompson et al. 1974 (Experiment 2, 6 F)	–	+	+	+	++	–	+	+	First
Wada et al. 2015	+	+	+	+	++	+	+	++	First
Wang et al. 1997	–	+	++	+	++	–	++	++	First
Oral intermediate-duration exposure									
Bull et al. 1986 (GO)	+	+	++	+	+	–	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Bull et al. 1986 (GW)	+	+	++	+	+	–	+	+	First
Chu et al. 1982a	–	+	+	+	–	–	+	++	First
Chu et al. 1982b	–	+	+	+	+	–	+	+	First
Eschenbrenner and Miller 1945	–	+	+	+	+	–	–	+	Second
NTP 1988a	++	+	++	+	++	+	+	++	First
Heywood et al. 1979	–	+	+	+	+	–	+	–	First
EPA 1980 (mouse)	++	+	–	+	+	+	+	++	First
EPA 1980 (rat)	++	+	–	+	+	+	+	++	First
Larson et al. 1994b (GO)	+	+	+	+	+	+	+	+	First
Larson et al. 1994b (W)	+	+	+	+	+	+	+	+	First
Larson et al. 1994d	+	+	++	+	+	+	+	++	First
Larson et al. 1995a (GO)	+	+	++	+	+	+	+	+	First
Larson et al. 1995a (W)	+	+	++	+	+	+	+	+	First
Larson et al. 1995b	+	+	++	+	++	+	+	+	First
Melnick et al. 1998	–	+	++	+	+	+	+	++	First
Mostafa et al. 2009	–	+	– –	+	++	–	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings: Selection bias — Was administered dose or exposure level adequately randomized?	Selection bias — Was the allocation to study groups adequately concealed?	Performance bias — Were the experimental conditions identical across study groups?	Performance bias — Were the research personnel blinded to the study group during the study?	Attrition / exclusion bias — Were outcome data complete without attrition or exclusion from analysis?	Detection bias — Is there confidence in the exposure characterization?	Detection bias — **Is there confidence in the outcome assessment?***	Selective reporting bias — Were all measured outcomes reported?	Risk of bias tier
Munson et al. 1982	–	+	+	+	–	–	+	+	First
Sehata et al. 2002 (CB6F1)	–	+	++	+	+	+	++	++	First
Oral chronic-duration exposure									
Heywood et al. 1979	–	+	+	+	–	++	–	++	Second
Dunnick and Melnick 1993; NCI 1976 (mouse)	+	+	++	+	++	+	+	++	First
Dunnick and Melnick 1993; NCI 1976 (rat)	+	+	++	+	++	+	+	++	First
Roe et al. 1979 (Experiment 1)	+	+	–	+	–	++	+	+	First
Outcome: Kidney effects									
Inhalation acute-duration exposure									
Baeder and Hofmann 1988	–	+	++	+	++	+	–	++	Second
Constan et al. 1999 (Sv/129 mice)	+	+	++	+	++	+	+	++	First
Constan et al. 1999 (B6C3F1 mice)	+	+	++	+	++	+	+	++	First
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	++	First
Larson et al. 1994c; Mery et al. 1994 (mouse)	–	+	++	+	++	+	+	++	First
Larson et al. 1994c; Mery et al. 1994 (rat)	–	+	++	+	++	+	+	++	First
Larson et al. 1996	–	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Templin et al. 1996b (4 days)	–	+	++	+	++	+	+	++	First
Templin et al. 1996c (2 weeks)	+	+	++	+	+	+	+	++	First
Templin et al. 1996c (4days)	+	+	++	+	+	+	+	++	First
Inhalation intermediate-duration exposure									
Kasai et al. 2002 (mouse)	–	+	++	+	++	++	+	++	First
Kasai et al. 2002 (rat)	–	+	++	+	++	++	+	++	First
Larson et al. 1996 (13 weeks; 5 days/week)	–	+	++	+	++	–	+	++	First
Larson et al. 1996 (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Larson et al. 1996 (3 weeks)	–	+	++	+	++	+	+	++	First
Larson et al. 1996 (6 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 5 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (3 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (6 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1998 (13 weeks)	+	+	++	+	++	+	+	++	First
Templin et al. 1998 (3 weeks)	+	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Templin et al. 1998 (7 weeks)	+	+	++	+	++	+	+	++	First
Torkelson et al. 1976 (rat 1–4 hours/day)	–	+	++	+	–	++	+	++	First
Torkelson et al. 1976 (rat 7 hours/day)	–	+	++	+	–	++	+	++	First
Inhalation chronic-duration exposure									
Yamamoto et al. 2002 (mouse)	–	+	++	+	++	++	++	+	First
Yamamoto et al. 2002 (rat)	–	+	++	+	++	++	++	+	First
Oral acute-duration exposure									
Chu et al. 1982b	–	+	++	+	–	+	+	+	First
Ewaid et al. 2020	+	+	–	+	–	+	+	+	First
Keegan et al. 1998	–	+	++	+	++	+	+	++	First
Larson et al. 1993 (rat)	+	+	++	+	++	+	+	+	First
Larson et al. 1993 (mouse)	+	+	++	+	++	+	+	+	First
Larson et al. 1994b (GO)	+	+	+	+	+	+	++	+	First
Larson et al. 1994b (W)	+	+	+	+	+	+	++	+	First
Larson et al. 1994d	+	+	++	+	+	+	+	++	First
Larson et al. 1995a (DW)	+	+	++	+	+	+	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Larson et al. 1995a (G)	+	+	++	+	+	+	+	+	First
Larson et al. 1995b	+	+	++	+	++	+	+	+	First
Lilly et al. 1997	–	+	++	+	++	+	++	+	First
Liu et al. 2013	–	+	+	+	+	–	++	++	First
Miyagawa et al. 1998	–	+	+	+	+	–	+	+	First
Moore et al. 1982 (G)	+	+	+	+	+	–	+	++	First
Moore et al. 1982 (GO)	+	+	+	+	+	–	+	++	First
Potter et al. 1996	+	+	++	+	+	–	+	++	First
Templin et al. 1996a (Fischer 344)	+	+	++	+	++	+	+	++	First
Templin et al. 1996a (Osborne-Mendel)	+	+	++	+	++	+	+	++	First
Thompson et al. 1974 (Experiment 2, 6 F)	–	+	+	+	++	–	+	+	First
Oral intermediate-duration exposure									
Chu et al. 1982a	–	+	+	+	–	–	+	–	Second
Chu et al. 1982b	–	+	+	+	+	–	+	+	First
NTP 1988a	++	+	++	+	++	+	+	++	First
Heywood et al. 1979	–	+	+	+	+	–	–	–	Second

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Hooth et al. 2002; McDorman et al. 2003a, 2003b	+	+	++	+	++	+	+	++	First
EPA 1980 (mouse)	++	+	–	+	+	+	+	++	First
EPA 1980 (rat)	++	+	–	+	+	+	+	++	First
Larson et al. 1994b (GO)	+	+	+	+	+	+	++	+	First
Larson et al. 1994b (W)	+	+	+	+	+	+	++	+	First
Larson et al. 1994d	+	+	++	+	+	+	+	++	First
Larson et al. 1995a (GO)	+	+	++	+	+	+	+	+	First
Larson et al. 1995a (W)	+	+	++	+	+	+	+	+	First
Larson et al. 1995b	+	+	++	+	++	+	+	+	First
Lipsky et al. 1993 (GO)	–	+	++	+	+	–	+	+	First
Lipsky et al. 1993 (GW)	–	+	++	+	+	–	+	+	First
Sehata et al. 2002 (CB6F1)	–	+	++	+	+	+	++	+	First
Oral chronic-duration exposure									
Heywood et al. 1979	–	+	+	+	–	++	–	++	Second
Hard et al. 2000; Jorgenson et al. 1985 (rat)	+	+	++	+	–	++	++	++	First
Nagano et al. 2006	+	+	++	+	+	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Dunnick and Melnick 1993; NCI 1976 (rat)	+	+	++	+	++	+	+	++	First
Dunnick and Melnick 1993; NCI 1976 (mouse)	+	+	++	+	++	+	+	++	First
Roe et al. 1979 (Experiment 1)	+	+	–	+	–	++	+	++	First
Roe et al. 1979 (Experiment 3)	+	+	–	+	–	++	+	+	First
Outcome: Neurological effects									
Inhalation acute-duration exposure									
Constan et al. 1999 (Sv/129 mice)	+	–	++	–	++	+	–	++	Second
Constan et al. 1999 (B6C3F1 mice)	+	–	++	–	++	+	–	++	Second
DHA 2022	–	+	++	+	++	++	+	++	First
EPA 1978	–	+	++	–	++	–	–	++	Second
Gehring 1968	–	+	++	–	++	–	–	++	Second
Larson et al. 1994c; Mery et al. 1994 (rat)	–	+	++	+	++	+	+	++	First
Lehmann and Flury 1943 (cat)	–	–	–	–	–	–	–	–	Third
Lehmann and Flury 1943 (mouse)	–	–	–	–	–	–	–	–	Third
Inhalation intermediate-duration exposure									
Larson et al. 1996 (13 weeks; 7 days/week)	–	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Larson et al. 1996 (3 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (13 weeks)	–	+	++	+	++	+	+	++	First
Templin et al. 1996b (3 weeks)	–	+	++	+	++	+	+	++	First
Inhalation chronic-duration exposure									
Yamamoto et al. 2002 (mouse)	–	+	++	–	++	++	+	+	First
Yamamoto et al. 2002 (rat)	–	+	++	–	++	++	+	+	First
Oral acute-duration exposure									
Balster and Borzelleca 1982 (14 days)	+	+	+	+	++	–	++	++	First
Balster and Borzelleca 1982 (once)	+	+	+	+	+	–	++	+	First
Bowman et al. 1978	–	+	+	+	–	–	–	–	Third
NTP 1988a	++	+	++	+	+	+	+	–	First
Jones et al. 1958	– –	+	–	+	–	–	–	+	Third
Landauer et al. 1982	+	+	++	+	+	–	+	+	First
Oral intermediate-duration exposure									
Balster and Borzelleca 1982 (30 days)	+	+	+	+	–	–	++	+	First
Balster and Borzelleca 1982 (60 days)	+	+	+	+	–	–	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Balster and Borzelleca 1982 (90 days)	+	+	+	+	–	–	++	+	First
Chu et al. 1982a	–	+	–	+	–	–	+	–	Second
Chu et al. 1982b	–	+	–	+	+	–	+	+	First
Dorman et al. 1997	– –	+	++	+	+	+	+	++	First
Sehata et al. 2002 (CB6F1)	–	+	++	+	+	+	+	+	First
Wada et al. 2015	+	++	+	++	++	+	++	+	First
Oral chronic-duration exposure									
Heywood et al. 1979	–	–	+	–	–	++	–	+	Third
Dunnick and Melnick 1993; NCI 1976 (rat)	+	+	++	–	++	++	+	++	First
Dunnick and Melnick 1993; NCI 1976 (mouse)	+	+	++	–	++	++	+	++	First
Roe et al. 1979 (Experiment 1)	+	–	–	–	–	++	+	+	Second
Outcome: Developmental Effects									
Inhalation acute-duration exposure									
Baeder and Hofmann 1988	–	+	++	+	++	+	+	++	First
EPA 1978	–	+	++	+	++	–	+	–	Second
Murray et al. 1979 (GDs 1–7)	–	+	++	+	++	+	+	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Murray et al. 1979 (GDs 6–15)	–	+	++	+	++	+	+	++	First
Murray et al. 1979 (GDs 8–15)	–	+	++	+	++	+	+	++	First
Schwetz et al. 1974	–	+	++	+	–	+	+	++	First
Oral acute-duration exposure									
Ruddick et al. 1983	+	+	++	+	++	+	++	–	First
Thompson et al. 1974 (Experiment 1, 25 F)	–	+	++	+	++	–	+	++	First
Thompson et al. 1974 (Experiment 2, 6 F)	–	+	++	+	++	–	+	++	First
Thompson et al. 1974 (rabbit, 1 time/day)	–	+	++	+	++	–	+	++	First
Thompson et al. 1974 (rabbit, 2 times/day)	–	+	++	+	++	–	+	++	First
Oral intermediate-duration exposure									
Burkhalter and Balster 1979	+	+	++	++	–	+	++	++	First
NTP 1988a	+	+	++	+	+	++	++	++	First

Table C-9. Summary of Risk of Bias Assessment for Chloroform—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition / exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were the experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	**Is there confidence in the outcome assessment?***	Were all measured outcomes reported?	
Lim et al. 2004 (5 weeks)	+	+	++	+	–	– –	++	++	First
Lim et al. 2004 (8 weeks)	+	+	++	+	–	– –	++	++	First

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias; (DW) = drinking water; F = females; (G) = gavage; GD = gestation day; (GO) = gavage in oil; (GW) = gavage in water; (W) = water

*Key question

C.6 RATE THE CONFIDENCE IN THE BODY OF EVIDENCE FOR EACH RELEVANT OUTCOME

Confidences in the bodies of human and animal evidence were evaluated independently for each potential outcome. ATSDR did not evaluate the confidence in the body of evidence for carcinogenicity; rather, the Agency defaulted to the cancer weight-of-evidence assessment of other agencies including HHS, EPA, and IARC. The confidence in the body of evidence for an association or no association between exposure to chloroform and a particular outcome was based on the strengths and weaknesses of individual studies. Four descriptors were used to describe the confidence in the body of evidence for effects or when no effect was found:

- **High confidence:** the true effect is highly likely to be reflected in the apparent relationship
- **Moderate confidence:** the true effect may be reflected in the apparent relationship
- **Low confidence:** the true effect may be different from the apparent relationship
- **Very low confidence:** the true effect is highly likely to be different from the apparent relationship

Confidence in the body of evidence for a particular outcome was rated for each type of study: case-control, case series, cohort, population, human-controlled exposure, and experimental animal. In the absence of data to the contrary, data for a particular outcome were collapsed across animal species, routes of exposure, and exposure durations. If species (or strain), route, or exposure duration differences were noted, then the data were treated as separate outcomes.

C.6.1 Initial Confidence Rating

In ATSDR's modification to the OHAT approach, the body of evidence for an association (or no association) between exposure to chloroform and a particular outcome was given an initial confidence rating based on the key features of the individual studies examining that outcome. The presence of these key features of study design was determined for individual studies using four "yes or no" questions, which were customized for epidemiology, human controlled exposure, or experimental animal study designs. Separate questionnaires were completed for each outcome assessed in a study. The key features for observational epidemiology (cohort, population, and case-control) studies, human controlled exposure, and experimental animal studies are presented in Tables C-11, C-12, C-13, respectively. The initial confidence in the study was determined based on the number of key features present in the study design:

- **High Initial Confidence:** Studies in which the responses to the four questions were "yes".

- **Moderate Initial Confidence:** Studies in which the responses to only three of the questions were "yes".

- **Low Initial Confidence:** Studies in which the responses to only two of the questions were "yes".

- **Very Low Initial Confidence:** Studies in which the response to one or none of the questions was "yes".

Table C-10. Key Features of Study Design for Observational Epidemiology Studies

Exposure was experimentally controlled
Exposure occurred prior to the outcome
Outcome was assessed on individual level rather than at the population level
A comparison group was used

Table C-11. Key Features of Study Design for Human-Controlled Exposure Studies

A comparison group was used or the subjects served as their own control
A sufficient number of subjects were tested
Appropriate methods were used to measure outcomes (i.e., clinically-confirmed outcome versus self-reported)
Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis

Table C-12. Key Features of Study Design for Experimental Animal Studies

A concurrent control group was used
A sufficient number of animals per group were tested
Appropriate parameters were used to assess a potential adverse effect
Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis

The presence or absence of the key features and the initial confidence levels for studies examining respiratory, hepatic, renal, neurological, and developmental endpoints observed in the observational epidemiology and animal experimental studies are presented in Tables C-14 and C-15, respectively.

Table C-13. Presence of Key Features of Study Design for Chloroform—Observational Epidemiology Studies

	Key features				
Reference	Controlled exposure	Exposure prior to outcome	Outcomes assessed on an individual level	Comparison group	Initial study confidence
Outcome: Respiratory effects					
Cross-sectional studies					
Font-Ribera et al. 2010	No	Yes	Yes	Yes	Moderate

Table C-13. Presence of Key Features of Study Design for Chloroform—Observational Epidemiology Studies

Reference	Key features				Initial study confidence
	Controlled exposure	Exposure prior to outcome	Outcomes assessed on an individual level	Comparison group	
Outcome: Hepatic effects					
Cohort studies					
Aiking et al. 1994	No	Yes	Yes	Yes	Moderate
Bomski et al. 1967	No	Yes	Yes	Yes	Moderate
Challen et al. 1958	No	Yes	Yes	Yes	Moderate
Li et al. 1993	No	Yes	Yes	Yes	Moderate
Outcome: Renal effects					
Cohort studies					
Aiking et al. 1994	No	Yes	Yes	Yes	Moderate
Li et al. 1993	No	Yes	Yes	Yes	Moderate
Outcome: Neurological effects					
Cohort studies					
Challen et al. 1958	No	Yes	Yes	Yes	Moderate
Li et al. 1993	No	Yes	Yes	Yes	Moderate
Outcome: Developmental effects					
Cohort studies					
Botton et al. 2015	No	No	Yes	Yes	Low
Cao et al. 2016	No	No	Yes	Yes	Low
Costet et al. 2011	No	Yes	Yes	Yes	Moderate
Dodds and King 2001	No	Yes	Yes	Yes	Moderate
Grazuleviciene et al. 2011	No	Yes	Yes	Yes	Moderate
Grazuleviciene et al. 2013	No	Yes	Yes	Yes	Moderate
Hinckley et al. 2005	No	No	Yes	Yes	Low
Hoffman et al. 2008	No	Yes	Yes	Yes	Moderate
Liu et al. 2021	No	Yes	Yes	Yes	Moderate
Rivera-Núñez and Wright 2013	No	No	Yes	Yes	Low
Sun et al. 2020	No	Yes	Yes	Yes	Moderate
Villanueva et al. 2018	No	Yes	Yes	Yes	Moderate
Villanueva et al. 2011	No	No	Yes	Yes	Low
Zhu et al. 2022	No	Yes	Yes	Yes	Moderate
Population studies					
Porter et al. 2005	No	Yes	Yes	Yes	Moderate
Wright et al. 2004	No	Yes	Yes	Yes	Moderate

Table C-13. Presence of Key Features of Study Design for Chloroform—Observational Epidemiology Studies

Reference	Key features				Initial study confidence
	Controlled exposure	Exposure prior to outcome	Outcomes assessed on an individual level	Comparison group	
Case-control studies					
Bonou et al. 2017	No	Yes	Yes	Yes	Moderate
Kaufman et al. 2018	No	Yes	Yes	Yes	Moderate
Kaufman et al. 2020	No	Yes	Yes	Yes	Moderate
Kramer et al. 1992	No	Yes	Yes	Yes	Moderate
Levallois et al. 2012	No	Yes	Yes	Yes	Moderate
Summerhayes et al. 2012	No	Yes	Yes	Yes	Moderate
Swartz et al. 2015a, 2015b	No	No	Yes	Yes	Low
Zaganjor et al. 2020	No	Yes	Yes	Yes	Moderate

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				Initial study confidence
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	
Outcome: Respiratory effects					
Inhalation acute-duration exposure					
Constan et al. 1999 (Sv/129 mice)	Yes	Yes	Yes	Yes	High
Constan et al. 1999 (B6C3F1 mice)	Yes	Yes	Yes	Yes	High
de Oliveira et al. 2015	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (mouse)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (4 days)	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Inhalation intermediate-duration exposure					
Kasai et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 5 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 7 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (3 weeks)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (6 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (3 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (6 weeks)	Yes	Yes	Yes	Yes	High
Inhalation chronic-duration exposure					
Yamamoto et al. 2002 (mouse); additional information from unpublished study (MHLW 1994a, 1994b)	Yes	Yes	Yes	Yes	High
Yamamoto et al. 2002 (rat); additional information from unpublished study (MHLW 1994a, 1994b)	Yes	Yes	Yes	Yes	High
Oral acute-duration exposure					
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Templin et al. 1996a (Fischer 344)	Yes	Yes	Yes	Yes	High
Templin et al. 1996a (Osborne-Mendel)	Yes	Yes	Yes	Yes	High
Oral intermediate-duration exposure					
Chu et al. 1982a	Yes	Yes	Yes	No	Moderate
Chu et al. 1982b	Yes	Yes	Yes	No	Moderate
Dorman et al. 1997	Yes	Yes	Yes	Yes	High
NTP 1988a	Yes	Yes	Yes	Yes	High
EPA 1980 (mouse)	Yes	Yes	Yes	Yes	High
EPA 1980 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Sehata et al. 2002 (CB6F1)	Yes	Yes	Yes	Yes	High
Oral chronic-duration exposure					
Dunnick and Melnick 1993; NCI 1976 (mouse)	Yes	Yes	Yes	No	Moderate

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features: Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Dunnick and Melnick 1993; NCI 1976 (rat)	Yes	Yes	Yes	No	Moderate
Roe et al. 1979 (Experiment 1)	Yes	Yes	No	No	Low
Outcome: Liver effects					
Inhalation acute-duration exposure					
Baeder and Hofmann 1988	Yes	Yes	No	Yes	Moderate
Constan et al. 1999 (Sv/129 mice)	Yes	Yes	Yes	Yes	High
Constan et al. 1999 (B6C3F1 mice)	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (mouse)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (4 days)	Yes	Yes	Yes	Yes	High
Templin et al. 1996c (2 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1996c (4 days)	Yes	No	Yes	Yes	Moderate
Inhalation intermediate-duration exposure					
Kasai et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 5 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 7 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (3 weeks)	Yes	No	Yes	Yes	Moderate
Larson et al. 1996 (6 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (3 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1996b (6 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1998 (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1998 (3 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1998 (7 weeks)	Yes	No	Yes	Yes	Moderate
Torkelson et al. 1976 (rat 1–4 hours/day)	Yes	Yes	Yes	Yes	High
Torkelson et al. 1976 (rat 7 hours/day)	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Inhalation chronic-duration exposure					
Yamamoto et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Yamamoto et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Oral acute-duration exposure					
Chu et al. 1982b	Yes	Yes	Yes	No	Moderate
Ewaid et al. 2020	Yes	No	Yes	No	Low
Jones et al. 1958	No	Yes	Yes	No	Low
Keegan et al. 1998	Yes	Yes	Yes	Yes	High
Larson et al. 1993 (mouse)	Yes	Yes	Yes	No	Moderate
Larson et al. 1993 (rat)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994d	Yes	Yes	Yes	No	Moderate
Larson et al. 1995a (DW)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995a (G)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Lilly et al. 1997	Yes	Yes	Yes	Yes	High
Miyagawa et al. 1998	Yes	Yes	Yes	No	Moderate
Moore et al. 1982 (G)	Yes	Yes	Yes	Yes	High
Moore et al. 1982 (GO)	Yes	Yes	Yes	Yes	High
Munson et al. 1982	Yes	Yes	Yes	Yes	High
Templin et al. 1996a (Fischer 344)	Yes	Yes	Yes	Yes	High
Templin et al. 1996a (Osborne-Mendel)	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (Experiment 2, 6 F)	Yes	Yes	Yes	No	Moderate
Wada et al. 2015	Yes	Yes	Yes	Yes	High
Wang et al. 1997	Yes	Yes	Yes	Yes	High
Oral intermediate-duration exposure					
Bull et al. 1986 (GO)	Yes	Yes	Yes	No	Moderate
Bull et al. 1986 (GW)	Yes	Yes	Yes	No	Moderate
Chu et al. 1982a	Yes	Yes	Yes	Yes	High
Chu et al. 1982b	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Eschenbrenner and Miller 1945	Yes	Yes	No	Yes	Moderate
NTP 1988a	Yes	Yes	Yes	Yes	High
Heywood et al. 1979	Yes	Yes	Yes	No	Moderate
EPA 1980 (mouse)	Yes	Yes	Yes	Yes	High
EPA 1980 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1994b (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994d	Yes	Yes	Yes	Yes	High
Larson et al. 1995a (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995a (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Melnick et al. 1998	Yes	Yes	Yes	Yes	High
Mostafa et al. 2009	No	Yes	Yes	No	Low
Munson et al. 1982	Yes	Yes	Yes	No	Moderate
Sehata et al. 2002 (CB6F1)	Yes	Yes	Yes	Yes	High
Oral chronic-duration exposure					
Heywood et al. 1979	Yes	Yes	Yes	No	Moderate
Dunnick and Melnick 1993; NCI 1976 (mouse)	Yes	Yes	Yes	No	Moderate
Dunnick and Melnick 1993; NCI 1976 (rat)	Yes	Yes	Yes	No	Moderate
Roe et al. 1979 (Experiment 1)	Yes	Yes	Yes	No	Moderate
Outcome: Kidney effects					
Inhalation acute-duration exposure					
Baeder and Hofmann 1988	Yes	Yes	No	Yes	Moderate
Constan et al. 1999 (Sv/129 mice)	Yes	Yes	Yes	Yes	High
Constan et al. 1999 (B6C3F1 mice)	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (mouse)	Yes	Yes	Yes	Yes	High
Larson et al. 1994c; Mery et al. 1994 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (4 days)	Yes	Yes	Yes	Yes	High
Templin et al. 1996c (2 weeks)	Yes	No	Yes	Yes	Moderate

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features: Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Templin et al. 1996c (4 days)	Yes	No	Yes	Yes	Moderate
Inhalation intermediate-duration exposure					
Kasai et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Kasai et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 5 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (13 weeks; 7 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (3 weeks)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (6 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (3 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (6 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1998 (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1998 (3 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1998 (7 weeks)	Yes	Yes	Yes	Yes	High
Torkelson et al. 1976 (rat 1–4 hours/day)	Yes	Yes	Yes	Yes	High
Torkelson et al. 1976 (rat 7 hours/day)	Yes	Yes	Yes	Yes	High
Inhalation chronic-duration exposure					
Yamamoto et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Yamamoto et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Oral acute-duration exposure					
Chu et al. 1982b	Yes	Yes	Yes	No	Moderate
Ewaid et al. 2020	Yes	No	Yes	No	Low
Keegan et al. 1998	Yes	Yes	No	Yes	Moderate
Larson et al. 1993 (rat)	Yes	Yes	Yes	No	Moderate
Larson et al. 1993 (mouse)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994d	Yes	Yes	Yes	Yes	High
Larson et al. 1995a (DW)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995a (G)	Yes	Yes	Yes	No	Moderate

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Lilly et al. 1997	Yes	Yes	Yes	Yes	High
Liu et al. 2013	Yes	Yes	Yes	Yes	High
Miyagawa et al. 1998	Yes	Yes	Yes	No	Moderate
Moore et al. 1982 (G)	Yes	Yes	Yes	Yes	High
Moore et al. 1982 (GO)	Yes	Yes	Yes	Yes	High
Potter et al. 1996	Yes	Yes	Yes	Yes	High
Templin et al. 1996a (Fischer 344)	Yes	Yes	Yes	Yes	High
Templin et al. 1996a (Osborne-Mendel)	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (Experiment 2, 6 F)	Yes	Yes	Yes	No	Moderate
Oral intermediate-duration exposure					
Chu et al. 1982a	Yes	Yes	Yes	Yes	High
Chu et al. 1982b	Yes	Yes	Yes	Yes	High
NTP 1988a	Yes	Yes	Yes	Yes	High
Heywood et al. 1979	Yes	Yes	No	No	Low
Hooth et al. 2002; McDorman et al. 2003a, 2003b	Yes	Yes	Yes	Yes	High
EPA 1980 (mouse)	Yes	Yes	Yes	Yes	High
EPA 1980 (rat)	Yes	Yes	Yes	Yes	High
Larson et al. 1994b (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994b (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1994d	Yes	Yes	Yes	Yes	High
Larson et al. 1995a (GO)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995a (W)	Yes	Yes	Yes	No	Moderate
Larson et al. 1995b	Yes	Yes	Yes	No	Moderate
Lipsky et al. 1993 (GO)	Yes	Yes	Yes	No	Moderate
Lipsky et al. 1993 (GW)	Yes	Yes	Yes	No	Moderate
Sehata et al. 2002 (CB6F1)	Yes	Yes	Yes	Yes	High
Oral chronic-duration exposure					
Heywood et al. 1979	Yes	Yes	Yes	No	Moderate
Hard et al. 2000; Jorgenson et al. 1985 (rat)	Yes	Yes	Yes	Yes	High
Nagano et al. 2006	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features: Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Dunnick and Melnick 1993; NCI 1976 (rat)	Yes	Yes	Yes	No	Moderate
Dunnick and Melnick 1993; NCI 1976 (mouse)	Yes	Yes	Yes	No	Moderate
Roe et al. 1979 (Experiment 1)	Yes	Yes	Yes	No	Moderate
Roe et al. 1979 (Experiment 3)	Yes	Yes	Yes	Yes	High
Outcome: Neurological effects					
Inhalation acute-duration exposure					
Constan et al. 1999 (Sv/129 mice)	Yes	Yes	No	Yes	Moderate
Constan et al. 1999 (B6C3F1 mice)	Yes	Yes	No	Yes	Moderate
DHA 2022	Yes	Yes	Yes	Yes	High
EPA 1978	Yes	Yes	No	Yes	Moderate
Gehring 1968	Yes	Yes	No	Yes	Moderate
Larson et al. 1994c; Mery et al. 1994 (rat)	Yes	Yes	Yes	Yes	High
Lehmann and Flury 1943 (cat)	No	No	No	No	Very low
Lehmann and Flury 1943 (mouse)	No	No	No	No	Very low
Inhalation intermediate-duration exposure					
Larson et al. 1996 (13 weeks; 7 days/week)	Yes	Yes	Yes	Yes	High
Larson et al. 1996 (3 weeks)	Yes	No	Yes	Yes	Moderate
Templin et al. 1996b (13 weeks)	Yes	Yes	Yes	Yes	High
Templin et al. 1996b (3 weeks)	Yes	No	Yes	Yes	Moderate
Inhalation chronic-duration exposure					
Yamamoto et al. 2002 (mouse)	Yes	Yes	Yes	Yes	High
Yamamoto et al. 2002 (rat)	Yes	Yes	Yes	Yes	High
Oral acute-duration exposure					
Balster and Borzelleca 1982 (14 days)	Yes	Yes	Yes	Yes	High
Balster and Borzelleca 1982 (once)	No	Yes	Yes	Yes	Moderate
Bowman et al. 1978	No	Yes	No	No	Low
NTP 1988a	Yes	Yes	Yes	No	Moderate
Jones et al. 1958	No	Yes	Yes	No	Low
Landauer et al. 1982	Yes	Yes	Yes	Yes	High
Oral intermediate-duration exposure					
Balster and Borzelleca 1982 (30 days)	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features				
	Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Balster and Borzelleca 1982 (60 days)	Yes	Yes	Yes	Yes	High
Balster and Borzelleca 1982 (90 days)	Yes	Yes	Yes	Yes	High
Chu et al. 1982a	Yes	Yes	Yes	Yes	High
Chu et al. 1982b	Yes	Yes	Yes	Yes	High
Dorman et al. 1997	Yes	Yes	Yes	Yes	High
Sehata et al. 2002 (CB6F1)	Yes	Yes	Yes	Yes	High
Wada et al. 2015	Yes	Yes	Yes	Yes	High
Oral chronic-duration exposure					
Heywood et al. 1979	Yes	Yes	Yes	No	Moderate
Dunnick and Melnick 1993; NCI 1976 (rat)	Yes	Yes	Yes	No	Moderate
Dunnick and Melnick 1993; NCI 1976 (mouse)	Yes	Yes	Yes	No	Moderate
Roe et al. 1979 (Experiment 1)	Yes	Yes	Yes	No	Moderate
Outcome: Developmental Effects					
Inhalation acute-duration exposure					
Baeder and Hofmann 1988	Yes	Yes	Yes	Yes	High
EPA 1978	Yes	Yes	Yes	No	Moderate
Murray et al. 1979 (GDs 1–7)	Yes	Yes	Yes	Yes	High
Murray et al. 1979 (GDs 6–15)	Yes	Yes	Yes	Yes	High
Murray et al. 1979 (GDs 8–15)	Yes	Yes	Yes	Yes	High
Schwetz et al. 1974	Yes	No	Yes	Yes	Moderate
Oral acute-duration exposure					
Ruddick et al. 1983	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (Experiment 1, 25 F)	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (Experiment 2, 6 F)	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (rabbit, 1 time/day)	Yes	Yes	Yes	Yes	High
Thompson et al. 1974 (rabbit, 2 times/day)	Yes	Yes	Yes	Yes	High
Oral intermediate-duration exposure					
Burkhalter and Balster 1979	Yes	No	Yes	Yes	Moderate
NTP 1988a	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Chloroform—Experimental Animal Studies

Reference	Key features: Concurrent control group	Sufficient number of subjects	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Lim et al. 2004 (5 weeks)	Yes	Yes	Yes	Yes	High
Lim et al. 2004 (8 weeks)	Yes	Yes	Yes	Yes	High

(DW) = drinking water; F = females; (G) = gavage; GD = gestation day; (G) = gavage in water; (GW) = gavage in water; (W) = water

A summary of the initial confidence ratings for each outcome is presented in Table C-15. If individual studies for a particular outcome and study type had different study quality ratings, then the highest confidence rating for the group of studies was used to determine the initial confidence rating for the body of evidence; any exceptions were noted in Table C-16.

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Outcome: Respiratory effects		
Inhalation acute-duration exposure		
Human studies		
Font-Ribera et al. 2010	Moderate	Moderate
Animal studies		
Constan et al. 1999 (Sv/129 mice)	High	High
Constan et al. 1999 (B6C3F1 mice)	High	
de Oliveira et al. 2015	High	
Kasai et al. 2002 (rat)	High	
Larson et al. 1994c; Mery et al. 1994 (mouse)	High	
Larson et al. 1994c; Mery et al. 1994 (rat)	High	
Larson et al. 1996	High	
Templin et al. 1996b (4 days)	High	
Inhalation intermediate-duration exposure		
Animal studies		
Kasai et al. 2002 (mouse)	High	High
Kasai et al. 2002 (rat)	High	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Larson et al. 1996 (13 weeks; 5 days/week)	High	
Larson et al. 1996 (13 weeks; 7 days/week)	High	
Larson et al. 1996 (3 weeks)	High	
Larson et al. 1996 (6 weeks)	High	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (3 weeks)	High	
Templin et al. 1996b (6 weeks)	High	
Inhalation chronic-duration exposure		
Animal studies		
Yamamoto et al. 2002 (mouse)	Moderate	Moderate
Yamamoto et al. 2002 (rat)	Moderate	
Oral acute-duration exposure		
Animal studies		
Larson et al. 1995b	Moderate	
Templin et al. 1996a (Fischer 344)	High	High
Templin et al. 1996a (Osborne-Mendel)	High	
Oral intermediate-duration exposure		
Animal studies		
Chu et al. 1982a	Moderate	
Chu et al. 1982b	Moderate	
Dorman et al. 1997	High	
NTP 1988a	High	
EPA 1980 (mouse)	High	High
EPA 1980 (rat)	High	
Larson et al. 1995b	Moderate	
Sehata et al. 2002 (CB6F1)	High	
Oral chronic-duration exposure		
Animal studies		
Dunnick and Melnick 1993; NCI 1976 (mouse)	Moderate	
Dunnick and Melnick 1993; NCI 1976 (rat)	Moderate	Moderate
Roe et al. 1979 (Experiment 1)	Low	
Outcome: Hepatic effects		
Inhalation acute-duration exposure		
Human studies		
Aiking et al. 1994	Moderate	Moderate
Animal studies		
Baeder and Hofmann 1988	Moderate	
Constan et al. 1999 (Sv/129 mice)	High	
Constan et al. 1999 (B6C3F1 mice)	High	High
Kasai et al. 2002 (rat)	High	
Larson et al. 1994c; Mery et al. 1994 (mouse)	High	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Larson et al. 1994c; Mery et al. 1994 (rat)	High	
Larson et al. 1996	High	
Templin et al. 1996b (4 days)	High	
Templin et al. 1996c (2 weeks)	Moderate	
Templin et al. 1996c (4 days)	Moderate	
Inhalation intermediate-duration exposure		
Animal studies		
Kasai et al. 2002 (mouse)	High	
Kasai et al. 2002 (rat)	High	
Larson et al. 1996 (13 weeks; 5 days/week)	High	
Larson et al. 1996 (13 weeks; 7 days/week)	High	
Larson et al. 1996 (3 weeks)	Moderate	
Larson et al. 1996 (6 weeks)	Moderate	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (13 weeks)	High	High
Templin et al. 1996b (3 weeks)	Moderate	
Templin et al. 1996b (6 weeks)	Moderate	
Templin et al. 1998 (13 weeks)	High	
Templin et al. 1998 (3 weeks)	Moderate	
Templin et al. 1998 (7 weeks)	Moderate	
Torkelson et al. 1976 (rat 1–4 hours/day)	High	
Torkelson et al. 1976 (rat 7 hours/day)	High	
Inhalation chronic-duration exposure		
Human studies		
Bomski et al. 1967	Moderate	
Challen et al. 1958	Moderate	Moderate
Li et al. 1993	Moderate	
Animal studies		
Yamamoto et al. 2002 (mouse)	High	High
Yamamoto et al. 2002 (rat)	High	
Oral acute-duration exposure		
Animal studies		
Chu et al. 1982b	Moderate	
Ewaid et al. 2020	Low	
Jones et al. 1958	Low	
Keegan et al. 1998	High	
Larson et al. 1993 (mouse)	Moderate	High
Larson et al. 1993 (rat)	Moderate	
Larson et al. 1994b (GO)	Moderate	
Larson et al. 1994b (W)	Moderate	
Larson et al. 1994d	Moderate	
Larson et al. 1995a (DW)	Moderate	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Larson et al. 1995a (G)	Moderate	
Larson et al. 1995b	Moderate	
Lilly et al. 1997	High	
Miyagawa et al. 1998	Moderate	
Moore et al. 1982 (G)	High	
Moore et al. 1982 (GO)	High	
Munson et al. 1982	High	
Templin et al. 1996a (Fischer 344)	High	
Templin et al. 1996a (Osborne-Mendel)	High	
Thompson et al. 1974 (Experiment 2, 6 F)	Moderate	
Wada et al. 2015	High	
Wang et al. 1997	High	
Oral intermediate-duration exposure		
Animal studies		
Bull et al. 1986 (GO)	Moderate	
Bull et al. 1986 (GW)	Moderate	
Chu et al. 1982a	High	
Chu et al. 1982b	High	
Eschenbrenner and Miller 1945	Moderate	
NTP 1988a	High	
Heywood et al. 1979	Moderate	
EPA 1980 (mouse)	High	
EPA 1980 (rat)	High	
Larson et al. 1994b (GO)	Moderate	High
Larson et al. 1994b (W)	Moderate	
Larson et al. 1994d	High	
Larson et al. 1995a (GO)	Moderate	
Larson et al. 1995a (W)	Moderate	
Larson et al. 1995b	Moderate	
Melnick et al. 1998	High	
Mostafa et al. 2009	Low	
Munson et al. 1982	Moderate	
Sehata et al. 2002 (CB6F1)	High	
Oral chronic-duration exposure		
Animal studies		
Heywood et al. 1979	Moderate	
Dunnick and Melnick 1993; NCI 1976 (mouse)	Moderate	Moderate
Dunnick and Melnick 1993; NCI 1976 (rat)	Moderate	
Roe et al. 1979 (Experiment 1)	Moderate	
Outcome: Renal effects		
Inhalation acute-duration exposure		
Animal studies		

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Baeder and Hofmann 1988	Moderate	High
Constan et al. 1999 (Sv/129 mice)	High	
Constan et al. 1999 (B6C3F1 mice)	High	
Kasai et al. 2002 (rat)	High	
Larson et al. 1994c; Mery et al. 1994 (mouse)	High	
Larson et al. 1994c; Mery et al. 1994 (rat)	High	
Larson et al. 1996	High	
Templin et al. 1996b (4 days)	High	
Templin et al. 1996c (2 weeks)	Moderate	
Templin et al. 1996c (4 days)	Moderate	
Inhalation intermediate-duration exposure		
Animal studies		
Kasai et al. 2002 (mouse)	High	High
Kasai et al. 2002 (rat)	High	
Larson et al. 1996 (13 weeks; 5 days/week)	High	
Larson et al. 1996 (13 weeks; 7 days/week)	High	
Larson et al. 1996 (3 weeks)	High	
Larson et al. 1996 (6 weeks)	High	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (3 weeks)	High	
Templin et al. 1996b (6 weeks)	High	
Templin et al. 1998 (13 weeks)	High	
Templin et al. 1998 (3 weeks)	High	
Templin et al. 1998 (7 weeks)	High	
Torkelson et al. 1976 (rat 1–4 hours/day)	High	
Torkelson et al. 1976 (rat 7 hours/day)	High	
Inhalation chronic-duration exposure		
Human studies		
Aiking et al. 1994	Moderate	Moderate
Li et al. 1993	Moderate	
Animal studies		
Yamamoto et al. 2002 (mouse)	High	High
Yamamoto et al. 2002 (rat)	High	
Oral acute-duration exposure		
Animal studies		
Chu et al. 1982b	Moderate	High
Ewaid et al. 2020	Low	
Keegan et al. 1998	Moderate	
Larson et al. 1993 (rat)	Moderate	
Larson et al. 1993 (mouse)	Moderate	
Larson et al. 1994b (GO)	Moderate	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Larson et al. 1994b (W)	Moderate	
Larson et al. 1994d	High	
Larson et al. 1995a (DW)	Moderate	
Larson et al. 1995a (G)	Moderate	
Larson et al. 1995b	Moderate	
Lilly et al. 1997	High	
Liu et al. 2013	High	
Miyagawa et al. 1998	Moderate	
Moore et al. 1982 (G)	High	
Moore et al. 1982 (GO)	High	
Potter et al. 1996	High	
Templin et al. 1996a (Fischer 344)	High	
Templin et al. 1996a (Osborne-Mendel)	High	
Thompson et al. 1974 (Experiment 2, 6 F)	Moderate	
Oral intermediate-duration exposure		
Animal studies		
Chu et al. 1982a	High	
Chu et al. 1982b	High	
NTP 1988a	High	
Heywood et al. 1979	Low	
Hooth et al. 2002; McDorman et al. 2003a, 2003b	High	
EPA 1980 (mouse)	High	
EPA 1980 (rat)	High	
Larson et al. 1994b (GO)	Moderate	High
Larson et al. 1994b (W)	Moderate	
Larson et al. 1994d	High	
Larson et al. 1995a (GO)	Moderate	
Larson et al. 1995a (W)	Moderate	
Larson et al. 1995b	Moderate	
Lipsky et al. 1993 (GO)	Moderate	
Lipsky et al. 1993 (GW)	Moderate	
Sehata et al. 2002 (CB6F1)	High	
Oral chronic-duration exposure		
Animal studies		
Heywood et al. 1979	Moderate	
Hard et al. 2000; Jorgenson et al. 1985 (rat)	High	
Nagano et al. 2006	High	
Dunnick and Melnick 1993; NCI 1976 (rat)	Moderate	Moderate
Dunnick and Melnick 1993; NCI 1976 (mouse)	Moderate	
Roe et al. 1979 (Experiment 1)	Moderate	
Roe et al. 1979 (Experiment 3)	High	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Outcome: Neurological effects		
Inhalation acute-duration exposure		
Animal studies		
Constan et al. 1999 (Sv/129 mice)	Moderate	High
Constan et al. 1999 (B6C3F1 mice)	Moderate	
DHA 2022	High	
EPA 1978	Moderate	
Gehring 1968	Moderate	
Larson et al. 1994c; Mery et al. 1994 (rat)	High	
Lehmann and Flury 1943 (cat)	Very low	
Lehmann and Flury 1943 (mouse)	Very low	
Inhalation intermediate-duration exposure		
Animal studies		
Larson et al. 1996 (13 weeks; 7 days/week)	High	High
Larson et al. 1996 (3 weeks)	Moderate	
Templin et al. 1996b (13 weeks)	High	
Templin et al. 1996b (3 weeks)	Moderate	
Inhalation chronic-duration exposure		
Human studies		
Challen et al. 1958	Moderate	Moderate
Li et al. 1993	Moderate	
Animal studies		
Yamamoto et al. 2002 (mouse)	High	High
Yamamoto et al. 2002 (rat)	High	
Oral acute-duration exposure		
Animal studies		
Balster and Borzelleca 1982 (14 days)	High	High
Balster and Borzelleca 1982 (once)	Moderate	
Bowman et al. 1978	Low	
NTP 1988a	Moderate	
Jones et al. 1958	Low	
Landauer et al. 1982	High	
Oral intermediate-duration exposure		
Animal studies		
Balster and Borzelleca 1982 (30 days)	High	High
Balster and Borzelleca 1982 (60 days)	High	
Balster and Borzelleca 1982 (90 days)	High	
Chu et al. 1982a	High	
Chu et al. 1982b	High	
Dorman et al. 1997	High	
Sehata et al. 2002 (CB6F1)	High	
Wada et al. 2015	High	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Oral chronic-duration exposure		
Animal studies		
Heywood et al. 1979	Moderate	Moderate
Dunnick and Melnick 1993; NCI 1976 (rat)	Moderate	
Dunnick and Melnick 1993; NCI 1976 (mouse)	Moderate	
Roe et al. 1979 (Experiment 1)	Moderate	
Outcome: Developmental effects		
Inhalation acute-duration exposure		
Animal studies		
Baeder and Hofmann 1988	High	High
EPA 1978	Moderate	
Murray et al. 1979 (GDs 1–7)	High	
Murray et al. 1979 (GDs 6–15)	High	
Murray et al. 1979 (GDs 8–15)	High	
Schwetz et al. 1974	Moderate	
Inhalation chronic-duration exposure		
Human studies		
Swartz et al. 2015a, 2015b	Low	Low
Oral acute-duration exposure		
Animal studies		
Ruddick et al. 1983	High	High
Thompson et al. 1974 (Experiment 1, 25 F)	High	
Thompson et al. 1974 (Experiment 2, 6 F)	High	
Thompson et al. 1974 (rabbit, 1 time/day)	High	
Thompson et al. 1974 (rabbit, 2 times/day)	High	
Oral chronic-duration exposure		
Human studies		
Bonou et al. 2017	Moderate	Moderate
Botton et al. 2015	Low	
Cao et al. 2016	Low	
Costet et al. 2011	Moderate	
Dodds and King 2001	Moderate	
Grazuleviciene et al. 2011	Moderate	
Grazuleviciene et al. 2013	Moderate	
Hinckley et al. 2005	Low	
Hoffman et al. 2008	Moderate	
Kaufman et al. 2018	Moderate	
Kaufman et al. 2020	Moderate	
Kramer et al. 1992	Moderate	
Levallois et al. 2012	Moderate	
Liu et al. 2021	Moderate	
Porter et al. 2005	Moderate	

Table C-15. Initial Confidence Rating for Chloroform Health Effects Studies

	Initial study confidence	Initial confidence rating
Rivera-Núñez and Wright 2013	Low	
Summerhayes et al. 2012	Moderate	
Sun et al. 2020	Moderate	
Villanueva et al. 2011	Moderate	
Villanueva et al. 2018	Low	
Wright et al. 2004	Moderate	
Zaganjor et al. 2020	Moderate	
Zhu et al. 2022	Moderate	
Animal studies		
Burkhalter and Balster 1979	Moderate	High
NTP 1988a	High	
Lim et al. 2004 (5 weeks)	High	
Lim et al. 2004 (8 weeks)	High	

(DW) = drinking water; F = females; (G) = gavage; GD = gestation day; (G) = gavage in water; (GW) = gavage in water; (W) = water

Table C-16. Adjustments to the Initial Confidence in the Body of Evidence

	Initial confidence	Adjustments to the initial confidence rating	Final confidence
Outcome: Respiratory effects			
Human studies	Moderate	-1 Risk of bias	Low
Animal studies	High		High
Outcome: Hepatic effects			
Human studies	Moderate	-1 Risk of bias	Low
Animal studies	High	+1 Consistency in the body of evidence	High
Outcome: Renal effects			
Human studies	Moderate	-1 Risk of bias	Low
Animal studies	High	+1 Consistency in the body of evidence	High
Outcome: Neurological effects			
Human studies	Moderate	-1 Risk of bias	Low
Animal studies	High	-1 Risk of bias +1 Large magnitude of effect	High
Outcome: Developmental effects			
Human studies	Moderate	-1 Risk of bias -1 Unexplained inconsistencies	Very low
Animal studies	High	-1 Unexplained inconsistencies	Moderate

C.6.2 Adjustment of the Confidence Rating

The initial confidence rating was then downgraded or upgraded depending on whether there were substantial issues that would decrease or increase confidence in the body of evidence. The nine properties of the body of evidence that were considered are listed below. The summaries of the assessment of the confidence in the body of evidence for respiratory, hepatic, renal, neurological, and developmental effects are presented in Table C-17. For epidemiological data, if the confidence ratings for a particular outcome were based on more than one type of human study, then the highest confidence rating was used for subsequent analyses. The initial confidence reflects the body of evidence for the health outcome across all exposure routes and durations. Adjustments to the initial confidence are based on the properties discussed below and shown in Table C-16. If a property is not shown in Table C-16, ATSDR concluded that the property neither increases nor decreases confidence in the corresponding health outcome.

Table C-17. Confidence in the Body of Evidence for Chloroform

	Confidence in body of evidence	
Outcome	Human studies	Animal studies
Respiratory effects	Low	High
Hepatic effects	Low	High
Renal effects	Low	High
Neurological effects	Low	High
Developmental effects	Very low	Moderate

Five properties of the body of evidence were considered to determine whether the confidence rating should be downgraded:

- **Risk of bias.** Evaluation of whether there is substantial risk of bias across most of the studies examining the outcome. This evaluation used the risk of bias tier groupings for individual studies examining a particular outcome (Tables C-9 and C-10). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for risk of bias:
    - No downgrade if most studies are in the risk of bias first tier
    - Downgrade one confidence level if most studies are in the risk of bias second tier
    - Downgrade two confidence levels if most studies are in the risk of bias third tier

- **Unexplained inconsistency.** Evaluation of whether there is inconsistency or large variability in the magnitude or direction of estimates of effect across studies that cannot be explained. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for unexplained inconsistency:
    - No downgrade if there is little inconsistency across studies or if only one study evaluated the outcome
    - Downgrade one confidence level if there is variability across studies in the magnitude or direction of the effect
    - Downgrade two confidence levels if there is substantial variability across studies in the magnitude or direct of the effect

- **Indirectness.** Evaluation of four factors that can affect the applicability, generalizability, and relevance of the studies:
    - Relevance of the animal model to human health—unless otherwise indicated, studies in rats, mice, and other mammalian species are considered relevant to humans
    - Directness of the endpoints to the primary health outcome—examples of secondary outcomes or nonspecific outcomes include organ weight in the absence of histopathology or clinical chemistry findings in the absence of target tissue effects
    - Nature of the exposure in human studies and route of administration in animal studies—inhalation, oral, and dermal exposure routes are considered relevant unless there are compelling data to the contrary
    - Duration of treatment in animal studies and length of time between exposure and outcome assessment in animal and prospective human studies—this should be considered on an outcome-specific basis

  Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for indirectness:
    - No downgrade if none of the factors are considered indirect
    - Downgrade one confidence level if one of the factors is considered indirect
    - Downgrade two confidence levels if two or more of the factors are considered indirect

- **Imprecision.** Evaluation of the narrowness of the effect size estimates and whether the studies have adequate statistical power. Data are considered imprecise when the ratio of the upper to lower 95% CIs for most studies is ≥10 for tests of ratio measures (e.g., odds ratios) and ≥100 for absolute measures (e.g., percent control response). Adequate statistical power is determined if the study can detect a potentially biologically meaningful difference between groups (20% change from control response for categorical data or risk ratio of 1.5 for continuous data). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for imprecision:
    - No downgrade if there are no serious imprecisions
    - Downgrade one confidence level for serious imprecisions
    - Downgrade two confidence levels for very serious imprecisions

- **Publication bias.** Evaluation of the concern that studies with statistically significant results are more likely to be published than studies without statistically significant results.
    - Downgrade one level of confidence for cases where there is serious concern with publication bias

Four properties of the body of evidence were considered to determine whether the confidence rating should be upgraded:

- **Large magnitude of effect.** Evaluation of whether the magnitude of effect is sufficiently large so that it is unlikely to have occurred as a result of bias from potential confounding factors.
    - Upgrade one confidence level if there is evidence of a large magnitude of effect in a few studies, provided that the studies have an overall low risk of bias and there is no serious unexplained inconsistency among the studies of similar dose or exposure levels; confidence can also be upgraded if there is one study examining the outcome, provided that the study has an overall low risk of bias

- **Dose response.** Evaluation of the dose-response relationships measured within a study and across studies. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
    - Upgrade one confidence level for evidence of a monotonic dose-response gradient
    - Upgrade one confidence level for evidence of a non-monotonic dose-response gradient where there is prior knowledge that supports a non-monotonic dose-response and a non-monotonic dose-response gradient is observed across studies

- **Plausible confounding or other residual biases.** This factor primarily applies to human studies and is an evaluation of unmeasured determinants of an outcome such as residual bias towards the null (e.g., "healthy worker" effect) or residual bias suggesting a spurious effect (e.g., recall bias). Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
    - Upgrade one confidence level for evidence that residual confounding or bias would underestimate an apparent association or treatment effect (i.e., bias toward the null) or suggest a spurious effect when results suggest no effect

- **Consistency in the body of evidence.** Evaluation of consistency across animal models and species, consistency across independent studies of different human populations and exposure scenarios, and consistency across human study types. Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
    - Upgrade one confidence level if there is a high degree of consistency in the database

C.7 TRANSLATE CONFIDENCE RATING INTO LEVEL OF EVIDENCE OF HEALTH EFFECTS

In the seventh step of the systematic review of the health effects data for chloroform, the confidence in the body of evidence for specific outcomes was translated to a level of evidence rating. The level of evidence rating reflected the confidence in the body of evidence and the direction of the effect (i.e., toxicity or no toxicity); route-specific differences were noted. The level of evidence for health effects was rated on a five-point scale:

- **High level of evidence:** High confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Moderate level of evidence:** Moderate confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Low level of evidence:** Low confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Evidence of no health effect:** High confidence in the body of evidence that exposure to the substance is not associated with the health outcome
- **Inadequate evidence:** Low or moderate confidence in the body of evidence that exposure to the substance is not associated with the health outcome OR very low confidence in the body of evidence for an association between exposure to the substance and the health outcome

A summary of the level of evidence of health effects for chloroform is presented in Table C-18.

Table C-18. Level of Evidence of Health Effects for Chloroform

Outcome	Confidence in body of evidence	Direction of health effect	Level of evidence for health effect
Human studies			
Respiratory effects	Low	No Effect	Inadequate evidence
Hepatic effects	Low	Effect	Low
Renal effects	Low	Effect	Low
Neurological effects	Low	Effect	Low
Developmental effects	Very low	Effect	Inadequate evidence
Animal studies			
Respiratory effects	High	Effect	High
Hepatic effects	High	Effect	High
Renal effects	High	Effect	High
Neurological effects	High	Effect	High
Developmental effects	Moderate	Effect	Moderate

C.8 INTEGRATE EVIDENCE TO DEVELOP HAZARD IDENTIFICATION CONCLUSIONS

The final step involved the integration of the evidence streams for the human studies and animal studies to allow for a determination of hazard identification conclusions. For health effects, there were four hazard identification conclusion categories:

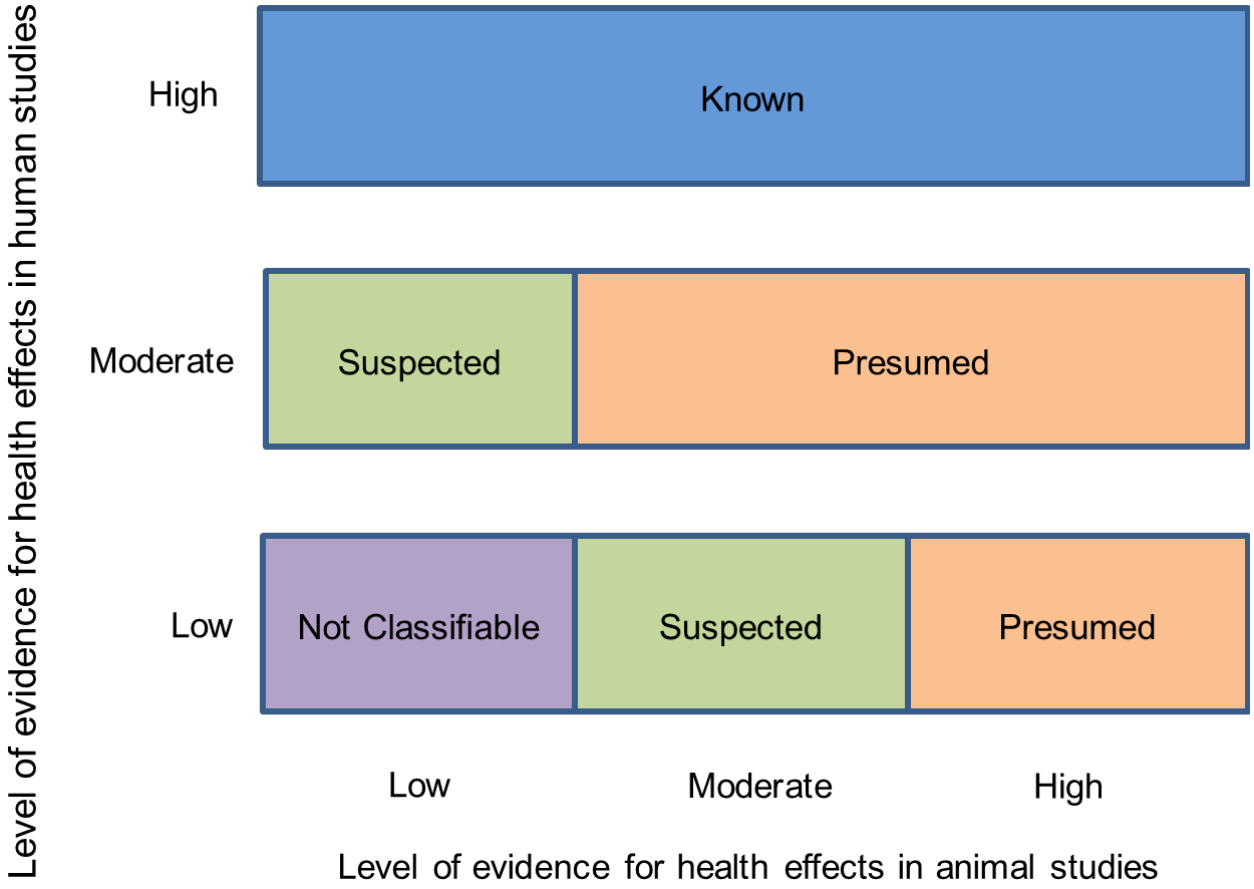
- **Known** to be a hazard to humans
- **Presumed** to be a hazard to humans
- **Suspected** to be a hazard to humans
- **Not classifiable** as to the hazard to humans

The initial hazard identification was based on the highest level of evidence in the human studies and the level of evidence in the animal studies; if there were no data for one evidence stream (human or animal), then the hazard identification was based on the one data stream (equivalent to treating the missing evidence stream as having low level of evidence). The hazard identification scheme is presented in Figure C-1 and described below:

- **Known:** A health effect in this category would have:
  - High level of evidence for health effects in human studies **AND** a high, moderate, or low level of evidence in animal studies.
- **Presumed:** A health effect in this category would have:
  - Moderate level of evidence in human studies **AND** high or moderate level of evidence in animal studies **OR**
  - Low level of evidence in human studies **AND** high level of evidence in animal studies
- **Suspected:** A health effect in this category would have:
  - Moderate level of evidence in human studies **AND** low level of evidence in animal studies **OR**
  - Low level of evidence in human studies **AND** moderate level of evidence in animal studies

- **Not classifiable:** A health effect in this category would have:
  - Low level of evidence in human studies **AND** low level of evidence in animal studies

Figure C-1. Hazard Identification Scheme



Other relevant data such as mechanistic or mode-of-action data were considered to raise or lower the level of the hazard identification conclusion by providing information that supported or opposed biological plausibility.

Two hazard identification conclusion categories were used when the data indicated that there may be no health effect in humans:

- **Not identified** to be a hazard in humans
- **Inadequate** to determine hazard to humans

If the human level of evidence conclusion of no health effect was supported by the animal evidence of no health effect, then the hazard identification conclusion category of "not identified" was used. If the human or animal level of evidence was considered inadequate, then a hazard identification conclusion category of "inadequate" was used. As with the hazard identification for health effects, the impact of other relevant data was also considered for no health effect data.

The hazard identification conclusions for chloroform are listed below and summarized in Table C-19.

Known Health Effects

- Hepatic effects: There is a low level of evidence for hepatic effects from limited epidemiological data and a high level of evidence from a large number of animal studies with consistent findings. The Hazard Identification conclusion for hepatic effects was increased from "Presumed" to "Known" based on other relevant data consisting of the extensive database of case reports and case series documenting hepatic effects of chloroform in exposed humans.
  - Evidence from epidemiological studies: There is some evidence of adverse hepatic effects in humans with occupational exposure to chloroform (Bomski et al. 1967), while other studies of occupational exposure did not find any hepatic effects (Challen et al. 1958; Li et al. 1993).
  - Evidence from animal studies: Hepatic lesions have been observed in numerous animal studies, including 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 (Section 2.9). Typical lesion progression begins with mild histopathological damage after low and/or brief exposures (e.g., lipid accumulation, cellular swelling and vacuolation, scattered necrosis, hepatocellular proliferation) and progresses to widespread and severe necrosis and degeneration with high and/or long-term exposure.
  - Other relevant data: The hepatic findings are strengthened by a large number of case reports and case-series reports indicating that the liver is a primary target following high-level chloroform exposure. Acute liver failure and/or severe liver damage are common findings in fatal exposures via inhalation (Giusti and Chiarotti 1981; Lionte 2010; Royston 1924; Townsend 1939) or oral (Dettling et al. 2016; Piersol et al. 1933) exposure. Reversible clinical signs of hepatotoxicity are commonly observed in nonfatal case studies of chloroform toxicity following inhalation exposure (Dettling et al. 2016; Gosselink et al. 2012; Hutchens and Kung 1985; Kang et al. 2014; Lin et al. 2005; Lunt 1953; Minor et al. 2018; Phoon et al. 1983; Smith et al. 1973). Similarly, reversible hepatotoxicity is a common finding in nonfatal cases of attempted suicide via chloroform ingestion (Choi et al. 2006; Dell'Aglio et al. 2010; Jayaweera et al. 2017; Kim 2008; Rao et al. 1993; Schroeder 1965;) and other cases of accidental or unspecified oral poisoning (Hakim et al. 1992; Sridhar et al. 2011; Storms 1973). One nonfatal dermal case also reported reversible hepatotoxicity (Vlad et al. 2014). Experimental studies demonstrate that hepatic effects are attributable to reactive intermediates produced during metabolism of chloroform (Brown et al. 1974a; Constan et al. 1999; Fang et al. 2008; Gopinath and Ford 1975).
- Neurological effects: There is a low level of evidence for neurological effects from limited epidemiological data and a high level of evidence from a large number of animal studies with consistent findings. The Hazard Identification conclusion for neurological effects was increased from "Presumed" to "Known" based on: (a) the historical use of chloroform as a general anaesthetic; (b) case reports and case series documenting marked neurological effects of chloroform in exposed humans; and (c) a plausible mechanism of action.
  - Evidence from epidemiological studies: There is limited evidence of neurological impairments (e.g., impaired hand-eye coordination, slowed reaction time) and subjective neurological complaints (e.g., dizziness, fatigue, depression) following occupational exposure to chloroform (Challen et al. 1958; Li et al. 1993).
  - Evidence from animal studies Chloroform is a CNS depressant in animals exposed via inhalation (Constan et al. 1999; EPA 1978; Gehring 1968; Lehmann and Flury 1943) or oral routes (Bowman et al. 1978; NTP 1988a; Jones et al. 1958). At exposure levels below those associated with CNS depression, there is limited evidence for altered neurobehavior following oral exposure in animals, including altered motor activity, impaired coordination, and altered operant learning (Balster and Borzelleca 1982; DHA 2022; Landauer et al. 1982; Wada et al. 2015). The only histopathological change reported in the neurological system is olfactory nerve loss in rats following acute-duration inhalation exposure (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.

- Other relevant data: Chloroform was used as a general anesthetic beginning in the late 1800s and was widely used for more than 100 years (Davison 1959), providing clear evidence for its neurological effects after inhalation exposure. Case reports also show that chloroform induces CNS depression at high inhalation exposure levels in humans (Featherstone 1947; Smith et al. 1973; Whitaker and Jones 1965). CNS depression has also been reported in individuals who intentionally or accidentally ingested the chemical (Piersol et al. 1933; Schroeder 1965; Storms 1973). Chloroform may cause CNS depression via perturbation of the lipophilic cell membrane, which results 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).

Presumed Health Effects

- Respiratory effects: There is inadequate evidence for respiratory effects from a single epidemiological study and a high level of evidence from several animal studies with consistent findings. Other relevant data (case reports) were not sufficient to merit an increase in the hazard identification conclusion.
  - Evidence from epidemiological studies: A single epidemiological study reported no change in respiratory function in adults after a 40-minute swim in a chlorinated pool (Font-Ribera et al. 2010); no other epidemiology studies of this endpoint were located, and no studies evaluating nasal effects in humans following exposure to chloroform were identified.
  - Evidence from animal studies: In animals, the nasal epithelium is a sensitive target of toxicity following inhalation and oral exposure (Section 2.4). Damage to the lower respiratory tract in animals was generally only observed at lethal exposure levels (Bowman et al. 1978; Kasai et al. 2002; NCI 1976). There is limited evidence of inflammatory responses in the lung at low inhalation exposure levels in mice (de Oliveira et al. 2015).
  - Other relevant data: Lung damage has been reported in several fatal cases of inhalation or oral exposure (Ago et al. 2011; Featherstone 1947; Giusti and Chiarotti 1981; Harada et al. 1997; Piersol et al. 1933; Royston 1924; Schroeder 1965). Changes in respiratory rate and/or respiratory arrest have been reported in human case reports of high exposure (Cui et al. 2022; Jayaweera et al. 2017; Storms 1973; Whitaker and Jones 1965) but these effects are likely secondary to CNS depression.
- Renal effects: There is inadequate evidence for renal effects from limited epidemiological data and a high level of evidence from a large number of animal studies with consistent findings. Other relevant data (case reports) were not sufficient to merit an increase in the hazard identification conclusion.
  - Evidence from epidemiological studies: No changes in renal clinical chemistry values were observed in one occupational cohort (Li et al. 1993) or in a group of competitive swimmers exposed to chlorinated water during training (Aiking et al. 1994).
  - Evidence from animal studies: The kidney is a clear target of toxicity in animals. Renal lesions have been observed in numerous studies 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 (Section 2.10). Typical lesion progression begins with mild histopathological damage after low and/or brief exposures (e.g., tubular dilation, single-cell necrosis, renal cell proliferation) and progresses to severe nephropathy characterized by widespread necrosis and degeneration with higher and/or long-term exposure.
  - Other relevant data: Case reports of fatal chloroform exposures have reported renal damage (Piersol et al. 1933; Royston 1924). Additionally, reversible changes in renal clinical chemistry and urinalysis have been reported in nonfatal cases (Dettling et al. 2016; Gosselink

et al. 2012; Piersol et al. 1933; Schroeder 1965; Sridhar et al. 2011; Wallace 1950). Experimental studies demonstrate that renal effects are attributable to reactive intermediates produced during metabolism of chloroform (Constan et al. 1999; Culliford and Hewitt 1957; Liu et al. 2013; Weir et al. 2005).

Suspected Health Effects

- Developmental effects: There is inadequate evidence for developmental effects from epidemiological data and a moderate level of evidence from animal studies with some inconsistent findings. Other relevant data were limited and did influence the hazard conclusion.
  - Evidence from epidemiological studies: Impaired growth (e.g., low birth weight, small for gestational age, decreased postnatal weight gain) has been associated with chloroform exposure from tap water in some epidemiological studies (Botton et al. 2015; Grazuleviciene et al. 2011; Kramer et al. 1992; Summerhayes et al. 2012; Sun et al. 2020; Wright et al. 2004; Zaganjor et al. 2020). However, these findings were not observed in other studies (Bonou et al. 2017; Cao et al. 2016; Hinckley et al. 2005; Liu et al. 2021; Porter et al. 2005; Villanueva et al. 2011). No clear associations were observed between chloroform exposure and birth defects (Dodds and King 2001; Grazuleviciene et al. 2013; Hoffman et al. 2008; Kaufman et al. 2018, 2020; Levallois et al. 2012; Rivera-Núñez and Wright 2013) or neurodevelopmental outcomes (Villanueva et al. 2018). A meta-analysis identified a slight (5%) increase in risk of small for gestational age associated with increased chloroform levels in maternal drinking water; however, this increase in risk paralleled the increase (7%) observed for total trihalomethanes in drinking water (Summerhayes et al. 2021).
  - Evidence from animal studies: In animals, maternal inhalation during gestation was associated with birth defects in rats, such as missing ribs and acaudate fetuses with imperforate anus, and cleft palate in mice (Murray et al. 1979; Schwetz et al. 1974). These defects were not observed in additional developmental studies in rats exposed via inhalation (Baeder and Hofmann 1988; EPA 1978) or rats or rabbits exposed orally (Ruddick et al. 1983; Thompson et al. 1974). However, delayed ossification and decreased fetal growth were reported in many developmental studies after inhalation or oral exposure, generally at maternally toxic exposure levels (Baeder and Hofmann 1988; Murray et al. 1979; Ruddick et al. 1983; Schwetz et al. 1974; Thompson et al. 1974).
  - Other relevant data: Chloroform is known to cross the placenta (Danielsson et al. 1986).

Table C-19. Hazard Identification Conclusions for Chloroform

Outcome	Hazard identification
Respiratory effects	Presumed
Hepatic effects	Known
Renal effects	Presumed
Neurological effects	Known
Developmental effects	Suspected

APPENDIX D. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page D-5)

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), food intake (FI), gross necropsy (GN), hematology (HE), histopathology (HP), immune function (IX), lethality (LE), neurological function (NX), organ function (OF), ophthalmology (OP), organ weight (OW), reproductive function (RX), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

FIGURE LEGEND

See Sample LSE Figure (page D-6)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the chronic exposure period are illustrated.

(14) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

(15) Levels of exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(17) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

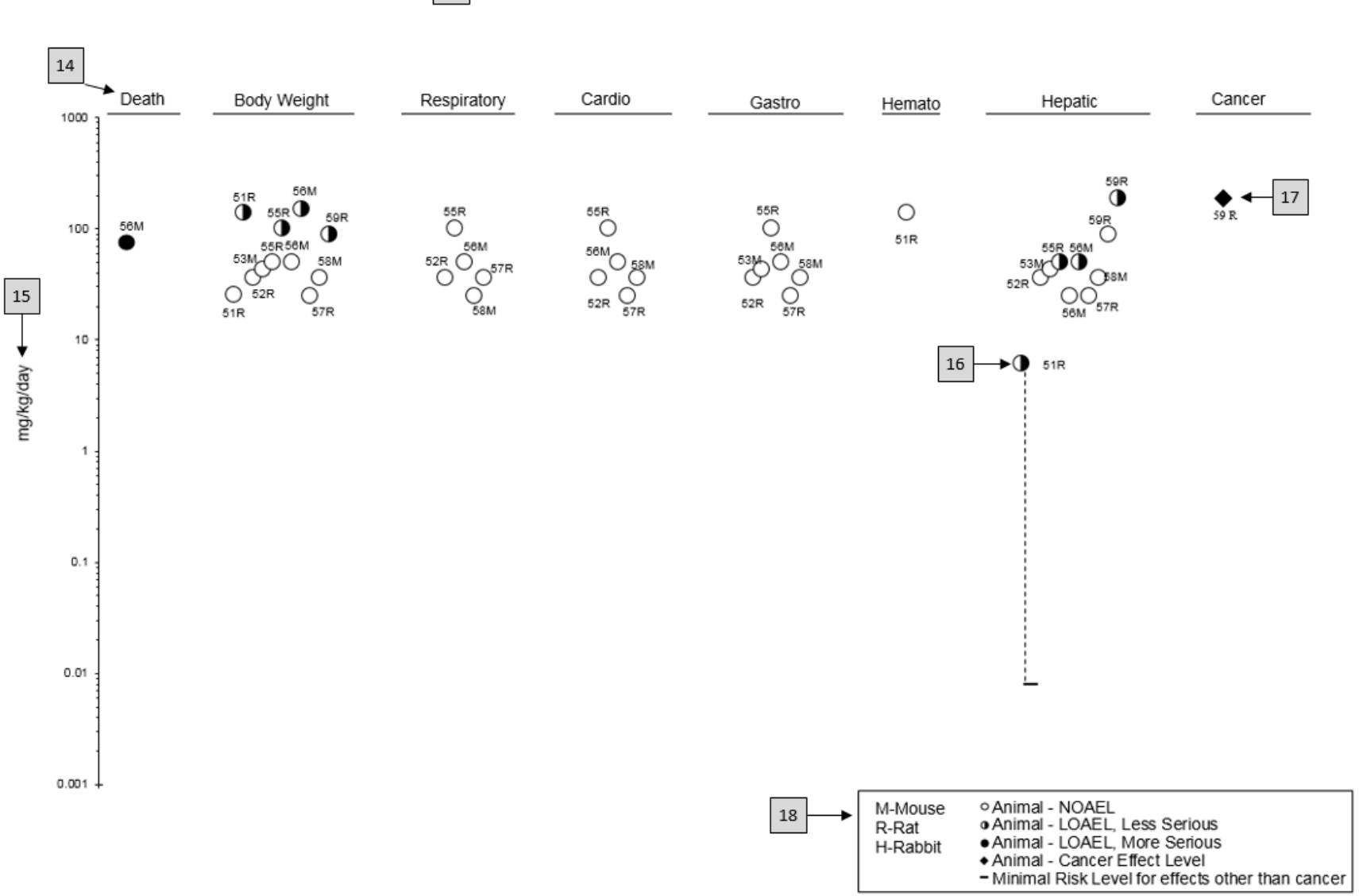
(18) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral (1)

Figure key[a] (4)	Species (strain) No./group (4)	Exposure parameters (5)	Doses (mg/kg/day) (5)	Parameters monitored (6)	Endpoint (7)	NOAEL (mg/kg/day) (8)	Less serious LOAEL (mg/kg/day) (9)	Serious LOAEL (mg/kg/day) (9)	Effect
(2) **CHRONIC EXPOSURE**									
51 (3)	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4	CS, WI, BW, OW, HE, BC, HP	Bd wt	25.5	138.0		Decreased body weight gain in males (23–25%) and females (31–39%)
					Hemato	138.0			
					Hepatic		6.1[c]		Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure
(10) **Aida et al. 1992**									
52	Rat (F344) 78 M	104 weeks (W)	0, 3.9, 20.6, 36.3	CS, BW, FI, BC, OW, HP	Hepatic	36.3			
					Renal	20.6	36.3		Increased incidence of renal tubular cell hyperplasia
					Endocr	36.3			
George et al. 2002									
59	Rat (Wistar) 58M, 58F	Lifetime (W)	M: 0, 90 F: 0, 190	BW, HP	Cancer		190 F		Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided
Tumasonis et al. 1985									

(11) [a]The number corresponds to entries in Figure 2-x.
[b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).
[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral



APPENDIX E. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Relevance to Public Health: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

Chapter 2: Health Effects: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting.

Pediatrics:
Section 3.2 Children and Other Populations that are Unusually Susceptible
Section 3.3 Biomarkers of Exposure and Effect

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

ATSDR develops educational and informational materials for health care providers categorized by hazardous substance, clinical condition, and/or by susceptible population. The following additional materials are available online:

Clinician Briefs and Overviews discuss health effects and approaches to patient management in a brief/factsheet style. They are narrated PowerPoint presentations with Continuing Education credit available (see https://www.atsdr.cdc.gov/emes/health_professionals/clinician-briefs-overviews.html).

Managing Hazardous Materials Incidents is a set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.asp).

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 400 7th Street, S.W., Suite 5W, Constitution Center, Washington, DC 20024 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

APPENDIX F. GLOSSARY

Absorption—The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD) or Benchmark Concentration (BMC)—is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a BMD_{10} would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

Cancer Effect Level (CEL)—The lowest dose of a chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or malignant tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

Clastogen—A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

Epidemiology—The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Excretion—The process by which metabolic waste products are removed from the body.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

Health Advisory—An estimate of acceptable drinking water levels for a chemical substance derived by EPA and based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunotoxicity—Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Metabolism—Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

Minimal LOAEL—Indicates a minimal adverse effect or a reduced capacity of an organ or system to absorb additional toxic stress that does not necessarily lead to the inability of the organ or system to function normally.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

Mortality—Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

No-Observed-Adverse-Effect Level (NOAEL)—The exposure level of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Although effects may be produced at this exposure level, they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) ≥1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio/Relative Risk—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Serious LOAEL—A dose that evokes failure in a biological system and can lead to morbidity or mortality.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxicokinetic—The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

Toxics Release Inventory (TRI)—The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX G. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AAPCC	American Association of Poison Control Centers
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ACMT	American College of Medical Toxicology
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Level
AIC	Akaike's information criterion
AIHA	American Industrial Hygiene Association
ALT	alanine aminotransferase
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BCF	bioconcentration factor
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
C	centigrade
CAA	Clean Air Act
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
cm	centimeter
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DWEL	drinking water exposure level
EAFUS	Everything Added to Food in the United States
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
ERPG	emergency response planning guidelines
F	Fahrenheit
F1	first-filial generation
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	Federal Register

FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GGT	γ-glutamyl transferase
GRAS	generally recognized as safe
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substances Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Level of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
MRL	Minimal Risk Level
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
Mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NCEH	National Center for Environmental Health
ND	not detected
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences

NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEHSU	Pediatric Environmental Health Specialty Unit
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
pg	picogram
PND	postnatal day
POD	point of departure
ppb	parts per billion
ppbv	parts per billion by volume
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure limit
REL-C	recommended exposure level-ceiling value
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification
SMR	standardized mortality ratio
sRBC	sheep red blood cell
STEL	short term exposure limit
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
USNRC	U.S. Nuclear Regulatory Commission

VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result