2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of 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.

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

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure --inhalation, oral, and dermal; and then by health effect--death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods--acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant, dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt

at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

Levels of exposure associated with the carcinogenic effects (Cancer Effect Levels, CELs) of chloroform are indicated in Figure 2-2. Because cancer effects could occur at lower exposure levels, Figures 2-l and 2-2 also show a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

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

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

asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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

2.2.1 Inhalation Exposure

Most of the data regarding inhalation exposure to chloroform in humans were obtained from clinical reports describing health effects in patients under anesthesia. In some instances, the results may have been confounded by the concurrent administration of other drugs with chloroform or by artificial respiration of patients under chloroform anesthesia. Furthermore, most of the studies did not provide any information regarding actual exposure levels for observed effects. Nonetheless, chloroforminduced effects in humans are supported by those observed in animals under experimental conditions.
The human studies cited in the profile provide qualitative information on chloroform toxicity in humans.

2.2.1.1 Death

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 may be misleading, in that many of these patients had pre-existing health conditions that may have contributed to the cause of death and that chloroform toxicity may not have been the only factor involved in the death of the patient. Older clinical case reports suggested that concentrations of ≈40,000 ppm, if continued for several minutes, may be an overdose (Featherstone 1947). When a cohort of 1,502 patients, ranging in age from 1 to 80 years, exposed under anesthesia to less than 22,500 ppm chloroform was evaluated, no indication of increased mortality was found (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. Several studies reported deaths in women after childbirth when chloroform anesthesia had been used (Royston 1924; Townsend 1939). No levels of actual exposure were provided in either study. Death was caused by acute hepatotoxicity. Prolonged labor with starvation, dehydration, and exhaustion contributed to the chloroform-induced hepatotoxicity.

Levels of acute 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. An inhalation LC_{50} (lethal concentration, 50% kill) of 9,770 ppm for a 4-hour exposure was reported for female rats (Lundberg et al. 1986). One rat died after 6 exposures in one report where groups of 9-12 pregnant female rats exposed to chloroform at doses as high as 4,117 ppm for 1 hour a day for 8 days (Newell and Dilley 1978). However, exposure to 8,000 ppm for 4 hours was lethal to albino rats (Smyth et al. 1962). Male mice appear to be more sensitive than female mice. Following exposure to 1,024 ppm for l-3 hours, 15 of 18 male mice died within 11 days; however, most of the female mice similarly exposed survived for several months (Deringer et al. 1953). Male mice that died had kidney and liver damage, while females did not. An exposure as low as 692 ppm for 1-3 hours resulted in the death of 3 of 6 male mice within 8 days. When exposed to 4,500 ppm chloroform for 9 hours, 10 of 20 female mice died (Gehring 1968). Increased mortality was observed in male rats exposed to 85 ppm chloroform for 6 months (Torkelson et al. 1976). The deaths were attributed to interstitial pneumonia. Rats of either sex exposed to 50 ppm survived. Exposure to 85 ppm for 6 months did not increase mortality in rabbits and guinea pigs. Similarly, no deaths were reported in dogs exposed to 25 ppm chloroform for the same time period.

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

2.2.1.2 Systemic Effects

No studies were located regarding dermal or ocular effects in humans or animals after inhalation exposure to chloroform.

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

Respiratory Effects. Changes in respiratory rate were observed in patients exposed to chloroform via anesthesia (exposure less than 22,500 ppm) (Whitaker and Jones 1965). Increased respiratory rates were observed in 44% of 1,502 patients who were exposed to light chloroform anesthesia. Respiratory rates were depressed, however, during deep and prolonged anesthesia when chloroform concentrations

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

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	Rat (Albino)	4 hr				8000 (5/6 died)	Smyth et al. 1962
2	Rat (Sprague-Dawley)	4 hr				9770 F (LC_{50})	Lundberg et al. 1986
3	Rat (Sprague-Dawley)	8 d Gd 7-14 1 hr/d				4117 F (death; 1 animal on Gd 12)	Newell and Dilley 1978
4	Mouse (Swiss-Webster)	9 hr				4500 F (10/20 died)	Gehring 1968
5	Mouse (C3H)	1-3 hr				692 M (3/6 died)	Deringer et al. 1953
Systemic							
6	Human	113 min	Cardio			8000 (arrhythmia)	Smith et al. 1973
			Gastro		8000 (vomiting)		
			Hemato		8000 (increased prothrombin time)		
			Hepatic			8000 (increased sulfobromophthalein sodium retention)	

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

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference
7	Human	0.5-2 hr	Resp		22,500	(changes in respiratory rate)			Whitaker and Jones 1965
			Cardio				22,500	(cardiac arrhythmia, bradycardia)	
			Gastro		22,500	(vomiting)			
			Hepatic		22,500	(transient jaundice in 1 patient)			
8	Rat (Sprague-Dawley)	8 d Gd 7-14 1 hr/d	Bd Wt	2232 F			4117 F	(60% decreased maternal body weight gain)	Newell and Dilley 1978
9	Rat (Sprague-Dawley)	4 hr	Hepatic	76 F	153 F	(SDH-enzyme levels increased)			Lundberg et al. 1986
10	Rat (Wistar)	10 d Gd 7-16 7 hr/d	Hepatic	300 F					Baeder and Hofmann 1988
			Renal	300 F					
			Bd Wt		30 F	(18% decreased weight gain of dams)	100 F	(24% decreased weight gain of dams)	
			Other		30 F	(decrease in feed consumption)			
11	Rat (Wistar)	8 hr	Hepatic		50 M	(elevated liver triglycerides and liver GSH)			Ikatsu and Nakajima 1992
12	Rat (Fischer- 344)	7 d 6 hr/d	Resp	3.1 M	10.4 M	(degeneration of Bowman's gland; new bone formation; increased number of S-phase nuclei)			Mery et al. 1994
			Bd Wt	100 M	271 M	(unspecified decrease in body weight)			

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

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
13	Rat (Fischer- 344)	7 d 6 hr/d	Resp	3.1 M	10.4 M (epithelial goblet cell hyperplasia and degeneration of Bowman's glands in olfactory mucosa)		Larson et al. 1994c
			Hepatic	100 M	271 M (swelling and mild centrilobular vacuolation)		
			Renal	10.4 M	29.3 M (increased number of S-phase nuclei for tubule cells in the cortex)		
			Bd Wt	3.1 M	10.4 M (decreased weight gain)		
14	Rat (Wistar)	6 hr	Hepatic	100 M	500 M (increased plasma GOT and GPT; decreased hepatic GSH)		Wang et al. 1994
15	Rat (Wistar)	6 hr	Hepatic	100	500 (incr. plasma GOT activity)		Wang et al. 1995
16	Mouse (CBA; W.H.)	2 hr	Hepatic		246 (fatty changes)		Culliford and Hewitt 1957
			Renal	246 F		246 M (tubular necrosis in males)	
17	Mouse (NS)	4 hr	Hepatic		100 F (fatty changes)		Kylin et al. 1963
18	Mouse (C3H)	1-3 hr	Resp	1106			Deringer et al. 1953
			Hepatic	1106 F		942 M (liver necrosis in males that died)	
			Renal	1106 F		692 M (tubular necrosis in males that died)	

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

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
19	Mouse (CF1)	8 d Gd 1-7, Gd 6-15, or Gd 8-15 7 hr/d	Hepatic		100 F (increased SGPT activity; increased absolute and relative liver weights)		Murray et al. 1979
			Bd Wt		100 F (unspecified decreased weight gain in dams)		
20	Mouse (Swiss-Webster)	9 hr	Hepatic		4500 F (increased SGPT activity)		Gehring 1968
21	Mouse (B6C3F1)	7 d 6 hr/d	Resp	3 F	10 F (increased number of S-phase nuclei)		Mery et al. 1994
			Bd Wt	29.5 F	101 F (unspecified decr. in body weight)		
22	Mouse (B6C3F1)	7 d 6 hr/d	Resp	288 F			Larson et al. 1994c
			Hepatic	3[b] F	10 F (mild to moderate vacuolar changes in centrilobular hepatocytes)	101 F (centrilobular hepatocyte necrosis and severe diffuse vacuolar degeneration of midzonal and periportal hepatocytes)	
			Renal	101 F	288 F (proximal tubules lined by regenerating epithelium)		
			Bd Wt	288 F			

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

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
23	Mouse (B6C3F1)	4 d 6 hr/d	Resp	2 F	10 F (mild, transient, proliferative responses & bone thickening in the periosteum and posterior ventral areas)		Larson et al. 1996
			Musc/skel	88 F			
			Hepatic	30 F	88 F (increased relative liver weights; hepatocyte lipid vacuolization & scattered individual hepatocyte necrosis; significant increase in hepatic LI)		
			Renal	88 F			
	Immunological/Lymphoreticular						
24	Rat (Wistar)	10 d Gd 7-16 7 hr/d		300 F			Baeder and Hofmann 1988
25	Mouse (C3H)	1-3 hr		1106			Deringer et al. 1953
	Neurological						
26	Human	3 min			920 (dizziness, vertigo)		Lehman and Hasegawa 1910
27	Human	113 min				8000 (narcosis)	Smith et al. 1973
28	Human	0.5-2 hr				22,500 (narcosis)	Whitaker and Jones 1965
29	Mouse (NS)	0.5-2 hr		2500		3100 (slight narcosis)	Lehmann and Flury 1943
30	Cat (NS)	5-93 min				7,200 (disturbed equilibrium)	Lehmann and Flury 1943

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

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
Reproductive							
31	Rat (Sprague-Dawley)	10 d Gd 6-15 7 hr/d		100 F		300 F (73% decreased conception rate; increased incidence of fetal resorptions)	Schwetz et al. 1974
32	Rat (Wistar)	10 d Gd 7-16 7 hr/d				30 F (empty implantations in 2/20 dams)	Baeder and Hofmann 1988
33	Mouse (CF1)	8 d Gd 1-7, Gd 6-15, or Gd 8-15 7 hr/d				100 F (increased incidence of resorptions; decrease in % pregnancies)	Murray et al. 1979
34	Mouse (C57B1/ C3H)F1	5 d 4 hr/d				400 M (increased percentage of abnormal sperm)	Land et al. 1979
Developmental							
35	Rat (Sprague-Dawley)	10 d Gd 6-15 7 hr/d			30 (delayed ossification and wavy ribs)	100 (missing ribs; acaudate fetuses with imperforate anus)	Schwetz et al. 1974
36	Rat (Wistar)	10 d Gd 7-16 7 hr/d			30 (slight growth retardation)		Baeder and Hofmann 1988
37	Mouse (CF1)	8 d Gd 1-7, Gd 6-15, or Gd 8-15 7 hr/d				100 (cleft palate, decreased ossification)	Murray et al. 1979

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

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
INTERMEDIATE EXPOSURE							
Death							
38	Rat (NS)	6 mo 5 d/wk 7 hr/d				85 M (increased mortality; 4/10 died)	Torkelson et al. 1976
Systemic							
39	Human	1-6 mo	Gastro		14 (nausea; vomiting)		Phoon et al. 1983
			Hepatic		14[c] (toxic hepatitis)		
40	Rat (NS)	6 mo 5 d/wk 7 hr/d	Resp	50 M 85 F	85 M (interstitial pneumonia)		Torkelson et al. 1976
			Hemato	85			
			Hepatic		25 (degenerative changes)		
			Renal		25 (cloudy swelling)		
			Bd Wt	25 M 85 F	50 M (decreased body weight in males)		
41	Mouse (B6C3F1)	3 wk 7 d/wk 6 hr/d	Resp	88			Larson et al. 1996
			Musc/skel	88			
			Hepatic	10	29.6 (hepatocyte vacuolation and swelling; variations in nuclear size)		
			Renal	10 M 88 F	29.6 M (nephropathy of epithelial cells of the proximal convoluted tubules)		
			Bd Wt	88			

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

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
42	Mouse (B6C3F1)	6 wk 7 d/wk 6 hr/d	Resp	88			Larson et al. 1996
			Musc/skel	88			
			Hepatic	10 F	29.6 F (16.1% increase in relative liver weights; mild degenerative changes in hepatocytes; increase in liver LI)		
			Renal	88			
			Bd Wt	88 F			
43	Mouse (B6C3F1)	13 wk 7 d/wk 6 hr/d	Resp	88			Larson et al. 1996
			Musc/skel	88			
			Hepatic	10	29.6 (vacuolation and swelling of hepatocytes; variations in nuclear size)		
			Renal	1.99 M	10 M (nephropathy & enlarged nuclei of the epithelial cells of the proximal convoluted tubules; mineralization in the cortex)		
				88 F			
			Bd Wt	88			

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

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
44	Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d	Resp	88			Larson et al. 1996
			Musc/skel	88			
			Hepatic	29.6	88 (increased relative liver weights; vacuolation & swelling of hepatocytes & enlarged nuclei; increased liver LI)		
			Renal	1.99 M	10 M (nephropathies and enlarged nuclei in epithelial cells of the proximal convoluted tubules; mineralization within the cortex; increased cortical and medullary tissue LIs)		
				88 F			
			Bd Wt	29.6			
45	Dog (NS)	6 mo 5 d/wk 7 hr/d	Hemato	25			Torkelson et al. 1976
			Hepatic	25			
			Renal	25 M	25 F (cloudy swelling of tubular epithelium)		
46	Rabbit (NS)	6 mo 5 d/wk 7 hr/d	Resp	50 M	25 F (interstitial pneumonia) 85 M (pneumonitis)		Torkelson et al. 1976
			Hemato	85			
			Hepatic		25 (centrilobular granular degeneration and necrosis)		
			Renal		25 (interstitial nephritis)		

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

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
47	Gn pig (NS)	6 mo 5 d/wk 7 hr/d	Hemato	85			Torkelson et al. 1976
			Hepatic		25 (centrilobular granular degeneration)		
			Renal		25 (tubular and interstitial nephritis)		
Immunological/Lymphoreticular							
48	Rat (NS)	6 mo 5 d/wk 7 hr/d		25			Torkelson et al. 1976
Reproductive							
49	Rat (NS)	6 mo 5 d/wk 7 hr/d		25 M			Torkelson et al. 1976

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

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
CHRONIC EXPOSURE							
Systemic							
50	Human	10-24 mo	Gastro		22 F (nausea)		Challen et al. 1958
			Hepatic	71			
51	Human	1-4 yr	Hepatic		2[d] (hepatomegaly)		Bomski et al. 1967
52	Human	3-10 yr	Gastro		77 F (nausea)		Challen et al. 1958
			Hepatic	237 F			
53	Human	1-15 yr	Hepatic		29.5 (elevated serum prealbumin and transferrin)		Li et al. 1993
			Renal	13.5			
Immunological/Lymphoreticular							
54	Human	1-4 yr			2 (splenomegaly)		Bomski et al. 1967
Neurological							
55	Human	3-10 yr			77 F (exhaustion, irritability, depression, lack of concentration)		Challen et al. 1958
56	Human	10-24 mo			22 F (exhaustion)		Challen et al. 1958

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

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
57	Human	1-15 yr			13.49 (dizziness, fatigue, somnolence, insomnia, increased dreaming, hypomnesia, anorexia, and palpitations)		Li et al. 1993

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

[b]Used to derive an acute-duration inhalation minimal risk level (MRL) of 0.1 ppm; concentration is converted to a human equivalent concentration and divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive an intermediate-duration inhalation MRL of 0.05 ppm; concentration is divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability).

[d]Used to derive a chronic-duration inhalation MRL of 0.02 ppm; concentration is divided by an uncertainty factor 100 (10 for use of a LOAEL and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); F = female; Gastro = gastrointestinal; Gd = gestational day; Gn pig = guinea pig; GOT = glutamic oxalotransaminase; GPT = glutamic pyruvic transaminase; GSH = glutathione; Hemato = hematological; hr = hour(s); LC_{50} = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; LI = labeling index; M = male; min = minute(s); mo = moth(s); musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; SDH = sorbitol dehydrogenase; SGPT = serum glutamic pyruvic transaminase; wk = week; yr = year(s)

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

Acute (≤14 days)

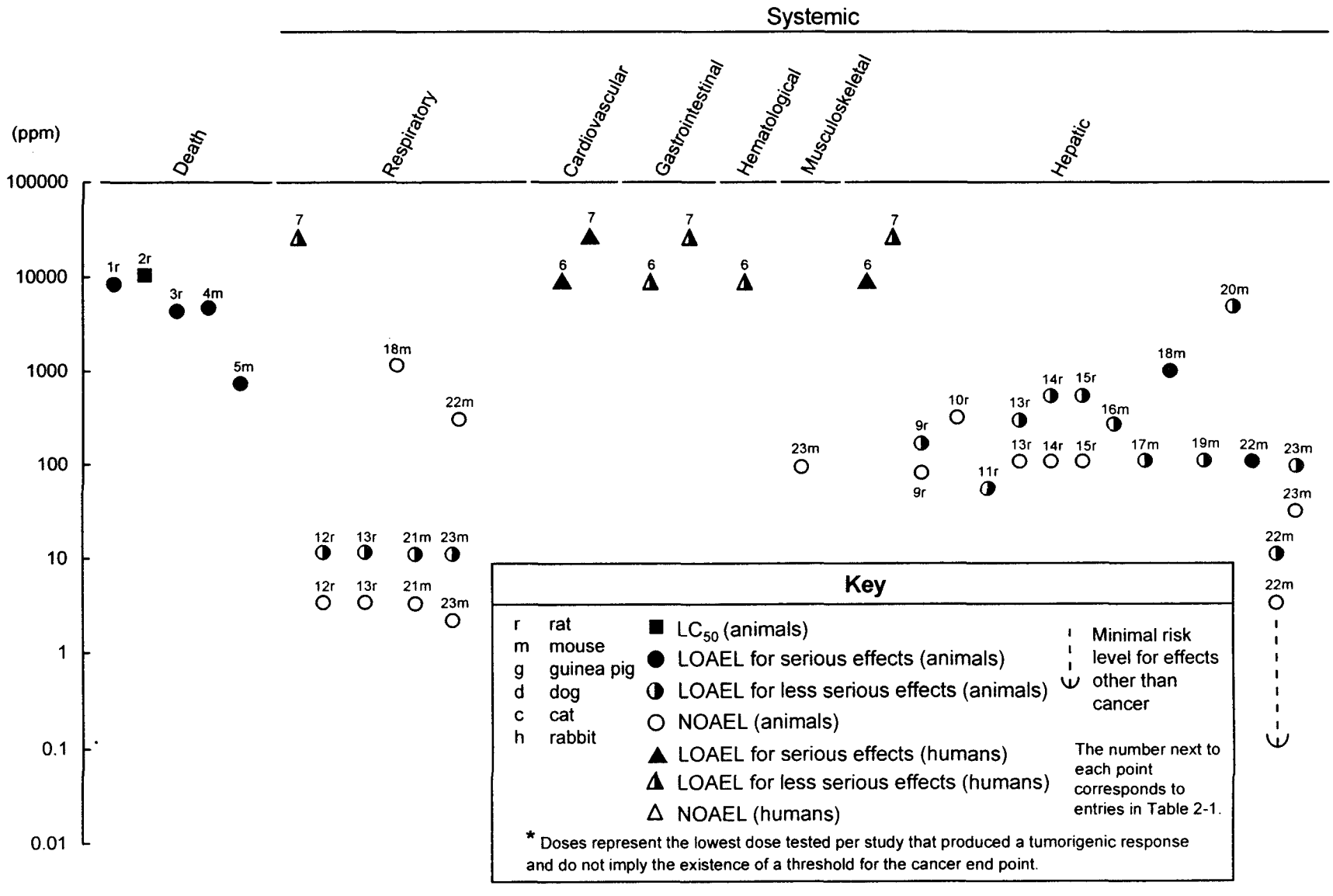


Figure 2-1. Levels of Significant Exposure to Chloroform - Inhalation (cont.)

Acute (≤14 days)

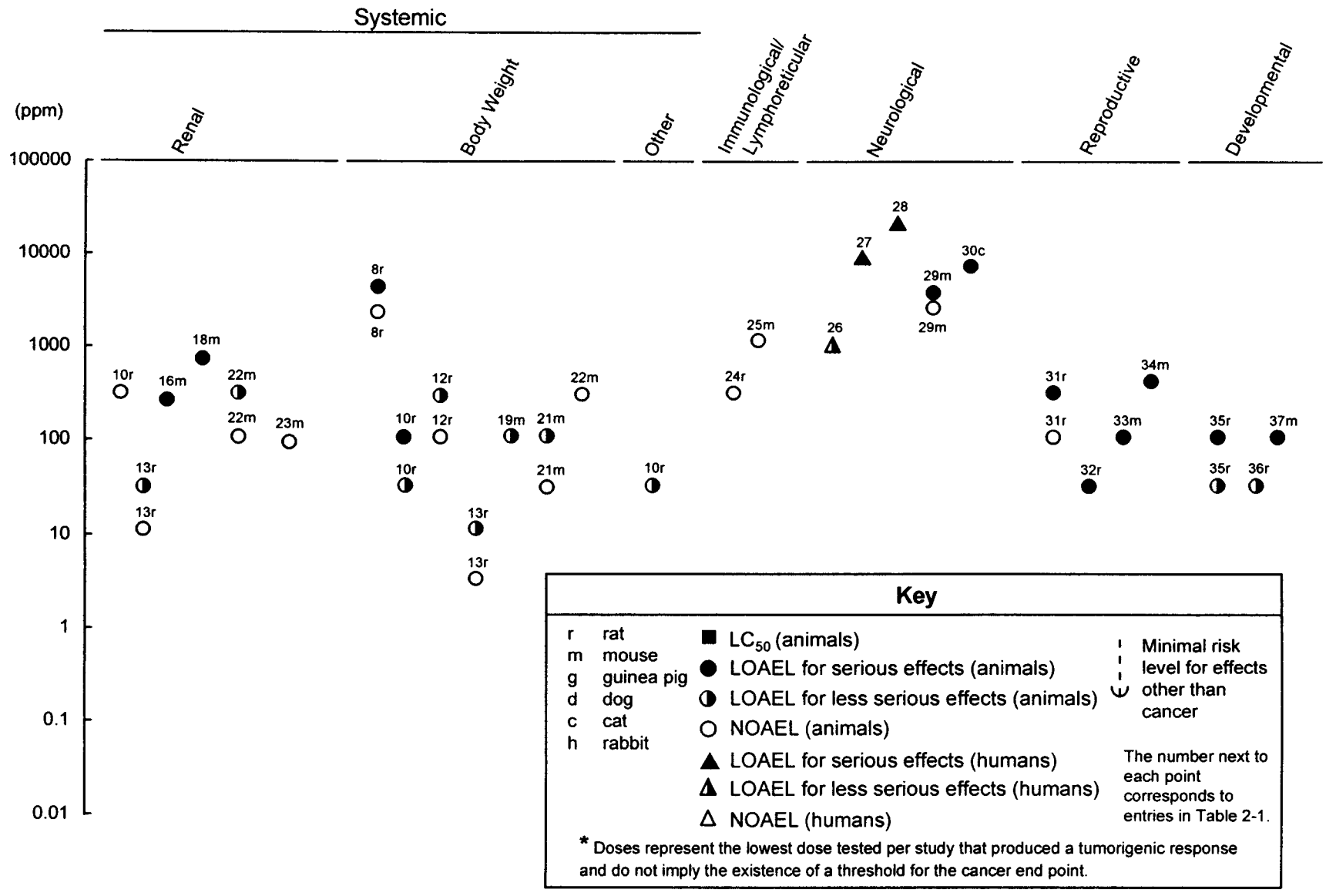


Figure 2-1. Levels of Significant Exposure to Chloroform - Inhalation (cont.)

Intermediate (15-364 days)

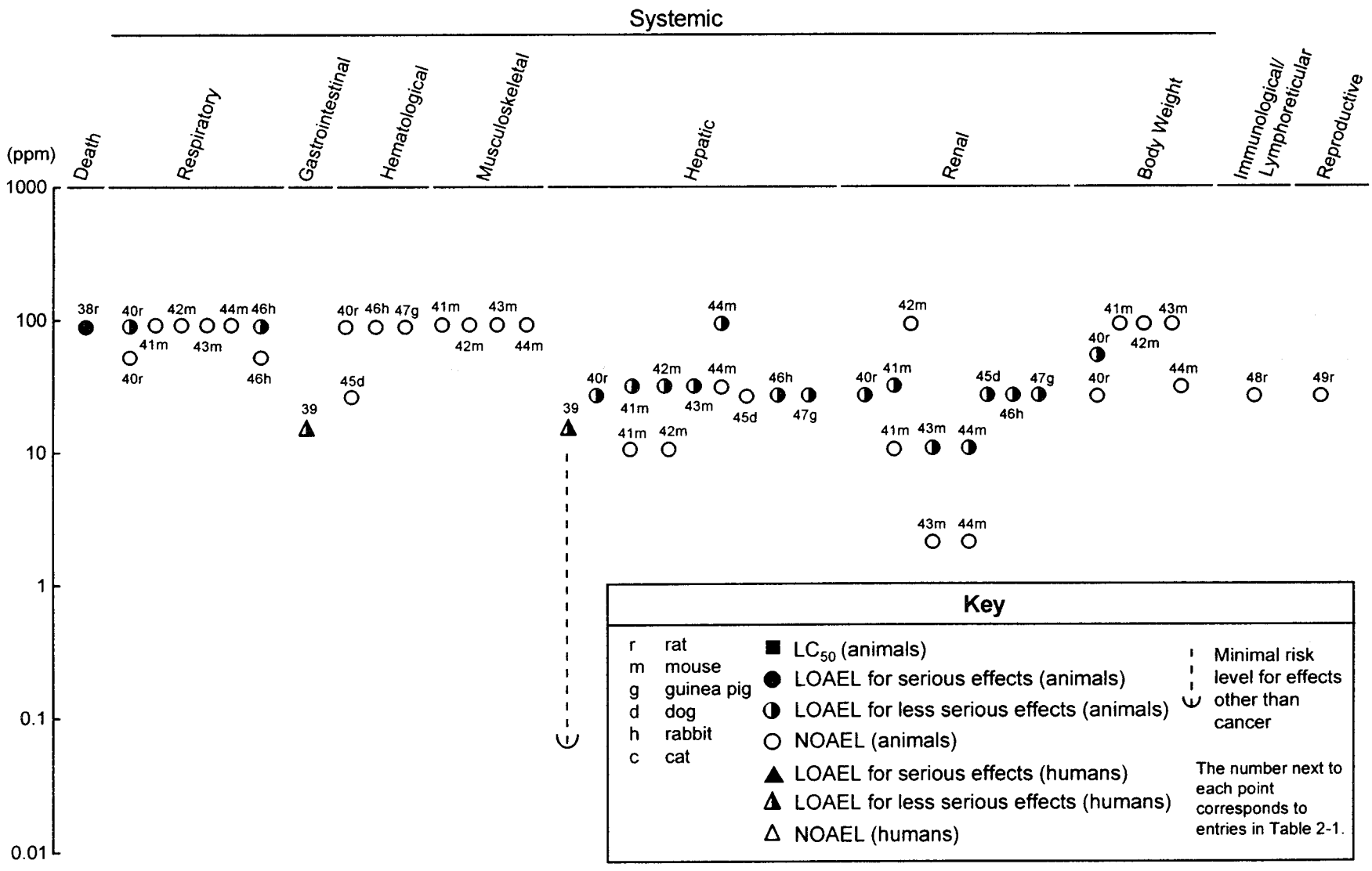
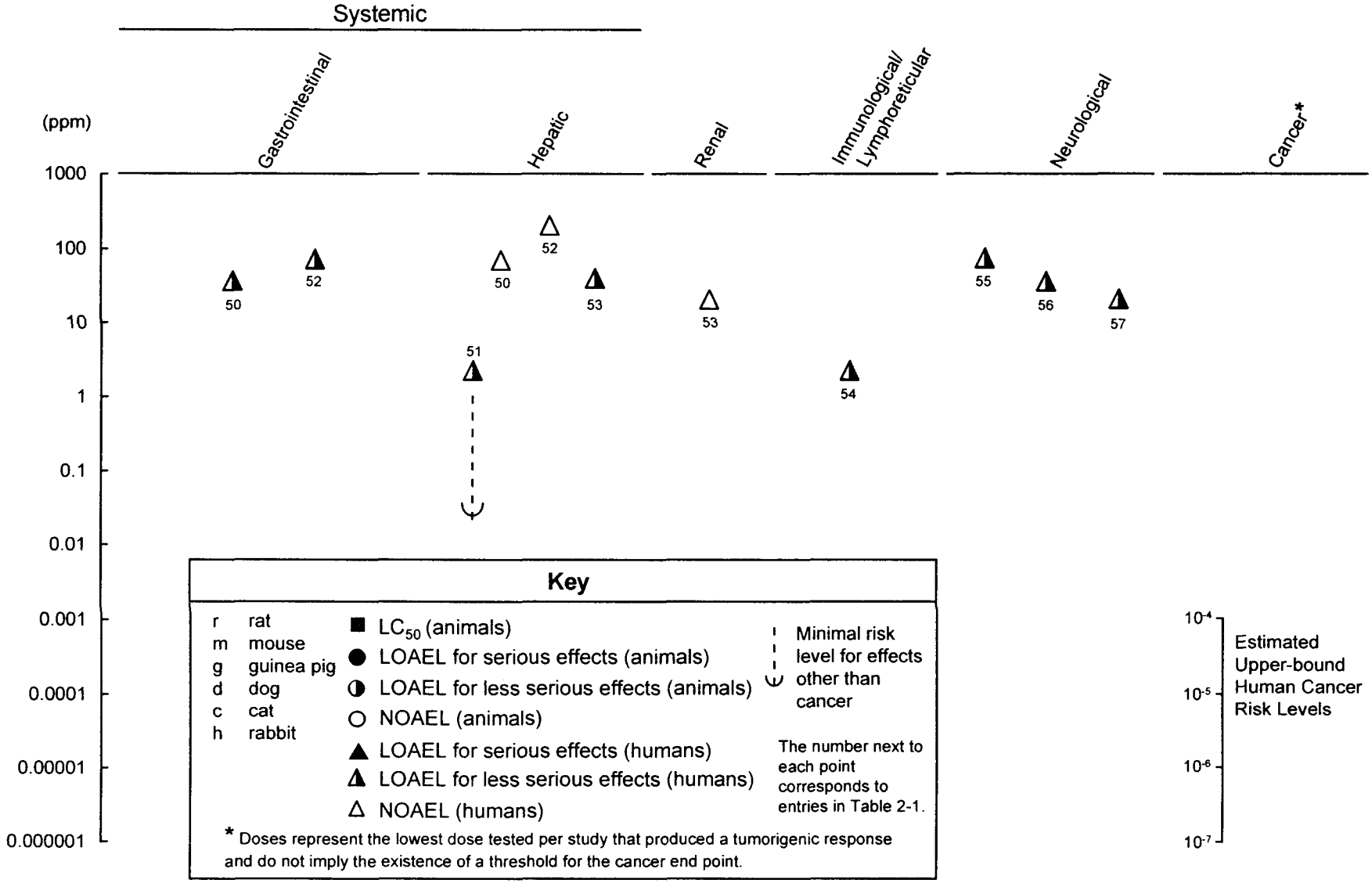


Figure 2-1. Levels of Significant Exposure to Chloroform - Inhalation (cont.)

Chronic (≥365 days)



did not exceed 2.25%. No other studies were located regarding respiratory effects in humans after inhalation exposure to chloroform.

Larson et al. (1996) investigated the ability of acute exposure to chloroform vapors to produce toxicity and regenerative cell proliferation in the liver, kidneys, and nasal passage of female B6C3F_1 mice. Groups of 5 animals were exposed to 0, 0.3, 2, 10, 30, or 90 ppm chloroform via inhalation for 6 hours a day for 4 consecutive days. Animals were administered bromodeoxyuridine (BrdU) via implanted osmotic pump for the last 3.5 days. At necropsy, livers and kidneys were removed, weighed, examined macroscopically, and prepared for microscopic evaluation. The nasal cavities were also removed and prepared for microscopic evaluation. Cell proliferation was quantitated as the percentage of cells in S-phase (labeling index = LI) measured by immunohistochemical detection of BrdU-labeled nuclei. This study found no overt clinical signs of toxicity in female mice exposed to chloroform for 4 days; however, some mild, transient changes occurred in the posterior ventral areas of nasal tissue in female mice exposed to the 10, 30, and 90 ppm concentrations of chloroform. The lesions were characterized by mild proliferative responses in the periosteum consisting of a thickening of the bone. The adjacent lamina also exhibited loss of acini of Bowman's glands and vascular congestion. No microscopic changes were noted in nonnasal bones, nor were nonnasal bone LIs significantly different from those of controls.

Another similar study by Larson et al. (1994c) using a wider range of inhaled doses investigated the ability of chloroform vapors to produce toxicity and regenerative cell proliferation in the liver and kidneys of female B6C3F_1, mice and male Fischer 344 rats, respectively. Nasal passages were also examined for toxic response. Groups of 5 animals were exposed to 0, 1, 3, 10, 30, 100, or 300 ppm chloroform via inhalation for 6 hours a day for 7 consecutive days. Actual exposure concentrations measured were 0, 1.2, 3, 10, 29.5, 101, and 288 ppm for mice; and 0, 1.5, 3.1, 10.4, 29.3, 100, and 271 ppm and for rats. Animals were administered BrdU via implanted osmotic pump for the last 3.5 days to quantitate S-phase cell proliferation using an LI method. Necropsies were performed on day 8. No histopathological lesions were observed in the nasal passages of female mice at any exposure concentration. In the nasal passages of rats, chloroform concentrations of 10 ppm and above induced histopathological changes that exhibited a clear concentration-related response. These lesions consisted of respiratory epithelial goblet cell hyperplasia and degeneration of Bowman's glands in olfactory mucosa with an associated osseous hyperplasia. of the endo- and ectoturbinates in the periphery of the ethmoid region.

2. HEALTH EFFECTS

Acute exposure to chloroform clearly can induce site-specific as well as biochemical changes in the nasal region of female $B6C3F_1$, mice and male Fischer 344 rats (Mery et al. 1994). To demonstrate the biochemical alterations, mice were exposed to 1.2, 3, 10, 29.5, 101, and 288 ppm chloroform and rats were exposed to 1.5, 3.1, 10.4, 29.3, 100, and 27 1 ppm for 6 hours a day for 7 days to determine the nasal cavity site-specific lesions and the occurrence of cell induction/proliferation associated with these varying concentrations of chloroform. In male rats, the respiratory epithelium of the nasopharyngeal meatus exhibited an increase in the size of goblet cells at 100 and 271 ppm chloroform, in addition to an increase in both neutral and acidic mucopolysaccharides. Affected epithelium was up to twice its normal thickness. New bone formation within the nasal region was prominently seen at 10.4 ppm and above, and followed a concentration response curve. At 29.3 and 100 ppm, new osseous spicules were present at the beginning of the first endoturbinate, while at 271 ppm, the width of the new bone was almost doubled compared to controls. The Bowman's glands were markedly reduced in size. Cytochrome P-450-2El staining was most prominent in the cytoplasm of olfactory epithelial sustentacular cells and in the acinar cells of Bowman's glands in control animals. In general, increasing the chloroform concentration tended to decrease the amount of P-450 staining in exposed animals. Exposure to chloroform resulted in a dramatic increase in the number of S-phase nuclei, with the proliferative response confined to activated periosteal cells, including both osteogenic (round) and preosteogenic (spindle) cells. The proximal and central regions of the first endoturbinate had the highest increase of cell proliferation. Interestingly, the only detectable treatment-related histologic change observed in female mice was a slight indication of new bone growth in the proximal part of the first endoturbinate in one mouse exposed to 288 ppm chloroform. The S-phase response was observed at chloroform concentrations of 10.4 ppm and higher. If similar nasal cavity changes occur in humans, the sense of smell could potentially be altered.

In some animal species, the lung may be a target organ when inhalation exposure to chloroform is of intermediate duration. Interstitial pneumonitis was observed in male rats and rabbits exposed to 85 ppm and in female rabbits exposed to 25 ppm chloroform for 6 months (Torkelson et al. 1976). The NOAEL was 50 ppm for male rats and rabbits. No respiratory changes were reported in guinea pigs and dogs exposed to 85 and 25 ppm chloroform, respectively.

Larson et al. (1996) investigated the ability of intermediate exposure to chloroform vapors to produce toxicity and regenerative cell proliferation in the nasal passage of male and female $B6C3F_1$, mice. Groups of 8 animals of each sex were exposed to 0, 0.3, 2, 10, 30, or 90 ppm chloroform via

inhalation for 6 hours a day, 7 days a week for 3, 6, or 13 weeks; additional groups of 8 animals of each sex were exposed for 6 hours a day, 5 days a week for 13 weeks. Animals were administered BrdU via implanted osmotic pump and cell proliferation was quantitated as the percentage of cells in S-phase using LI (measuring by immunohistochemical detection of BrdU-labeled nuclei). At necropsy, the nasal cavities were removed and prepared for microscopic evaluation. No alterations in nasal tissues were noted at any exposure level in either sex after exposures of 3, 6, or 13 weeks.

Cardiovascular Effects. Epidemiologic studies indicate that chloroform causes cardiac effects in patients under anesthesia. In a cohort of 1,502 patients (exposure less than 22,500 ppm), dose-related bradycardia developed in 8% of the cases, and cardiac arrhythmia developed in 1.3% of the cases (Whitaker and Jones 1965). Hypotension was observed in 27% of the patients and was related to the duration of the anesthesia and to pretreatment with thiopentone. Chloroform anesthesia (exposure 8,000-10,000 ppm) caused arrhythmia (nodal rhythm, first degree atrio-ventricular block, or complete heart block) in 50% of the cases from the cohort of 58 patients and hypotension in 12% (Smith et al. 1973). It should be noted that the effects seen may be secondary to surgical stress or the underlying disease which necessitated the surgical procedure.

No studies were located regarding cardiovascular effects in animals after inhalation exposure to chloroform.

Gastrointestinal Effects. Nausea and vomiting were frequently observed side effects in humans exposed to chloroform via anesthesia (exposure 8,000-22,500 ppm) (Royston 1924; Smith et al. 1973; Townsend 1939; Whitaker and Jones 1965). Nausea and vomiting were observed in male and female workers exposed solely to 14-400 ppm chloroform for l-6 months (Phoon et al. 1983). Similarly, gastrointestinal symptoms (nausea, dry mouth, and fullness of the stomach) were reported in female workers occupationally exposed to 22-71 ppm chloroform for lo-24 months and 77-237 ppm chloroform for 3-10 years (Challen et al. 1958).

No studies were located regarding gastrointestinal effects in animals after inhalation exposure to chloroform.

Hematological Effects. The hematological system does not appear to be a significant target after inhalation exposure to chloroform. Except for increased prothrombin time in some individuals after

anesthesia exposure to 8,000 ppm, no hematological effects were observed in humans after inhalation exposure to chloroform (Smith et al. 1973). This effect reflects the hepatotoxicity of chloroform because prothrombin is formed in the liver.

No hematological effects were observed in rats, rabbits, and guinea pigs exposed to 85 ppm chloroform or in dogs exposed to 25 ppm chloroform for intermediate durations (Torkelson et al. 1976).

Musculoskeletal Effects. Few musculoskeletal effects have been reported in the literature after an acute, intermediate, or chronic exposure to chloroform in humans or in laboratory animals. Larson et al. (1996) investigated the ability of acute- and intermediate-duration exposure to chloroform vapor to produce toxicity and regenerative cell proliferation in various tissues of female $B6C3F_1$, mice. Using the methods described in previous sections of this profile, Larson et al. (1996) found that, after acute exposure, no microscopic changes were noted in nonnasal bones, nor were non nasal bone LIs different from those of controls. In the intermediate duration studies, no alterations in nonnasal bone tissues were noted at any exposure level in either sex after exposures of 3, 6, or 13 weeks.

Hepatic Effects. Chloroform-induced hepatotoxicity is one of the major toxic effects observed in both humans and animals after inhalation exposure. Increased sulfobromophthalein retention was observed in some patients exposed to chloroform via anesthesia (exposure 8,000-10,000 ppm), indicating impaired liver function (Smith et al. 1973). Serum transaminase, cholesterol, total bilirubin, and alkaline phosphatase levels were not affected. Transient jaundice has also been reported in one study (Whitaker and Jones 1965), while several earlier studies report acute hepatic necrosis in women exposed to chloroform via anesthesia (exact exposure not provided) during childbirth (Lunt 1953; Royston 1924; Townsend 1939). The effects observed in the women included jaundice, liver enlargement and tenderness, delirium, coma, and death. Centrilobular necrosis was found at autopsy in those who died. Workers exposed to 14-400 ppm chloroform for l-6 months developed toxic hepatitis and other effects including jaundice, nausea, and vomiting, without fever (Phoon et al. 1983). The workers were originally diagnosed with viral hepatitis; however, in light of epidemiologic data, the diagnosis was changed to toxic hepatitis. No clinical evidence of liver injury was observed in workers exposed to as much as 71 and 237 ppm chloroform for intermediate and chronic durations, respectively; however, liver function was not well characterized (Challen et al. 1958). In contrast, toxic hepatitis (with hepatomegaly, enhanced serum glutamic pyruvic transaminase [SGPT] and serum

glutamic oxaloacetic transaminase [SGOT] activities, and hypergammaglobulinemia) was observed in workers exposed to 2-205 ppm chloroform (Bomski et al. 1967). Co-exposure to trace amounts of other solvents was also detected, however. Elevated serum prealbumin and transferrin were noted in another study (Li et al. 1993); however, the data is questionable as the exposed individuals most likely received exposures to toxic substances other than just chloroform. An intermediate-duration inhalation MRL of 0.05 ppm was derived from the LOAEL of 14 ppm from the data presented by Phoon et al. (1983); a chronic-duration inhalation MRL of 0.02 ppm was derived from the LOAEL of 2 ppm from the data presented by Bomski et al. (1967). More information on these MRLs and how they were derived is located in the footnote to Table 2-1, Section 2.5 and in Appendix A of this profile.

A study by Aiking et al. (1994) examined the possible hepatotoxicity of chloroform exposure in competitive swimmers who trained in indoor chlorinated swimming pools (n=l0) compared to those who trained in outdoor chlorinated swimming pools (n=8). The actual amount of chloroform inhaled was not determined; however, the mean concentration of chloroform was determined to be 24 μg/L in the indoor pools and 18.4 μg/L in the outdoor pools. Mean blood chloroform concentration in the indoor pool swimmers was found to be 0.89 μg/L, while the control group and the outdoor pool swimmers had blood chloroform concentrations of less than 0.5 μg/L, suggesting that the chloroform could not be removed by environmental air currents (resulting in higher exposure dose) as it did in an outdoor pool environment. No significant differences in liver enzyme function was seen between any of the groups.

Chloroform-induced hepatotoxicity in various animal species has been reported in several studies. No changes in SGPT activity were observed in rats exposed to 300 ppm chloroform during gestation days (Gd) 6-15 (Schwetz et al. 1974). No changes in liver weights were found in pregnant Wistar rats that were exposed to 0, 30, 100, or 300 ppm chloroform via inhalation during Gd 7-16, followed by termination on day 21 (Baeder and Hofmann 1988). In contrast, serum sorbitol dehydrogenase (SDH) activity was increased in rats exposed to 153 ppm and above for 4 hours (Lundberg et al. 1986) and SGPT levels were increased in mice exposed to 100 ppm, 7 hours a day for 8 days during various stages of pregnancy (Murray et al. 1979) and 4,500 ppm for 9 hours (Gehring 1968). These increased enzyme levels in serum indicate hepatoceliular necrosis. Fatty changes were observed microscopically in male and female mice after acute exposure to chloroform concentrations ≥100 ppm (Culliford and Hewitt 1957; Kylin et al. 1963). Elevated liver triglycerides and liver glutathione (GSH) have also been reported (Ikatsu and Nakajima 1992). Liver necrosis was observed in female rats exposed to

4,885 ppm chloroform for 4 hours (Lundberg et al. 1986) and in male mice that died after acute exposure to 692-1,106 ppm chloroform (Deringer et al. 1953). Centrilobular granular degeneration was observed in rats, rabbits, and guinea pigs exposed to 25 ppm chloroform for 6 months, but not in dogs exposed to 25 ppm for the same time period (Torkelson et al. 1976); however, these pathological findings were not observed in the 50 ppm exposure group of rabbits and guinea pigs, or in the 85 ppm exposure group of guinea pigs. Although the liver effects in rabbits and guinea pigs were not doserelated, the small number of surviving animals in the higher exposure group may have biased the results of the study and may not fully describe the pathological effects of chloroform at the higher dose.

In two separate studies, Wang et al. (1994, 1995) investigated the effect of ethanol consumption or fasting, respectively, on the metabolism and toxicity of chloroform administered by inhalation of an acute duration. In the first study, male Wistar rats were pretreated with either ethanol, 2,000 mg/kg, or water. Eighteen hours later, the animals were exposed to air containing chloroform at concentrations of 0, 50, 100, or 500 ppm for 6 hours (5 rats a group). At 24 hours postexposure, animals were anesthetized, blood samples were collected for determination of SGOT and SGPT levels; livers were harvested and processed for determination of GSH levels. Chloroform produced dosedependent hepatotoxicity, and ethanol pretreatment enhanced this effect. In rats exposed by inhalation, hepatotoxicity was only evident at the highest dose (500 ppm); SGOT and SGPT values in treated rats were 47 and 24 international units per liter (IV/L) versus 30 and 16 IU/L in controls, respectively. GSH concentrations in rats exposed to chloroform were lowered in a dose-dependent manner. Significant ($p<0.05$) reductions were seen at the 500 ppm dose in rats exposed by inhalation. In the second study, rats were divided in two groups: those fasted over night and those allowed free access to food. The following day, 5 rats per group were exposed to chloroform once by inhalation at 0, 50, 100, or 500 ppm for 6 hours. Twenty-four hours after exposure, blood samples were collected for SGOT and SGPT determinations. Chloroform tended to produce hepatotoxicity in a dose-dependent manner, and fasting tended to potentiate the toxicity. Plasma SGOT activity was significantly elevated in the fasting group at 100 and 500 ppm and the fed group at 500 ppm as compared to controls. SGPT levels in the fasting group exposed to 500 ppm chloroform (212 IU/L) significantly exceeded those of the fasting control group (16 IU/L). GSH levels in the fasting group exposed to 500 ppm chloroform (2.22 mg/g) were significantly lower than those of the fasted control group (2.51 mg/g).

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Larson et al. (1996) investigated the ability of acute chloroform vapor exposure to produce toxicity and regenerative cell proliferation in the liver of female B6C3F$_1$ mice. Groups of 5 animals were exposed to 0, 0.3, 2, 10, 30, or 90 ppm chloroform via inhalation for 6 hours a day for 4 consecutive days. Animals were administered BrdU via an implanted osmotic pump, and cell proliferation was quantitated as the percentage of cells in S-phase (LI) measured by immunohistochemical detection of BrdU-labeled nuclei. At necropsy, livers were removed, weighed, examined macroscopically, and prepared for microscopic evaluation. 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 individual 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; $p<0.05$).

In an earlier study, Larson et al. (1994c) investigated the ability of chloroform vapors to produce toxicity and regenerative cell proliferation in the liver and kidneys of female B6C3F$_1$ mice and male Fischer 344 rats, respectively. Groups of 5 animals were exposed to 0, 1, 3, 10, 30, 100, or 300 ppm chloroform via inhalation for 6 hours a day for 7 consecutive days. Actual exposure concentrations measured for mice were 0, 1.2, 3, 10, 29.5, 101, and 288 ppm and for rats were 0, 1.5, 3.1, 10.4, 29.3, 100, and 271 ppm. Necropsies were performed on day 8. Animals were administered BrdU via implanted osmotic pump for the last 3.5 days in order to measure S-phase cell proliferation using an LI method. Female mice exposed to 101 or 298 ppm exhibited centrilobular hepatocyte necrosis and severe diffuse vacuolar degeneration of mid-zonal and periportal hepatocytes, while exposure to 10 or 29.5 ppm resulted in mild-to-moderate vacuolar changes in centrilobular hepatocytes. Specifically, decreased eosinophilia of the centrilobular and mid-zonal hepatocyte cytoplasm relative to periportal hepatocytes was observed at 29.5 ppm. Livers of mice in the 1 and 3 ppm groups did not differ from controls. Slight, dose-related increases in the hepatocyte LIs were observed in the 10 and 30 ppm dose groups, while the LI was increased more than 30-fold in the 101 and 288 ppm groups. Relative liver weights were increased in a dose-dependent manner at exposures of 3 ppm and above. Livers from mice exposed to 101 or 288 ppm were enlarged and pale. In male rats, swelling and mild centrilobular vacuolation was observed only in the livers of rats exposed to 271 ppm. Necrosis was minimal and confined to individual hepatocytes immediately adjacent to the central vein; livers were dark red and congested. The hepatocyte LI in rats were increased only at 101 and 271 ppm, 3- and 7-fold over controls, respectively. An acute-duration inhalation MRL of 0.1 ppm was based on the

NOAEL of 3 ppm for hepatic effects in mice. More information on this MRL and how it was derived is located in the footnote to Table 2-1, Section 2.5 in Appendix A this profile.

Larson and coworkers (1996) investigated the ability of intermediate-duration chloroform vapor exposure to produce toxicity and regenerative cell proliferation in the liver of male and female $B6C3F_1$ mice. Groups of 8 animals of each sex were exposed to 0, 0.3, 2, 10, 30, or 90 ppm chloroform via inhalation for 6 hours a day, 7 days a week for 3, 6, or 13 weeks; additional groups of 8 animals of each sex were exposed for 6 hours a day, 5 days a week for 13 weeks. Animals were administered BrdU via implanted osmotic pump and cell proliferation was quantitated as the percentage of cells in S-phase. At necropsy, livers were removed, examined macroscopically, and then prepared for microscopic evaluation. In mice exposed for 3 weeks, no changes in relative liver weights occurred in males at any dose level, whereas females exposed to 90 ppm chloroform experienced a significant increase (10.7%; $p<0.05$) in relative liver weights. Liver lesions were noted in males and females at exposures of 30 and 90 ppm. Lesions were characterized by vacuolation and swelling of hepatocytes and variations in nuclear size. Cell proliferation was elevated in the livers of females and males at 30 and 90 ppm exposures, respectively. In mice exposed for 6 weeks, exposure to 90 ppm chloroform resulted in a significant increase (16.1%; $p<0.05$) in relative liver weights. Liver lesions were noted in females at exposures of 30 and 90 ppm; these lesions were characterized by mild degenerative changes in hepatocytes. An increase in liver LI was also noted in the females exposed to 30 and 90 ppm chloroform. In mice exposed 7 days a week for 13 weeks, relative liver weights increased in males exposed to 30 and 90 ppm chloroform and in females exposed to 90 ppm chloroform ($p<0.05$). Liver lesions were elevated above background in males and females at exposures of 30 and 90 ppm. Lesions were characterized by vacuolation and swelling of hepatocytes and variations in nuclear size. Cell proliferation was elevated in the livers of females and males at exposed to 90 ppm chloroform. Hepatic alterations in mice exposed 5 days a week for 13 weeks were similar to those of mice exposed 7 days a week; however, the severity of the lesions was diminished with significant effects seen only at the 90 ppm exposure level.

Renal Effects. Several studies regarding kidney toxicity effects in humans after inhalation exposure to chloroform were found. No biochemical renal anomalies were reported in one study examining factory workers in China exposed to varying levels of chloroform (Li et al. 1993). One report was obtained from case reports of death among women exposed to chloroform via anesthesia during childbirth (Royston 1924). The fatty degeneration of kidneys observed at autopsy indicated

chloroform-induced damage. A study by Aiking et al. (1994) examined the possible renal toxicity of chloroform exposure in competitive swimmers who trained in indoor and outdoor chlorinated swimming pools in the Netherlands. Although no significant differences in liver enzyme function were seen between any of the groups, the study did determine that β-2-microglobulin was elevated in the indoor pool swimmers (after controlling for possible age bias using multiple regression analysis), suggesting some degree of renal damage due to higher inhaled air concentrations of chloroform present in the air of indoor swimming pools.

In animals, the kidney is one of the target organs of inhalation exposure to chloroform. Groups of 20 female Wistar rats were exposed to 0, 30, 100, or 300 ppm chloroform via inhalation during Gd 7-16 and terminated on day 21 showed no changes in kidney weights compared to control animals (Baeder and Hofmann 1988). Larson et al. (1996) investigated the ability of acute chloroform vapor exposure to produce toxicity and regenerative cell proliferation in the kidneys of female $B6C3F_1$ mice. Groups of 5 animals were exposed to 0, 0.3, 2, 10, 30, or 90 ppm chloroform via inhalation for 6 hours a day for 4 consecutive days. At necropsy, kidneys were removed, weighed, examined macroscopically, and prepared for microscopic evaluation. Relative kidney weights were similar to controls at all chloroform exposure levels. 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.

In an earlier study, Larson et al. (1994~) examined the ability of chloroform vapors to produce toxicity and regenerative cell proliferation in the liver and kidneys of female $B6C3F_1$ mice and male Fischer 344 rats, respectively. Groups of 5 animals were exposed to 0, 1, 3, 10, 30, 100, or 300 ppm chloroform via inhalation for 6 hours a day for 7 consecutive days. Actual exposure concentrations measured for mice were 0, 1.2, 3, 10, 29.5, 101, and 288 ppm and for rats were 0, 1.5, 3.1, 10.4, 29.3, 100, and 271 ppm. Necropsies were performed on day 8. The kidneys of mice were affected only at the 300 ppm exposure, with approximately half of the proximal tubules lined by regenerating epithelium and an increased LI of tubule cells of 8-fold over controls. In the kidneys of male rats exposed to 300 ppm, about 25-50% of the proximal tubules were lined by regenerating epithelium. The LI for tubule cells in the cortex was increased at 30 ppm and above.

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Tubular necrosis was observed in male mice after acute exposure to chloroform concentrations ≥246 ppm (Culliford and Hewitt 1957; Deringer et al. 1953). Tubular calcifications were observed in mice that survived the exposure and were terminated after a 12-month recovery period.

In a study of intermediate duration, increased kidney weight (both sexes) and cloudy swelling (males) were observed in rats exposed to chloroform concentrations ≥25 ppm chloroform (Torkelson et al. 1976). Results were not consistent in rabbits and guinea pigs under the same exposure conditions. Cloudy swelling, and tubular and interstitial nephritis were observed in groups of rats exposed to 25 ppm chloroform, but not in groups exposed to 50 ppm. The results in rabbits and guinea pigs, however, may be biased due to the low survival rate at the higher exposure level.

Larson and coworkers (1996) also investigated the ability of intermediate-duration chloroform vapor exposure to produce toxicity and regenerative cell proliferation in the kidneys of male and female B6C3F_1 mice. Groups of 8 animals of each sex were exposed to 0, 0.3, 2, 10, 30, or 90 ppm chloroform via inhalation for 6 hours a day, 7 days a week for 3, 6, or 13 weeks; additional groups of 8 animals of each sex were exposed for 6 hours a day, 5 days a week for 13 weeks. At necropsy, kidneys were removed, examined macroscopically, and prepared for microscopic evaluation. In mice exposed for 3 weeks, no changes were noted in relative kidney weights in either sex at any exposure level. While kidneys of female mice did not differ from those of controls at any dose, those of males were significantly affected by chloroform exposures of 30 ppm or more. Lesions were mainly in the epithelial cells of the proximal convoluted tubules, with 25 and 50% of cells affected in the 30 and 90 ppm groups, respectively. Cell proliferation was elevated in males at the 30 ppm exposure level, while female kidney LIs were not affected at any exposure level. In mice exposed for 6 weeks, no changes were noted in relative kidney weights at any exposure level. Kidneys from exposed females were not histologically different from controls at any exposure level, and kidney LIs were similar to control values at all exposure levels. In mice exposed 7 days a week for 13 weeks, no changes were noted in relative kidney weights in either sex at any exposure level. While kidneys of female mice did not differ from those of controls at any dose, those of males were significantly affected by chloroform exposures of 10 ppm or more. Lesions were mainly in the epithelial cells of the proximal convoluted tubules, with 25 and 50% of cells affected in the 30 and 90 ppm groups, respectively. Mineralization within the cortex and enlarged nuclei in the epithelial cells were also noted. Cell proliferation was elevated in the cortical tissues of males at the 30 and 90 ppm exposure level; in contrast, female

kidney LIs were not affected at any exposure level. In contrast, exposure to chloroform vapors 5 days a week for 13 weeks produced no adverse renal effects.

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

Larson et al. (1994c) noted that in female B6C3F$_1$ mice and male Fischer 344 rats exposed to 0, 1, 3, 10, 30, 100, or 300 ppm chloroform via inhalation for 6 hours a day for 7 consecutive days that body weight gains were significantly decreased relative to controls in mice exposed to 101 and 288 ppm (1% weight loss at 101 and 288 ppm). Body weight gain was significantly decreased in a concentration-dependent manner in rats exposed to 10 ppm of chloroform and above (2% weight loss at 271 ppm; weight gains of 9-12% at 10.4-101 ppm, as compared to 18% weight gain by controls).

A dose-dependent reduction in feed consumption, resulting in decreased body weight gain, was observed in pregnant female rats exposed to 30 ppm chloroform (7 hours a day for 10 days) and above during gestation (Baeder and Hofmann 1988). Newell and Dilley (1978) report that maternal body weights decreased in Sprague-Dawley rats when the chloroform concentration reached 4,117 ppm when exposed for 1 hour a day during Gd 7-14. Similarly, decreased body weight was observed in pregnant mice exposed to 100 ppm chloroform during gestation (Murray et al. 1979). Decreased body weight was reported in male rats exposed to chloroform at 271 ppm for 6 hours a day for 7 days; however, no discernable decrease in body weight was noted at concentrations from 1.5 to 100 ppm. Decreases in body weight were also noted in female mice exposed to 101 ppm chloroform for the same duration (Mery et al. 1994). Decreased body weight also occurred in male rats exposed to 50 ppm for 6 months (Torkelson et al. 1976).

Larson and coworkers (1996) also investigated the effect of intermediate exposure to chloroform vapor on body weight in male and female B6C3F$_1$ mice. Groups of 8 animals of each sex were exposed to 0, 0.3, 2, 10, 30, or 90 ppm chloroform via inhalation for 6 hours a day, 7 days a week for 3, 6, or 13 weeks; additional groups of 8 animals of each sex were exposed for 6 hours a day, 5 days a week for 13 weeks. In mice exposed 7 days a week for 3 weeks, body weights in females were unaffected, while those of males exposed to 90 ppm chloroform were significantly lower compared to controls (2% weight loss versus 6% weight gain). Exposure to chloroform 7 days a week for 6, or 13 weeks did not affect body weights in males or females; however, when exposed 5 days a week for 13 weeks,

body weight gains in males and females exposed to 90 ppm chloroform were slightly reduced compared to their respective controls (93 and 91%, respectively).

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

Gearhart et al. (1993) studied the interactions of chloroform exposure with body temperature in mice. Male mice were exposed to chloroform concentrations up to 5,500 ppm chloroform for 6 hours and their core body temperature monitored. The largest decrease in core body temperature was observed in the 5,500 ppm exposure group, followed by the 2,000, 800, and 100 ppm groups. There was no significant decrease in *in vitro* cytochrome P-450 activity at any temperature tested. The data collected were used to develop a PBPK model, which is discussed in more detail in Section 2.3.5. Decreased feed consumption also been reported at chloroform doses as low as 30 ppm in rats (Baeder and Hofmann 1988).

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding in-depth immunological effects in humans after inhalation exposure to chloroform. Only one study (Bomski et al. 1967) described the health effects of a group of 68 workers occupationally exposed to chloroform for 1-4 years in a pharmaceutical plant. Chloroform air concentrations ranged from 0.01 to 1 mg/L, and other solvents were also reported in the air in trace amounts. Splenomegaly was the only immunologically detected health effect in a small percentage of these cases.

Chloroform appears to have little effect on the spleen of laboratory animals. No histological changes were found in the spleen of mice exposed to chloroform concentrations as high as 1,106 ppm for l-3 hours (Deringer et al. 1953) or in male rats receiving 25 ppm of chloroform for 6 months (Torkelson et al. 1976). Female Wistar rats exposed to 0, 30, 100, or 300 ppm chloroform for 10 days via inhalation during Gd 7-16 also failed to show a change in maternal spleen organ weights (Baeder and Hofmann 1988).

Other information on the immunotoxicity of chloroform is limited to one study on effects of chloroform on host resistance in CD-l mice. A single exposure to 10.6 ppm chloroform for 3 hours

did not increase the mortality rate after streptococcal challenge and did not alter the ability of alveolar macrophages to destroy bacteria in these mice (Aranyi et al. 1986). After repeated chloroform exposure (3 hours a day for 5 days), the mortality rate significantly increased, but the bactericidal activity of macrophages was not suppressed compared to control animals.

2.2.1.4 Neurological Effects

The central nervous system is a major target for chloroform toxicity in humans and in animals. 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. Levels of 3,000-30,000 ppm were used to induce anesthesia (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). To induce anesthesia, increasing the concentration of chloroform gradually to 25,000 or 30,000 ppm during the first 2 or 3 minutes with maintenance at much lower levels was recommended. Concentrations <1,500 ppm are insufficient to induce anesthesia; concentration of 1,500-2,000 ppm cause light anesthesia (Goodman and Gilman 1980).

Dizziness and vertigo were observed in humans after exposure to 920 ppm chloroform for 3 minutes; headache and slight intoxication were observed at higher concentrations (Lehmann and Hasegawa 1910). Exhaustion was reported in 10 women exposed to ≥22 ppm chloroform during intermediateand chronic-duration occupational exposures (Challen et al. 1958). Chronic exposure to chloroform concentrations ≥77 ppm caused exhaustion, lack of concentration, depression, or irritability in 9 of 10 occupationally exposed women. 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.

A study of 61 workers exposed for l-l5 years (average 7.8 years) attempted to delineate a possible exposure-effect relationship and to determine the toxicity of chloroform after long-term exposures at a low concentrations in factories in China (Li et al. 1993). Concentrations of chloroform ranged from 0.87 to 28.9 ppm. Dizziness, fatigue, somnolence, insomnia, increased dreaming, hypomnesia,

anorexia, and palpitations were significantly elevated in these individuals. Depression, anger, and fatigue were also reported to be significantly elevated. Significant changes were found in neurologic testings of Simple Visual Reaction Time, Digital Symbol Substitution, Digit Span, Benten Retention and Aim Pursuiting in some workers. A limitation of this study was that the exposed group, based on information indicating where the exposed groups originated, indicated that these individuals probably inhaled much more than just chloroform (i.e., other solvents, drugs, pesticides, etc.) and all the effects attributed to chloroform may be attributable to other chemicals in addition to chloroform.

Evidence of central nervous system toxicity in animals includes disturbed equilibrium in cats exposed to 7,200 ppm chloroform for 5 minutes, deep narcosis in cats exposed to 21,500 ppm for 13 minutes, deep narcosis in mice exposed to 4,000 ppm for 30 minutes, slight narcosis in mice exposed to 3,100 ppm for 1 hour, and no obvious effects in mice exposed to 2,500 ppm for 2 hours (Lehmann and Flury 1943). Memory retrieval was affected in mice exposed to chloroform via anesthesia (concentration not specified) (Valzelli et al. 1988). The amnesic effect was not long-lasting.

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

2.2.1.5 Reproductive Effects

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

Several studies indicate that inhalation exposure to chloroform may cause reproductive effects in animals. Rats exposed to chloroform during gestation had decreased conception rates after exposure to 300 ppm, but not after exposure to 100 ppm (Schwetz et al. 1974). Studies by Baeder and Hofmann (1988) indicated that exposure to as little as 30 ppm chloroform resulted in increased fetal resorptions. Similarly, a decreased ability to maintain pregnancy, characterized by an increased number of fetal resorptions and decreased conception rates, was observed in mice exposed to 100 ppm chloroform (Murray et al. 1979). In addition to the reproductive effects described above, a significant increase in the percentage of abnormal sperm was observed in mice exposed to 400 ppm chloroform for 5 days (Land et al. 1979).

In males, groups of 10-12 male rats (strain not reported) were exposed to 0, 25, 50, or 85 ppm chloroform for 6 months. Adjusted testicular weights in the 50 and 85 ppm groups were greater than those of their respective controls, but were not different from those of other control groups within the same colony. Additionally, no histological changes were noted in testicular tissues of treated animals. The significant increase in testes weights reported in this study was considered to be spurious not likely a direct effect of chloroform exposure (Torkelson et al. 1976).

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

2.2.1.6 Developmental Effects

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

Chloroform-induced fetotoxicity and teratogenicity were observed in experimental animals. The offspring of rats exposed during gestation had delayed ossification and wavy ribs (30 ppm), acaudate fetuses with imperforate anus and missing ribs (100 ppm), and decreased fetal body weight and crownrump length, and increased fetal resorptions (300 ppm) (Schwetz et al. 1974). Slight growth retardation of live fetuses at 30 ppm was observed in rats exposed during gestation; no major teratogenic effects were observed (Baeder and Hofmann 1988). The offspring of mice exposed to 100 ppm chloroform during gestation had increased incidences of cleft palate, decreased ossification, and decreased fetal crown-rump length (Murray et al. 1979). The observed malformations occurred in the fetuses that were exposed during organogenesis (days 8-15). Increased resorptions were observed in dams exposed during Gd 1-7.

In another study using relatively higher doses, female Sprague-Dawley rats were exposed to 0, 942, 2,232, or 4,117 ppm chloroform 8 days during Gd 7-14, for 1 hour a day. The number of resorptions was enhanced (45% resorptions) and average fetal body weights declined in the highest exposure group only, with no adverse effects noted in the 2,232 ppm and lower doses. The average fetal weight was decreased at the highest dose. No gross teratologic effects or anomalies in ossification were observed in the offspring of exposed dams (Newell and Dilley 1978).

All reliable LOAEL values for developmental effects in each species in the acute-duration category are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after inhalation exposure to chloroform.

Inhalation exposure to 400 ppm chloroform for 5 days increased the percentage of abnormal sperm in mice (Land et al. 1979). Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

No studies were located regarding cancer in humans or animals after inhalation exposure to chloroform.

Studies in animals indicate that oral exposure to chloroform causes cancer (see Section 2.2.2.8). Because chloroform is carcinogenic in animals exposed orally and because chloroform has identical toxicological end points following oral or inhalation exposure, EPA (1985a) derived a q_1* for inhalation exposure to chloroform based on mouse liver tumor data from a chronic gavage study (NCI 1976). EPA considered the NCI (1976) study to be appropriate to use in the inhalation risk estimate because there are no inhalation cancer bioassays and no pharmacokinetic data to contraindicate the use of gavage data (IRIS 1995). The geometric mean of the estimates for male and female mice in the NCI (1976) study, $8x10^{-2}$ $(mg/kg/day)^{-1}$, was recommended as the inhalation q_l* for chloroform. EPA (1985a) combined the estimates for both data sets because the data for males includes observations at a lower dose, which appear to be consistent with the female data. Expressed in terms of air concentration, the q_l* is equal to $2.3x10^{-5}$ $(\mu g/m^3)^{-1}$ or $l.lx10^{-4}$ parts per billion $(ppb)^{-1}$

The air concentrations associated with individual, lifetime upper-bound risks of 10^{-4} to 10^{-7} are $4.3x10^{-3}$ to $4.3x10^{-6}$ mg/m^3 ($8.8x10^{-4}$ to $8.8x10^{-7}$ ppm), assuming that a 70-kg human breathes 20 m^3 air/day. The 10^{-4} to 10^{-7} levels are indicated in Figure 2- 1.

2.2.2 Oral Exposure

2.2.2.1 Death

Information regarding mortality in humans after oral exposure to chloroform is limited. In one report, a man died of severe hepatic injury 9 days after reportedly drinking ≈6 ounces of chloroform (3,755 mg/kg) (Piersol et al. 1933). He was admitted to a hospital in a deep coma within 15 minutes of ingestion. This man was also noted to be a long-time user of chloroform in his occupation and a heavy drinker, suggesting that damage inflicted by previous use of chloroform and alcohol over a long period of time may have been contributing factors in his death. In contrast, a patient who ingested 4 ounces (≈2,410 mg/kg) recovered from toxic hepatitis (Schroeder 1965). The recovery may have been due to better therapeutic handling of the case. Fatal doses have been reported to be as low as 10 mL (14.8 grams) or 212 mg/kg (Schroeder 1965).

Oral LD_{50}, (lethal dose, 50% kill) values in animals vary greatly. No deaths occurred in rats exposed to once 0.1-0.5 mL/kg chloroform by gavage in oil (Nakajima et al. 1995). Acute LD_{50} values of 2,000 mg/kg chloroform (Torkelson et al. 1976) and 2,180 mg/kg chloroform (Smyth et al. 1962) were reported for rats. LD_{50} values in male rats varied with age: 446 mg/kg for 14-day-olds, 1,337 mg/kg for young adults, and 1,188 mg/kg for old adults (Kimura et al. 1971). LD_{50} values were different for male rats (908 mg/kg/day) and female rats (1,117 mg/kg/day) (Chu et al. 1982b). Similarly, the LD_{50} for male mice was lower (1,120 mg/kg) than for female mice (1,400 mg/kg) (Bowman et al. 1978). In general, young adult males had lower survival rates. In another study, an acute oral LD_{50} value of 1,100 mg/kg/day was reported for male and female mice (Jones et al. 1958). Decreased survival rates were also observed in male mice exposed to 250 mg/kg/day chloroform for 14 days, but not in mice exposed to 100 mg/kg/day. Female mice, however, survived 500 mg/kg/day chloroform treatment (Gulati et al. 1988). Increased mortality was noted in 5 of 12 male mice exposed to 277 mg/kg/day in corn oil by gavage for 4 days (Larson et al. 1994d). Pregnant animals may be more susceptible to chloroform lethality. Increased mortality was observed in pregnant rats exposed to 516 mg/kg/day. Rabbits exposed to 63, 100, 159, 25 1, and 398 mg/kg/day chloroform during Gd 6-l 8 had increasing rates of mortality as the dose of chloroform increased (Thompson et al. 1974).

There was a high rate of mortality in rats exposed to 142 mg/kg/day chloroform in drinking water for 90 days and during a 90-day observation period. Histopathological examination revealed atrophy of

the liver and extensive squamous debris in the esophagus and gastric cardia. These changes suggested to the authors that the rats had died of starvation. Mortality was not increased in the 44 mg/kg/day group (Chu et al. 1982a). The vehicle and mode of administration may influence the lethality of chloroform in mice. In 90-day studies in mice, no effect on mortality was observed in groups treated by gavage with doses up to 250 mg/kg/day chloroform in oil (Munson et al. 1982) or with 435 mg/kg/day in drinking water (Jorgenson and Rushbrook 1980). The maximum tolerated dose of chloroform in drinking water was calculated as 306 mg/kg/day for mice (Klaunig et al. 1986). Survival was affected in mice exposed by gavage to 400 mg/kg/day chloroform in oil for 60 days, but not in those exposed to 100 mg/kg (Balster and Borzelleca 1982). Exposure to 150 mg/kg/day chloroform in toothpaste by gavage for 6 weeks caused death in 8 of 10 male mice (Roe et al. 1979). No death occurred in mice exposed to 149 mg/kg/day chloroform in oil for 30 days; there was an increased incidence of death in males exposed to 297 mg/kg/day (Eschenbrenner and Miller 1945a). No deaths occurred in dogs exposed to 120 mg/kg/day chloroform in toothpaste capsules for 12-18 weeks (Heywood et al. 1979).

Decreased survival was observed in rats exposed by gavage to concentrations ≥90 mg/kg/day chloroform in oil for 78 weeks and in female mice exposed to 477 mg/kg/day, but not in male mice exposed to 277 mg/kg/day time-weighted average (TWA) during the same time period (NCI 1976). In addition, no increase in compound-related mortality was observed in mice exposed by gavage to 60 mg/kg/day chloroform in toothpaste (Roe et al. 1979) in rats or mice exposed to ≥160 mg/kg/day chloroform in drinking water for chronic durations (Jorgenson et al. 1985; Klaunig et al. 1986). Similarly, mortality was not affected in dogs exposed to 30 mg/kg/day chloroform in toothpaste capsules for 7.5 years (Heywood et al. 1979).

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

2.2.2.2 Systemic Effects

No credible studies were located regarding ocular effects in humans or animals after oral exposure to chloroform. The other systemic effects of oral exposure to chloroform are discussed below. The highest NOAEL values and all reliable LOAEL values for each effect in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

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

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
	ACUTE EXPOSURE							
	Death							
1	Rat (Sprague-Dawley)	once (GO)				908 M (LD_{50})		Chu et al. 1982b
						1117 F (LD_{50})		
2	Rat (Sprague-Dawley)	once (G)				1337 M (LD_{50} for young adults)		Kimura et al. 1971
						1188 M (LD_{50} for old adults)		
						446 M (LD_{50} for 14-day olds)		
3	Rat (Wistar)	once (G)				2180 F (LD_{50})		Smyth et al. 1962
4	Rat (Sprague-Dawley)	10 d Gd 6-15 1-2 x/d (GO)				516 F (4/6 died)		Thompson et al. 1974
5	Rat (NS)	once (G)				2000 M (LD_{50})		Torkelson et al. 1976
6	Mouse (ICR Swiss)	once (GO)				1120 M (LD_{50})		Bowman et al. 1978
						1400 F (LD_{50})		
7	Mouse (CD-1)	14 d 1 x/d (GO)				250 M (5/8 died)		Gulati et al. 1988
8	Mouse (Swiss)	once (GO)				1100 (LD_{50})		Jones et al. 1958
9	Mouse (B6C3F1)	4 d 1 x/d (GO)				277 M (Unscheduled deaths; 5/12 mice)		Larson et al. 1994d

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
10	Rabbit (Dutch Belted)	13 d Gd 6-18 2 x/d (GO)				100 F (3/5 died)	Thompson et al. 1974
	Systemic						
11	Human	once (IN)	Resp		2410 M (respiratory tract obstruction)		Schroeder 1965
			Cardio			2410 M (arrhythmia)	
			Gastro		2410 M (vomiting)		
			Musc/skel		2410 M (muscle relaxation)		
			Hepatic			2410 M (jaundice and toxic hepatitis)	
			Renal			2410 M (oliguria)	
12	Rat (Sprague-Dawley)	once (GO)	Hemato		546 (reduced hemoglobin and hematocrit by 10-12%)		Chu et al. 1982b
			Renal		546 F (increased kidney weight)		
13	Rat (Fischer- 344)	once (GO)	Hepatic		34 M (elevated SDH, ALT and AST; scattered necrotic foci)		Larson et al. 1993
			Renal			34 M (renal proximal tubule necrosis)	
			Bd Wt	477 M			
14	Rat (Fischer- 344)	4 d 1 x/d (GO)	Hepatic	10 M	34 M (slight to mild centrilobular sinusoidal leukostasis)		Larson et al. 1995a
			Renal	10 M	34 M (degeneration of renal proximal tubules)		
			Bd Wt	90 M	180 M (decreased body weight gain)		

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
15	Rat (Fischer- 344)	4 d 1 x/d (W)	Hepatic	33.2 M	68.1 M (mild hepatocyte vacuolation)		Larson et al. 1995a
			Renal	57.5 M			
			Bd Wt		57.5 M (decreased body weight gain)		
16	Rat (Fischer- 344)	4 d (GO)	Resp		34 F (new bone formation; periosteal hypercellularity; degeneration of the olfactory epithelium and superficial Bowman's glands)		Larson et al. 1995b
			Hepatic	34 F		400 F (mild centrolobular vacuolization; sinusoidal leucostasis; mild to focally severe centrilobular hepatocyte degeneration and necrosis; diffuse centrolobular swelling)	
			Renal	100 F		200 F (distal nephrons with hyaline casts; proximal tubules lined with degenerated, necrotic or regenerating epithelium)	
			Bd Wt	34 F	400 F (weight loss approximately 14%)		
17	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)	Hemato		100 F (decreased hemoglobin and hematocrit)		Ruddick et al. 1983
			Hepatic		100 F (increased liver weight)		
			Renal	200 F	400 F (increased kidney weight)		
			Bd Wt			100 F (32% decreased body weight gain)	

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
18	Rat (Sprague-Dawley)	10 d Gd 6-15 1-2 x/d (GO)	Gastro			516 F (gastric erosions)	Thompson et al. 1974
			Hepatic			516 F *(acute toxic hepatitis)*	
			Renal			516 F (acute toxic nephrosis)	
			Bd Wt	79 F	126 F (decreased body weight gain)		
19	Rat (Sprague-Dawley)	10 d Gd 6-15 2 x/d (GO)	Dermal	50 F	126 F (alopecia)		Thompson et al. 1974
			Bd Wt	20 F	50 F (decreased maternal body weight gain)		
20	Rat (Wistar)	once (GO)	Hepatic		100 M (increased plasma GOT and GPT)		Wang et al. 1994
21	Rat (Wistar)	once (GO)	Hepatic		100 (increased plasma GOT & GPT activity)		Wang et al. 1995
22	Mouse (CD-1)	14 d 1x/d (GO)	Dermal	50	100 (rough hair coat)		Gulati et al. 1988
			Bd Wt	100 M	250 M (12% weight loss)	500 M (32% weight loss)	
23	Mouse (Swiss-Webster)	once (GO)	Hepatic		35 (midzonal fatty changes)	350 (centrilobular necrosis)	Jones et al. 1958
24	Mouse (B6C3F1)	once (GO)	Hepatic	34 F	238 F (small randomly scattered foci of hepatocyte necrosis)		Larson et al. 1993
25	Mouse (B6C3F1)	4 d 1 x/d (GO)	Hepatic	90 F	238 F (centrilobular vacuolar degeneration; increased hepatic cell proliferation)		*Larson et al. 1994b*

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
26	Mouse (B6C3F1)	4 d (W)	Hepatic	26[b] F	53 F (pale pink tinctorial changes in centrilobular hepatocytes)		Larson et al. 1994b
			Bd Wt		81 F (approximately 20% decreased body weight)		
27	Mouse (B6C3F1)	4 d 1 x/d (GO)	Hepatic		34 M (pale livers; mild centrilobular hepatocyte swelling, pale eosinophilc staining; periportal hepatocyte vacuolation)	138 M (centrilobular hepatocyte degeneration; scattered necrosis)	Larson et al. 1994d
			Renal			34 M (extensive acute necrosis, proximal convoluted tubule)	
			Bd Wt	277 M			
28	Mouse (CFLP-Swiss)	once (G)	Hepatic	59.2 M	199 M (increased SGPT)		Moore et al. 1982
			Renal	59.2 M		199 M (tubular necrosis; increased thymidine uptake)	
29	Mouse (CFLP Swiss)	once (GO)	Hepatic	65.6 M	273 M (increased thymidine uptake, increased SGOT)		Moore et al. 1982
			Renal	17.3 M		65.6 M (tubular necrosis)	
30	Mouse (CD-1)	14 d 1 x/d (GO)	Hemato	250			Munson et al. 1982
			Hepatic	125	250 (increased SGPT and SGOT levels)		
			Bd Wt	125 M	250 M (16% decreased body weight)		

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
31	Mouse (B6C3F1)	5 or 12 d 5 d/wk 1 x/d (GO)	Hepatic			263 F (necrotic, hydrotropic, swollen, and rounded hepatocytes; macrophage and neutrophil infiltration)	Pereira 1994
			Bd Wt	263 F			
32	Mouse (B6C3F1)	5 d 24 hr/d (W)	Hepatic		67.1 F (29% decreased relative liver weight; smaller hepatocytes with dense nonvacuolated and basophilic cytoplasm)		Pereira 1994
			Bd Wt	67.1 F			
			Other		67.1 F (decreased water consumption)		
33	Mouse (B6C3F1)	12 d 24 hr/d (W)	Hepatic		625.4 F (vacuolated hepatocytes)		Pereira 1994
			Bd Wt	625.4 F			
34	Rabbit (Dutch Belted)	13 d Gd 6-18 1 x/d (GO)	Gastro		20 F (diarrhea)		Thompson et al. 1974
			Bd Wt	35 F	50 F (decreased maternal body weight gain)		
	Immunological/Lymphoreticular						
35	Rat (Sprague-Dawley)	once (GO)		765 F	1071 F (reduced lymphocytes)		Chu et al. 1982b
36	Mouse (CD-1)	14 d 1 x/d (GO)			50 (suppressed humoral immunity)		Munson et al. 1982
	Neurological						
37	Human	once (IN)				2410 M (deep coma)	Schroeder 1965

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
38	Mouse (ICR)	14 d 1 x/d (GO)		31.1 M			Balster and Borzelleca 1982
39	Mouse (ICR)	once (GO)			484 M (calculated ED_{50} for motor performance)		Balster and Borzelleca 1982
40	Mouse (ICR Swiss)	once (GO)				500 (ataxia, incoordination, and anesthesia; brain hemorrhage)	Bowman et al. 1978
41	Mouse (CD-1)	14 d 1 x/d (GO)		100 M	250 M (hunched posture, inactivity)		Gulati et al. 1988
42	Mouse (Swiss)	once (GO)				350 (calculated ED_{50} for narcosis)	Jones et al. 1958
43	Mouse (CD-1)	10 d 1 x/d (GO)		10 M	30 M (taste aversion)		Landauer et al. 1982
	Reproductive						
44	Rat (Sprague-Dawley)	10 d Gd 6-15 1-2 x/d (GO)		300 F		316 F (increased resorptions)	Thompson et al. 1974
45	Rabbit (Dutch Belted)	13 d Gd 6-18 2 x/d (GO)		25 F		63 F (abortion; no viable concepti)	Thompson et al. 1974
	Developmental						
46	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)		200	400 (19% decreased fetal weight)		Ruddick et al. 1983

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
47	Rat (Sprague-Dawley)	10 d Gd 6-15 1-2 x/d (GO)		300	316 (decreased fetal weight)		Thompson et al. 1974
48	Rat (Sprague-Dawley)	10 d Gd 6-15 2 x/d (GO)		50	126 (decreased fetal weight)		Thompson et al. 1974
49	Rabbit (Dutch Belted)	13 d Gd 6-18 2 x/d (GO)		100			Thompson et al. 1974

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	INTERMEDIATE EXPOSURE						
	Death						
50	Rat (Sprague-Dawley)	90 d (W)				142.2 (high mortality during exposure and during recovery period)	Chu et al. 1982a
51	Mouse (Schofield)	6 wk 6 d/wk (G)				150 M (8/10 died)	Roe et al. 1979
	Systemic						
52	Rat (Sprague-Dawley)	90 d (W)	Hemato	149.8			Chu et al. 1982a
			Bd Wt	44.9	142.2 (25% decreased body weight gain)		
53	Rat (Sprague-Dawley)	28 d (W)	Hemato	22.8 M	193 M (decreased neutrophils)		Chu et al. 1982b
			Hepatic	193 M			
			Renal	193 M			
54	Rat (Osborne-Mendel)	90 d (W)	Resp	160 M			Jorgenson and Rushbrook 1980
			Gastro	160 M			
			Hemato	160 M			
			Hepatic	160 M			
			Renal	160 M			
			Bd Wt	81 M	160 M (11-17% decreased body weight)		

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
55	Rat (Fischer- 344)	3 wk 5 d/wk 1 x/d (GO)	Hepatic	90 M	180 M (degeneration of centrilobular hepatocytes)		Larson et al. 1995a
			Renal	90 M	180 M (progressive degeneration of the proximal tubules)		
			Bd Wt	180 M			
56	Rat (Fischer- 344)	3 wk 7 d/wk 1 x/d (W)	Hepatic	62.3 M	106 M (mild hepatocyte vacuolation)		Larson et al. 1995a
			Renal	6.0 M	17.4 M (increased numbers of focal areas of regenerating renal proximal tubular epithelium and cell proliferation)		
			Bd Wt	62.3 M	106 M (25% decrease in weight gain - taken from graph)		
57	Rat (Fischer- 344)	3 wk 5 d/wk (GO)	Resp		34 F (new bone formation; periosteal hypercellularity)		Larson et al. 1995b
			Hepatic	100 F	400 F (diffuse vacuolar change; focal centrilobular degeneration)		
			Renal	34 F	100 F (proximal tubule epithelial regeneration, dilation and mineralized concretions)		
			Bd Wt		100 F (significant decrease in weight gain)		

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference
58	Rat (Sprague-Dawley)	13 wk 7 d/wk 1 x/d (G)	Hemato	150	410	(increased cellular proliferation in bone marrow)			Palmer et al. 1979
			Hepatic	30	150	(increased relative liver weight)	410	(fatty changes, necrosis)	
			Renal	30	150	(increased relative kidney weight)			
59	Mouse (B6C3F1)	90 d 1 x/d (GO)	Hepatic		60	(fatty changes)	270	(cirrhosis)	Bull et al. 1986
			Bd Wt	130 M	270 M	(15% decreased body weight)			
60	Mouse (Strain A)	30 d 1 x/d (GO)	Hepatic	297			594	(cirrhosis)	Eschenbrenner and Miller 1945a
61	Mouse (CD-1)	105 d 1 x/d (GO)	Resp	41					Gulati et al. 1988
			Hepatic	16 F	41 F	(increased liver weight and hepatocellular degeneration)			
			Renal	41					
62	Mouse (B6C3F1)	90 d (W)	Resp	435 F					Jorgenson and Rushbrook 1980
			Gastro	435 F					
			Hemato	435 F					
			Hepatic	32 F	64 F	(fatty changes)			
			Renal	435 F					

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
63	Mouse (B6C3F1)	52 wk 7 d/wk (W)	Resp	257 M			Klaunig et al. 1986
			Hepatic		86 M (focal necrosis)		
			Renal			86 M (tubular necrosis)	
			Bd Wt		86 M (15% decreased body weight gain)		
64	Mouse (B6C3F1)	3 wk 5 d/wk 1 x/d (GO)	Hepatic		34 F (vacuolation of the centrilobular and midzonal hepatocytes; increased ALT and SDH)		Larson et al. 1994b
			Renal	477 F			
65	Mouse (B6C3F1)	3 wk 7 d/wk (W)	Hepatic		82 F (increased liver weight)		Larson et al. 1994b
			Renal	329 F			
66	Mouse (B6C3F1)	3 wk 5 d/wk 1 x/d (GO)	Hepatic	34 M	90 M (centrilobular hepatocyte swelling; loss of eosinophilia)	138 M (centrilobular and periportal hepatocyte degeneration and necrosis)	Larson et al. 1994d
			Renal		34 M (regenerating proximal convoluted tubules)	277 M (degeneration & necrosis of the proximal tubules)	
			Bd Wt	138 M	277 M (15-20% decrease in body weight)		
67	Mouse (CD-1)	90 d 1 x/d (GO)	Hepatic		50 (hydropic degeneration)		Munson et al. 1982
			Renal		50 (chronic inflammation of lymphocytes)		
			Bd Wt	250			

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
68	Mouse (B6C3F1)	33 or 159 d 5 d/wk 1 x/d (GO)	Hepatic		263 F	(30.1-38.2% increase in relative liver weight; focal areas of necrotic, swollen, rounded, and pale hepatocytes and a low number of mononuclear cells)	Pereira 1994
			Bd Wt	263 F			
69	Mouse (B6C3F1)	33 d 24 hr/d (W)	Hepatic	438.5 F			Pereira 1994
			Bd Wt	438.5 F			
70	Mouse (B6C3F1)	159 d 24 hr/d (W)	Hepatic		363.5 F	(31.4% increase in relative liver weight)	Pereira 1994
			Bd Wt	386 F			
71	Dog (Beagle)	6 wk 6 d/wk 1 x/d (C)	Hepatic	15[c]	30	(significantly increased SGPT activity)	Heywood et al. 1979
Immunological/Lymphoreticular							
72	Mouse (CD-1)	90 d 1 x/d (GO)			50	(depressed humoral immunity)	Munson et al. 1982
Neurological							
73	Mouse (ICR)	90 d 1x/d (GO)		31.1 M			Balster and Borzelleca 1982
74	Mouse (ICR)	60 d 1x/d (GO)			100 M	(operant behavior affected)	Balster and Borzelleca 1982

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Reproductive						
75	Rat (Osborne-Mendel)	90 d (W)		160 M			Jorgenson and Rushbrook 1980
76	Rat (Sprague-Dawley)	13 wk 7 d/wk 1 x/d (G)		150		410 (gonadal atrophy)	Palmer et al. 1979
77	Mouse (CD-1)	105 d 1 x/d (GO)		41			Gulati et al. 1988
	Developmental						
78	Mouse (ICR)	6-10 wk 1 x/d (GO)		31.1			Burkhalter and Balster 1979
79	Mouse (CD-1)	105 d 1 x/d (GO)			41 M (increased epididymal weights, degeneration of epididymal epithelium in F_1)		Gulati et al. 1988
80	Mouse (CD-1)	105 d 1 x/d (GO)			41 F (increased liver weight and hepatocellular degeneration in F_1 females)		Gulati et al. 1988
	Cancer						
81	Mouse (Strain A)	30 d 1 x/d (GO)				594 (CEL: hepatomas)	Eschenbrenner and Miller 1945a

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	CHRONIC EXPOSURE						
	Death						
82	Rat (Osborne-Mendel)	78 wk 5 d/wk 1 x/d (GO)				90 M (decreased survival)	NCI 1976
						100 F	
83	Mouse (B6C3F1)	78 wk 5 d/wk 1 x/d (GO)				477 F (decreased survival)	NCI 1976
	Systemic						
84	Human	1-5 yr	Hepatic	0.96			De Salva et al. 1975
			Renal	0.96			
85	Human	10 yr 1 x/d (IN)	Hemato		21 M (decreased erythrocytes)		Wallace 1950
			Hepatic			21 M (increased sulfobromophthalein sodium retention)	
			Renal			21 M (albuminuria)	
86	Rat (Osborne-Mendel)	104 wk 7 d/wk (W)	Renal	160 M			Jorgenson et al. 1985
			Bd Wt	38 M	81 M (decreased body weight)		

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
87	Rat (Osborne-Mendel)	78 wk 5 d/wk 1 x/d (GO)	Resp	200 F			NCI 1976
			Cardio	200 F			
			Gastro	200 F			
			Hemato	200 F			
			Musc/skel	200 F			
			Hepatic	100 F	200 F (necrosis of hepatic parenchyma)		
				180 M			
			Renal	200 F			
			Bd Wt		90 M (15% decreased weight gain)		
88	Rat (Wistar)	180 wk 7 d/wk (W)	Hepatic			200 (adenofibrosis)	Tumasonis et al. 1985, 1987
			Bd Wt			200 M (50% decreased body weight gain)	
89	Mouse (B6C3F1)	104 wk 7 d/wk (W)	Bd Wt	130 F	263 F (decreased body weight)		Jorgenson et al. 1985
90	Mouse (B6C3F1)	78 wk 5 d/wk 1 x/d (GO)	Resp		238 F (pulmonary inflammation)		NCI 1976
			Cardio		238 F (cardiac thrombosis)		
			Gastro	477 F			
			Hemato	477 F			
			Musc/skel	477 F			
			Hepatic			138 M (nodular hyperplasia of the liver)	
						238 F	
			Renal	477 F			
			Bd Wt	477 F			

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
91	Mouse (ICI)	80 wk 6 d/wk (G)	Resp	60			Roe et al. 1979
			Hepatic		17 (fatty degeneration)		
			Renal	60			
			Bd Wt	60			
92	Dog (Beagle)	7.5 yr 6 d/wk (C)	Cardio	30			Heywood et al. 1979
			Hemato	30			
			Hepatic		15[d] (increased SGPT activity)		
			Renal	15	30 (fatty changes)		
			Bd Wt	30			
	Neurological						
93	Rat (Osborne-Mendel)	78 wk 5 d/wk 1 x/d (GO)		200 F			NCI 1976
94	Mouse (B6C3F1)	78 wk 5 d/wk 1 x/d (GO)		477 F			NCI 1976
95	Mouse (ICI)	80 wk 6 d/wk (G)		60			Roe et al. 1979
	Reproductive						
96	Rat (Osborne-Mendel)	78 wk 5 d/wk 1 x/d (GO)		200 F 180 M			NCI 1976

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
97	Mouse (B6C3F1)	78 wk 5 d/wk 1 x/d (GO)		477 F 277 M				NCI 1976
98	Dog (Beagle)	7.5 yr 6 d/wk (C)		30				Heywood et al. 1979
	Cancer							
99	Rat (Osborne-Mendel)	104 wk 7 d/wk (W)				160 M	(CEL: tubular cell adenoma)	Jorgenson et al. 1985
100	Rat (Osborne-Mendel)	78 wk 5 d/wk 1 x/d (GO)				90 M 200 F	(CEL: tubular cell adenoma and carcinoma; tubular cell neoplasms in 4/50) (CEL: kidney tubular cell neoplasms in 2/48)	NCI 1976; Dunnick and Melnick 1993
101	Rat (Wistar)	180 wk 7 d/wk (W)				200 F	(CEL: hepatic neoplastic nodules)	Tumasonis et al. 1985, 1987
102	Mouse (B6C3F1)	78 wk 5 d/wk 1 x/d (GO)				138 M 238 F	(CEL: hepatocellular adenomas or carcinomas in 18/50 mice) (CEL: hepatocellular adenomas or carcinomas in 36/45 mice)	NCI 1976; Dunnick and Melnick 1993

Table 2-2. Levels of Significant Exposure to Chloroform - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
103	Mouse (ICI)	80 wk 6 d/wk (G)				60 M	(CEL: epithelial tumors of the kidney)	Roe et al. 1979

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

[b]Used to derive an acute oral minimal risk level (MRL) of 0.3 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability).

[c]Used to derive an intermediate oral MRL of 0.1 mg/kg/day; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability).

[d]Used to derive an chronic oral MRL of 0.01 mg/kg/day; dose adjusted for intermittent exposure and divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

ALT = alanine amino transferase; ST = aspartate amino transferase; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); ED_{50} = effective dose for a given effect in 50% of animals; F = female; F_1 = first filial generation; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; (GO) = gavage in oil; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; Hemato = hematological; hr = hour(s); (IN) = ingestion; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Musc/skel = musculoskeletal; NOAEL= no-observed-adverse-effect level; NS = not specified; Resp = respiratory; SDH = sorbitol dehydrogenase; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; (W) = drinking water; wk = week; x = time(s); yr = year(s)

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

Acute (≤14 days)

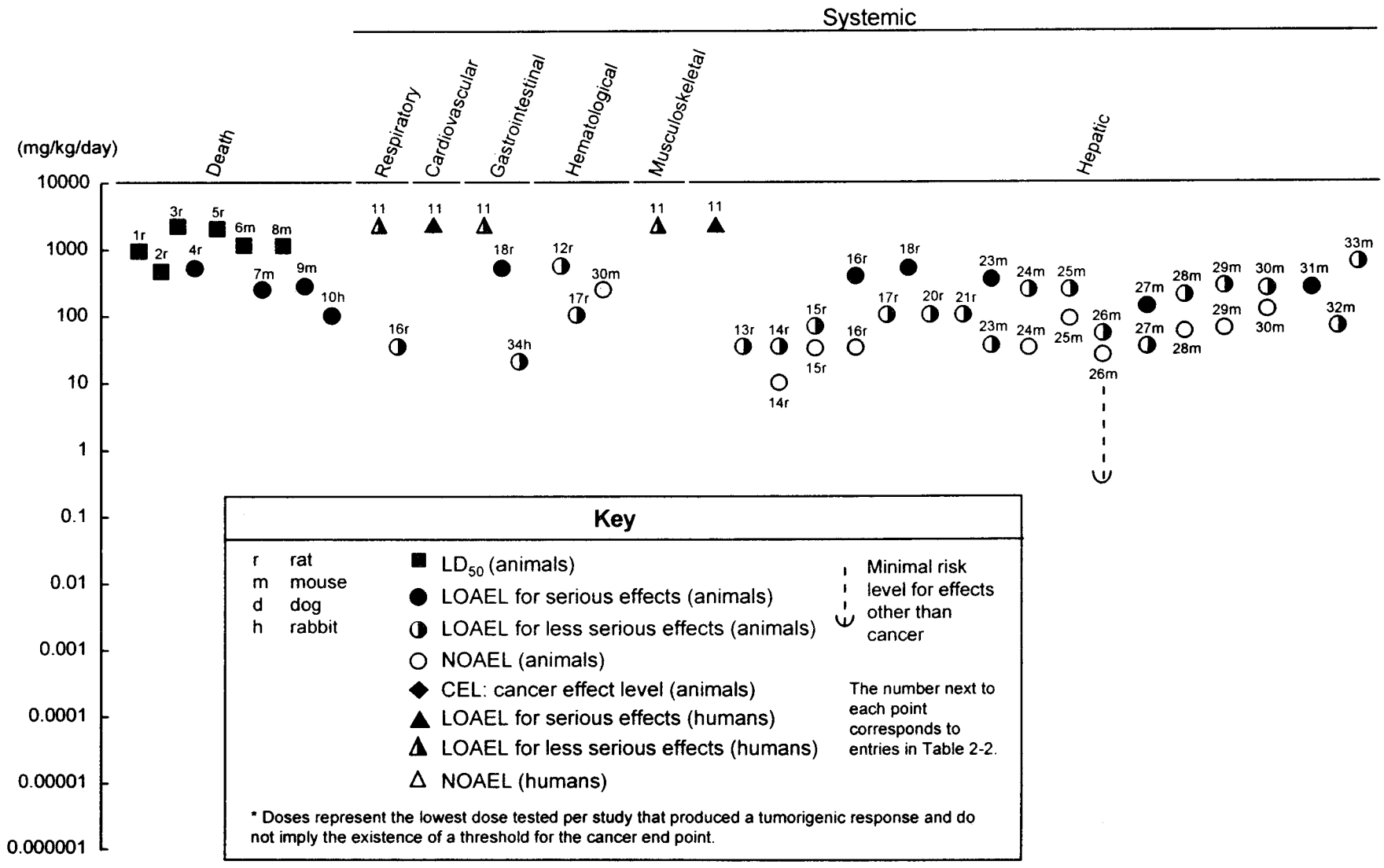


Figure 2-2. Levels of Significant Exposure to Chloroform - Oral (cont.)

Acute (≤14 days)

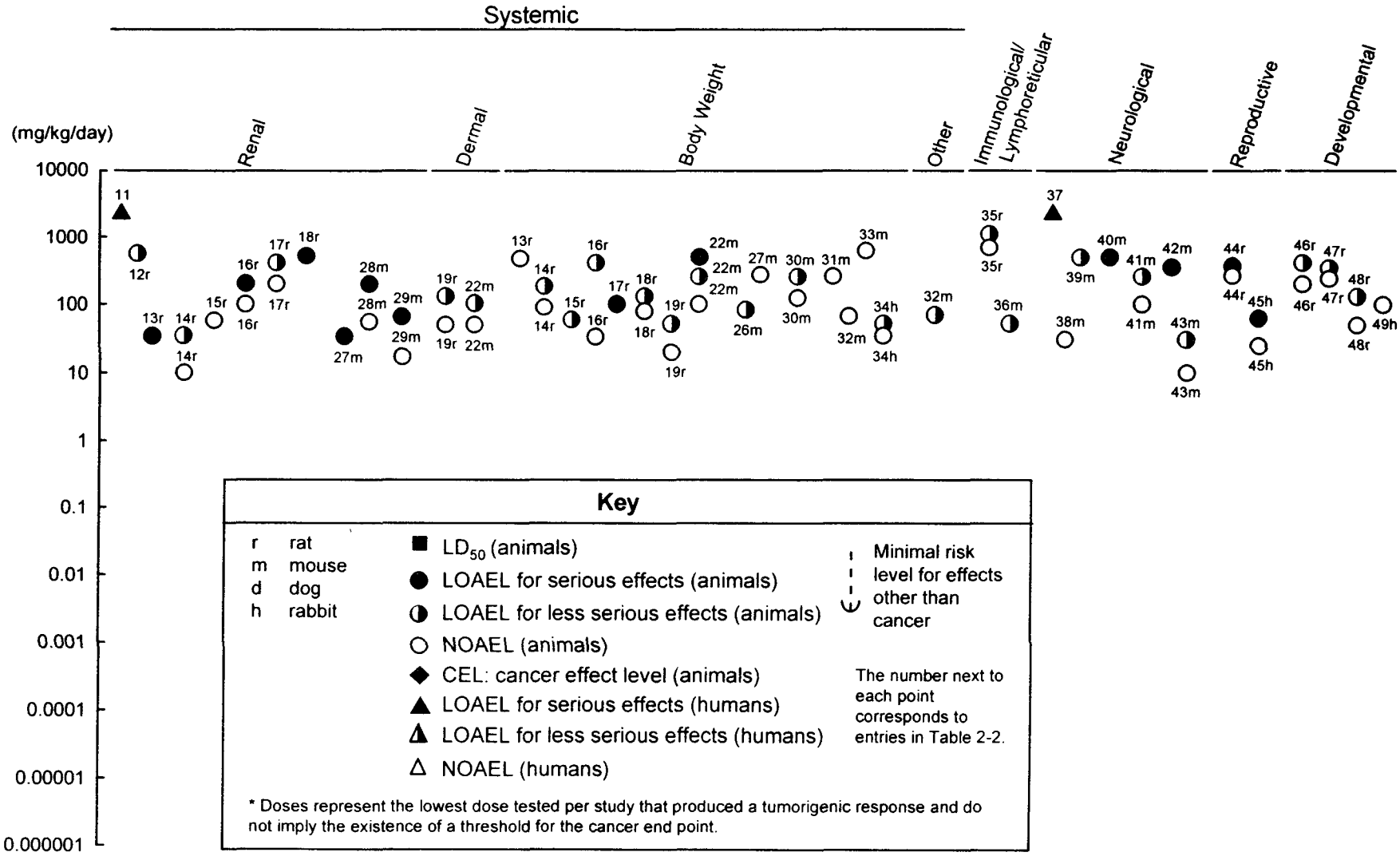


Figure 2-2. Levels of Significant Exposure to Chloroform - Oral (cont.)

Intermediate (15-364 days)

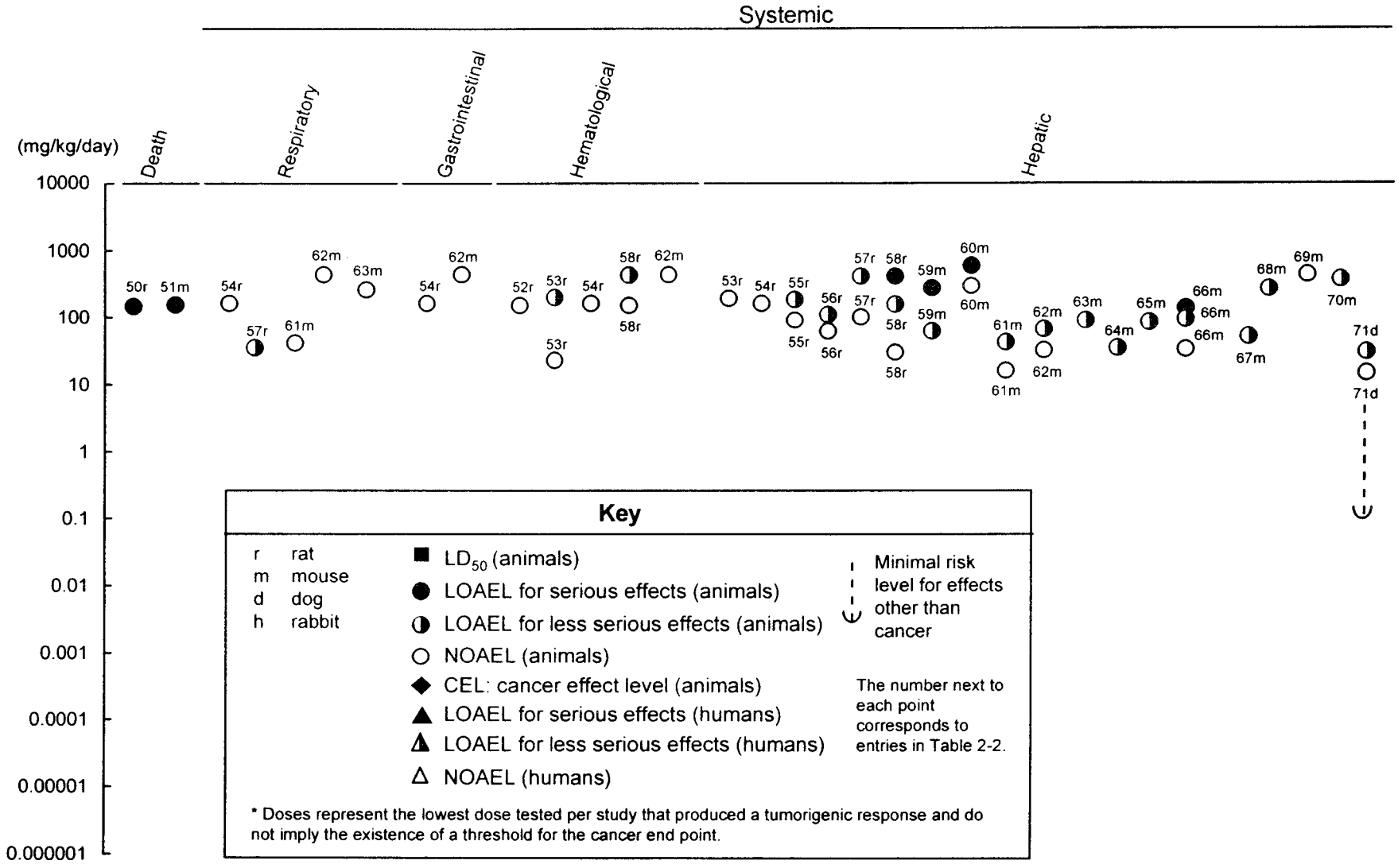


Figure 2-2. Levels of Significant Exposure to Chloroform - Oral (cont.)

Intermediate (15-364 days)

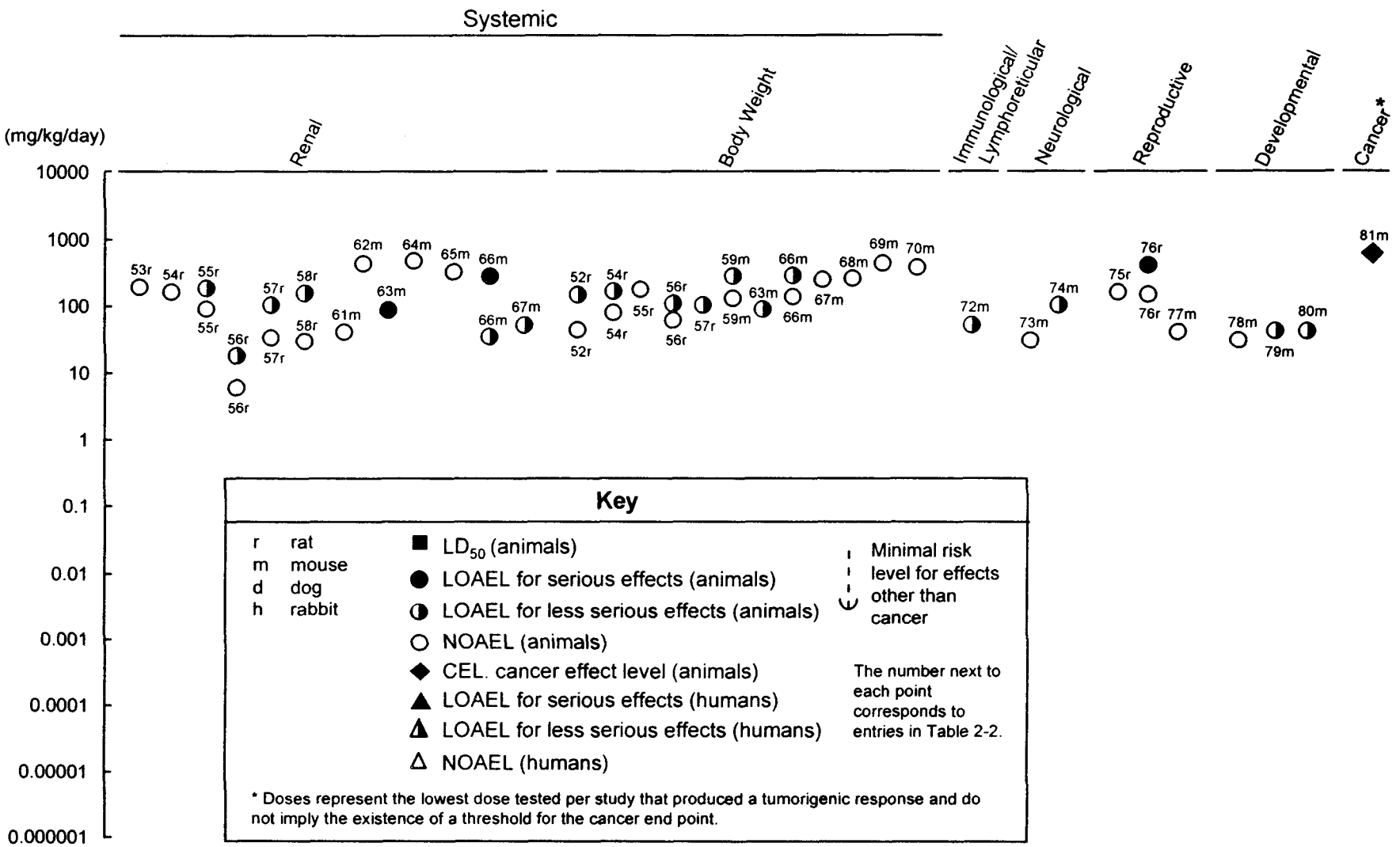
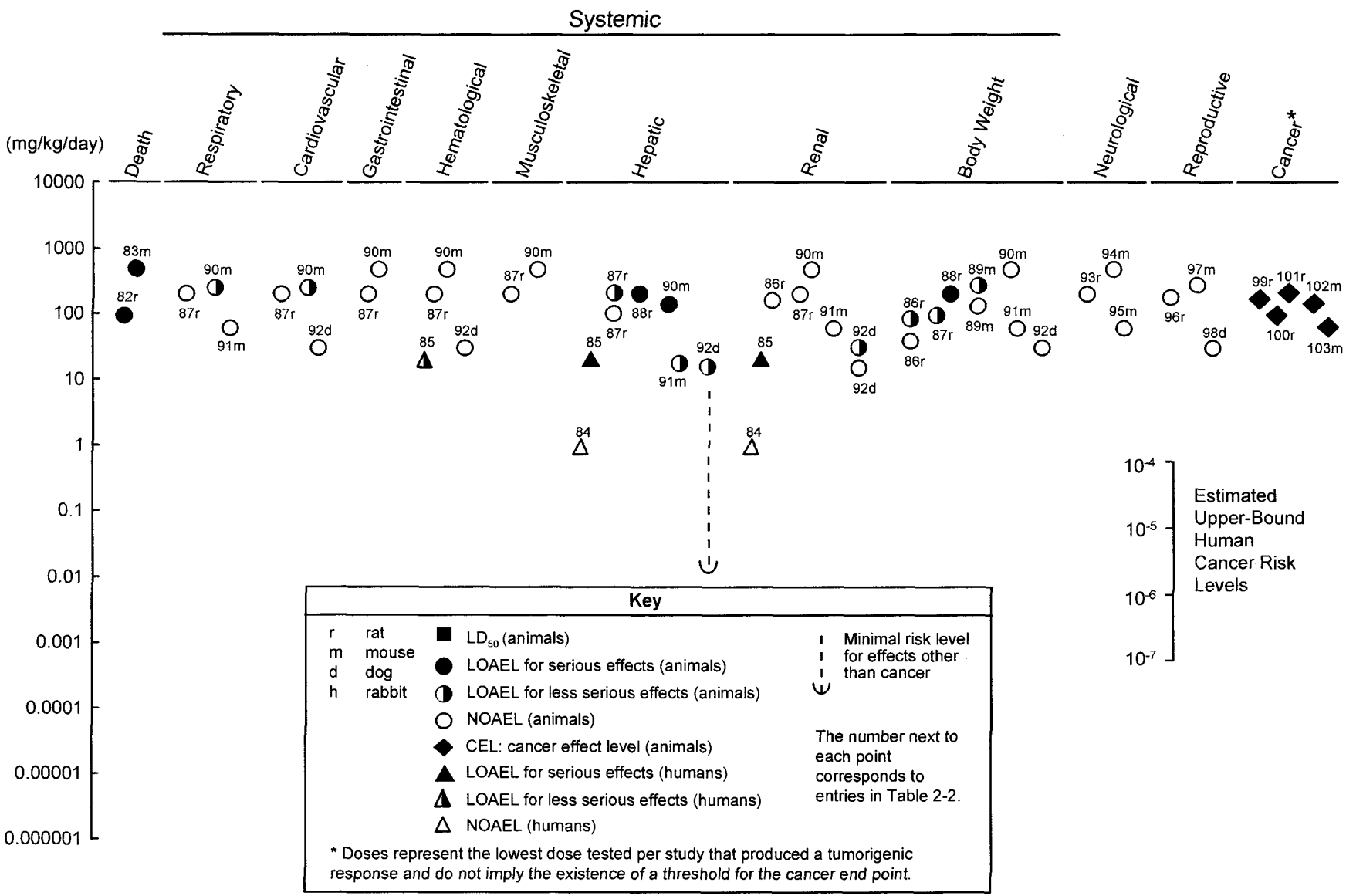


Figure 2-2. Levels of Significant Exposure to Chloroform - Oral (cont.)

Chronic (≥365 days)



2. HEALTH EFFECTS

Respiratory Effects. Information regarding respiratory effects in humans after oral exposure to chloroform is limited. Upper respiratory tract obstruction due to muscular relaxation was observed in a patient who accidentally ingested ≈2,410 mg/kg chloroform (Schroeder 1965). Congested lungs and scattered patches of pneumonic consolidation were found at autopsy in a man who committed suicide by drinking ≈6 ounces (3,755 mg/kg) of chloroform (Piersol et al. 1933). In both of these case studies, very large doses of chloroform were consumed. Thus, the respiratory effects noted may not be characteristic of those seen after ingestion of more moderate doses.

The majority of animal data suggest that the respiratory system is not a target of chloroform-induced toxicity after oral exposure. However, in one study, female Fisher 344 rats administered chloroform by gavage in corn oil at doses of 34, 100, 200, or 400 mg/kg/day for 4 days or 3 weeks did exhibit dose-dependent nasal lesions consisting of early phases of new bone formation, periosteal hypercellularity, and degeneration of the olfactory epithelium and superficial Bowman's glands (Larson et al. 1995b). In other studies, no treatment-related histopathological changes were found in the lungs of rats exposed to 160 mg/kg/day or mice exposed to 435 mg/kg/day chloroform in drinking water in a 90-day study (Jorgenson and Rushbrook 1980), in mice exposed by gavage to 41 mg/kg/day chloroform in oil for 105 days (Gulati et al. 1988), or in mice exposed to 257 mg/kg/day in drinking water for 1 year (Klaunig et al. 1986).

Following chronic exposure, no histopathological changes were observed in rats exposed by gavage to 200 mg/kg/day TWA chloroform in oil (NCI 1976). Respiratory disease was observed in all chloroform-exposed groups of rats (≥15 mg/kg/day); however, no histopathological changes were observed in a 60 mg/kg/day exposure group during another experiment by the same investigators (Palmer et al. 1979). No histopathological changes were observed in the lungs of male mice exposed by gavage to 277 mg/kg/day TWA chloroform in oil for 78 weeks (NCI 1976) or to 60 mg/kg/day in toothpaste for 80 weeks (Roe et al. 1979).

Cardiovascular Effects. Information regarding cardiovascular effects after oral exposure to chloroform is limited to case report studies. On admission to the hospital, the blood pressure was 140/90 mm Hg and pulse was 70 beats per minute (bpm) in a patient who accidentally ingested ≈2,410 mg/kg chloroform (Schroeder 1965). Electrocardiography showed occasional extra systoles and a slight S-T segment depression. The patient recovered with no persistent cardiovascular change, In another individual, blood pressure was 100/40 mm Hg and pulse was 108 bpm after ingestion of an

unknown quantity of chloroform and alcohol (Storms 1973). In both of these case studies, other factors (e.g., age, consumption of alcohol, suicidal/agitated state) may have contributed to the cardiovascular effects seen. Thus, it would appear that no studies exist which reliably indicate the presence of cardiovascular effects after oral chloroform exposure in humans.

Information regarding cardiovascular effects in animals after oral exposure to chloroform is limited; the data suggest that, at the doses utilized, the cardiovascular system was not a target for chloroform toxicity. No histopathological changes were observed in rats and mice chronically exposed by gavage to 200 and 477 mg/kg/day TWA chloroform, respectively, for 78 weeks (NCI 1976). In this study, cardiac thrombosis was observed in low-dose female mice, but was not seen in high-dose female or male mice or either sex of rat at any dose. Similarly, no cardiovascular changes were observed in dogs exposed to 30 mg/kg/day chloroform in toothpaste capsules for 7.5 years (Heywood et al. 1979).

Gastrointestinal Effects. Retrostemal soreness, pain on swallowing, and gastric distress with vomiting were reported in cases of intentional and accidental ingestion of chloroform (Piersol et al. 1933; Schroeder 1965). At autopsy, congestion with patchy necrosis of the mucosa was observed in the stomach and duodenum of a man who died after drinking ≈3,755 mg/kg chloroform (Piersol et al. 1933). The colonic mucosa was edematous, and the rectosigmoid junction was hemorrhagic. A 16-year-old female who ingested an unknown of amount of chloroform arrived at a hospital semiconscious and with repeated vomiting. She was treated with gastric lavage, antacids, intravenous glucose, and antiemetics. She had apparently recovered and was released. Seven days later, she presented with hepatomegaly, slightly depressed hemoglobin, and an abnormal liver sonogram, but no gastric side-effects (Hakim et al. 1992).

The effects of chronic oral exposure to chloroform, as a by-product of the chlorination of drinking water, were evaluated in four epidemiology studies (Alavanja et al. 1978; Cantor et al. 1978; Saurez-Varela et al. 1994; Young et al. 1981). The association between the incidence of gastrointestinal cancer in humans and the chlorination of drinking water is discussed in Section 2.2.2.8. The data from these studies should be viewed with caution as many other known or suspected carcinogens are known to exist in chlorinated drinking water.

Gastrointestinal irritation has been observed in some animals after oral exposure to chloroform. Gastric erosions were observed in pregnant rats gavaged with 516 mg/kg chloroform in oil during

gestation (Thompson et al. 1974). Rabbits exposed by gavage to 20 mg/kg/day chloroform in oil during gestation had diarrhea; no histological results were provided. In a 90-day drinking-water study, no histopathological changes were observed in rats and mice exposed to 160 and 435 mg/kg/day chloroform, respectively (Jorgenson and Rushbrook 1980). Vomiting was observed in dogs exposed to 30 mg/kg/day chloroform in toothpaste capsules for 12-18 weeks (Heywood et al. 1979). In a chronic exposure study, no histopathological changes in gastrointestinal tissue were observed in rats and mice exposed by gavage to 200 and 477 mg/kg/day TWA chloroform, respectively (NCI 1976).

Hematological Effects. The only information regarding hematological effects in humans after chronic oral exposure to chloroform was reported in a case study. Decreased erythrocytes and hemoglobin were observed in a subject who ingested ≈21 mg/kg/day chloroform in a cough medicine for 10 years (Wallace 1950). The lack of detail and the potential for confounding factors in this study does not allow a firm conclusion regarding the hematological effects of oral exposure to chloroform in humans.

Hematological effects have been observed in some animals after oral exposure to chloroform. Hemoglobin and hematocrit decreased in male and female rats after a single oral dose of 546 mg/kg chloroform in oil (Chu et al. 1982b) and in female rats exposed to 100 mg/kg/day chloroform during gestation (Ruddick et al. 1983). However, no hematological changes were observed in mice exposed to 250 mg/kg/day for 14 days (Munson et al. 1982). In an intermediate-duration study, decreased neutrophils were observed in rats exposed to 192.98 mg/kg/day in drinking water (Chu et al. 1982b); however, no hematological changes were observed in rats and mice exposed to 160 and 435 mg/kg/day chloroform, respectively, for 90 days in drinking water (Chu et al. 1982a; Jorgenson and Rushbrook 1980). Increased cellular proliferation in the bone marrow was observed in rats exposed by gavage for 13 weeks to 410 mg/kg chloroform in toothpaste (Palmer et al. 1979). No hematological changes were observed, however, in rats similarly exposed to 165 and 60 mg/kg/day chloroform by gavage for 52 and 80 weeks, respectively. Moreover, no histopathological changes in hematopoietic tissues were observed in rats and mice after chronic exposure to 200 and 477 mg/kg/day TWA chloroform in oil, respectively (NCI 1976). No hematological effects were observed in dogs exposed to 30 mg/kg/day chloroform in toothpaste in capsules for 7.5 years (Heywood et al. 1979). In conclusion, no consistent hematological effects were noted in human or animal studies of oral exposure to chloroform.

2. HEALTH EFFECTS

Musculoskeletal Effects. The only information regarding musculoskeletal effects in humans after oral exposure to chloroform was reported in a case study. Muscular relaxation of the jaw caused upper respiratory obstruction in a man who accidentally ingested approximately 2,410 mg/kg chloroform (Schroeder 1965), reflecting the neurological effects of chloroform exposure.

Only one report detailing musculoskeletal effects in animals after oral exposure to chloroform was located. In that study, no histopathological changes were observed in the musculoskeletal system of rats and mice after chronic gavage exposure to 200 and 477 mg/kg/day TWA chloroform in oil, respectively (NCI 1976).

Hepatic Effects. The liver is a primary target of chloroform toxicity in humans, with some evidence that suggests that the damage may be reversible (Wallace 1950). Hepatic injury occurred in patients within 1-3 days following chloroform ingestion (Piersol et al. 1933; Schroeder 1965; Storms 1973). Jaundice and liver enlargement and tenderness developed in all patients. The clinical observations were supported by blood biochemistry results with increased SGOT, SGPT, and lactate dehydrogenase (LDH) activities and increased bilirubin levels. At autopsy, fatty degeneration and extensive centrilobular necrosis were observed in one fatal case (Piersol et al. 1933).

A 16-year-old female who ingested an unknown of amount of chloroform and arrived at a hospital semiconscious and with repeated vomiting was reported by Hakim et al. (1992). She was treated with gastric lavage, antacids, intravenous glucose, and antiemetics. She had apparently recovered and was released. Seven days later, she presented with hepatomegaly, slightly depressed hemoglobin, and an abnormal liver sonogram, suggesting toxic hepatic disease due to chloroform toxicosis. A 33-year-old female had injected herself intravenously with 0.5 mL of chloroform and then became unconscious. When she awoke approximately 12 hours later, she then drank another 120 mL of chloroform. She was treated with hyperbaric oxygen, cimetidine (to inhibit cytochrome P-450 and formation of phosgene), and N-acetylcystine (to replenish GSH stores). Liver serum enzymes alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and LDH were elevated in a pattern that suggested liver cell necrosis. Generally, these enzymes were noted to peak by day 4 and decrease by day 11. Total bilirubin and direct bilirubin did not change appreciably. GGT (gamma glutamyltransferase, also known as gamma glutamyl transpeptidase), alpha-feto protein and retinol binding protein showed increases between 6 and 8 days after ingestion, but still within normal ranges for humans (Rao et al. 1993).

2. HEALTH EFFECTS

Increased sulfobromophthalein 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. Biochemical tests indicate that liver function in male and female humans was not affected by the use of mouthwash providing 0.96 mg/kg/day chloroform for ≤5 years (De Salva et al. 197.5).

The liver is also a target organ for chloroform toxicity in animals. In acute studies, hepatitis was observed in pregnant rats exposed by gavage to 516 mg/kg/day chloroform in oil (Thompson et al. 1974), while increased maternal liver weight without any histopathological changes was observed in pregnant rats similarly exposed to 100 mg/kg/day chloroform (Ruddick et al. 1983). Increased serum levels of transaminases, indicative of liver necrosis, were observed in mice treated with a single gavage dose of 199 mg/kg chloroform in toothpaste, 273 mg/kg in oil (Moore et al. 1982), or 250 mg/kg/day in oil for 14 days (Munson et al. 1982). Similar results were reported for rats treated with a single gavage dose of 100 mg/kg (Wang et al. 1994, 1995) or 0.1 mL/kg (Nakajima et al. 1995) Centrilobular necrosis of the liver with massive fatty changes was also observed in mice after a single dose of 350 mg/kg chloroform in oil (Jones et al. 1958). At a dose of 35 mg/kg, minimal lesions consisting of mid-zonal fatty changes were observed in mice.

Similar results were reported by Larson et al. (1993) in male rats in order to identify target tissues for the acute effects of chloroform in rats and mice and to establish the time-course of chloroform-induced histopathologic and proliferative responses. Rats were given 34, 180, or 477 mg/kg once in corn oil by gavage and sacrificed 24 hours after administration (acute-duration study). In a related time-course study (which focused on histologic changes in tissues over time), rats received 180 mg/kg chloroform in corn oil by gavage and were sacrificed at 0.5, 1, 2, 4, and 8 days after treatment, or received 477 mg/kg in corn oil by gavage and were sacrificed either 1 or 2 days after administration. In the acute study, gross liver to body weight ratios were unaffected at all doses. Histologically, chloroform caused hepatic injury, in a dose-related manner, producing morphologic changes generally limited to the centrilobular hepatocytes. Liver enzymes (SDH, ALT, and AST) were slightly elevated above controls in the 34 and 180 mg/kg group, but were significantly higher in the 477 mg/kg group for all three enzymes. In the time-course study, 1 day after dosing, about 50% of the hepatocytes adjacent to the central veins were degenerated or necrotic in the 180 mg/kg treatment group. Larger vessels had perivascular edema, influx of neutrophils and eosinophils. Only scattered hepatocyte necrosis was seen

by day 2 after treatment. By eight days however, the livers were not histologically different from controls.

In a similar study, mice were administered 34, 238, or 477 mg/kg once in corn oil by gavage and sacrificed 24 hours after administration (acute study) or 350 mg/kg chloroform in corn oil by gavage and sacrificed at 0.5, 1, 2, 4, and 8 days after treatment (time-course study). Livers of female mice were much more sensitive than the kidneys to the toxic effects of chloroform. In the acute study, livers of mice receiving 34 mg/kg chloroform were not histologically different from controls; however, those treated with 238 mg/kg had few small randomly scattered foci of hepatocyte necrosis. Livers from the 477 mg/kg group had centrilobular coagulative necrosis of 50% of the lobule. In the timecourse study, a significant increase in liver weights and liver to body-weight ratios was observed in mice at 2 and 4 days after treatment with the 350 mg/kg dose of chloroform. At 12 hours after treatment, mice had marked swelling of the centrilobular hepatocytes, affecting about 50% of the lobule. One day after treatment, the hepatocytes adjacent to the central vein were necrotic. Two days after chloroform treatment, centrilobular sinusoids were dilated with inflammatory cells associated with centrilobular necrosis. At eight days after treatment, the livers from the treated mice were not histologically different from those of control animals. Serum liver enzymes (SDH and ALT) were elevated in the groups sacrificed at 0.5, 1, 2, and 4 days after treatment, but not in controls or in those animals sacrificed 8 days after treatment.

Another study by Larson et al. (1994d) identified the relationships among chloroform-induced cytolethality, regenerative cell proliferation, and tumor induction in male B6C3F_1 mice dosed with 0, 34, 90, 138, or 277 mg/kg/day chloroform by gavage in corn oil. Mice exposed to 90 mg/kg/day experienced prominent centrilobular hepatocyte swelling with loss of cytoplasmic eosinophilia. Mice exposed to 138 and 277 mg/kg/day experienced increasing levels of centrilobular hepatocyte swelling and degeneration, as well as scattered necrosis and inflammatory cell accumulation. Dose-dependent increases in hepatocyte proliferation were seen in all dose groups after exposure to chloroform for 4 days.

Differences in chloroform toxicity have been noted in female mice when chloroform was administered in different vehicles and by different dosing regimes (Larson et al. 1994b). Mice were treated orally with 3, 10, 34, 90, 238, or 477 mg/kg/day of chloroform in corn oil, or with 16, 26, 53, 81, or 105 mg/kg/day in the drinking water, for 4 days. Chloroform treatment resulted in significant

increases in liver weights of mice at the 238 and 477 mg/kg/day dose levels. Mice treated with 238 mg/kg chloroform had moderate centrilobular vacuolar degeneration of hepatocytes and scattered centrilobular and subcapsular hepatocyte necrosis. At the 477 mg/kg dose, severe centrilobular coagulative necrosis with small number of inflammatory cells in the necrotic areas was also observed. Dose-dependent increases in both ALT and SDH were also observed. At daily doses of 90 mg/kg or less, no increase in hepatic cell proliferation was noted. Dose-dependent increases in hepatic cell proliferation and cells observed to be in S-phase occurred in the 238 and 477 mg/kg/day doses. For mice dosed with 16, 26, 53, 81, or 105 mg/kg/day in the drinking water, serum ALT or SDH were not different from controls at any dose. In the 53, 81, and 105 mg/kg/day treatment groups, the livers had changes that were characterized by pale cytoplasmic eosinophilic staining of centrilobular hepatocytes compared to the periportal hepatocytes and controls. Livers from mice treated with 26 mg/kg/day chloroform or less failed to showed significant histologic changes when compared to controls. Cell proliferations in the liver were not found at any dose or duration. An acute oral MRL of 0.3 mg/kg/day was calculated using the 26.4 mg/kg/day NOAEL based on the hepatic effects in these animals from this study. More information on this MRL and how it was derived is located in the footnote to Table 2-2, Section 2.5, and in Appendix A of this profile.

Larson et al. (1995a) examined the dose-response relationships for the induction of cytolethality and regenerative cell proliferation in the livers of male Fischer 344 rats given chloroform by gavage. Groups of 12 rats were administered oral doses of 0, 3, 10, 34, 90, and 180 mg/kg/day chloroform in corn oil by gavage for 4 days. BrdU was administered via an implanted osmotic pump to label cells in S-phase. Cells having incorporated BrdU were visualized in tissue sections immunohistochemically and the LI evaluated as the percentage of S-phase cells. Necropsies and histopathological examinations were performed at death. The relative liver weights were increased at doses of 10 mg/kg/day and above at 4 days posttreatment. Rats treated with 34, 90, or 180 mg/kg/day by gavage for 4 days had mild-to-moderate degeneration of centrilobular hepatocytes. The livers of rats given 90 mg/kg/day for 4 days had a slight increase in centrilobular pallor and necrosis of hepatocytes surrounding the central vein; the remaining central and some mid-zonal hepatocytes were swollen and displayed a cytoplasmic granularity. In the 180 mg/kg/day dose group, the livers had scattered individual cell necrosis throughout the central and mid-zonal regions. The cytoplasm of the centrilobular hepatocytes were pale, eosinophilic and mildly vacuolated. Dose-dependent increases in both ALT and SDH were observed at 4 days in the 90 and 180 mg/kg/day dose groups and at 3 weeks

in the 180 mg/kg/day dose group only. A dose-dependent increase in LI was seen in rat liver after 4 days of treatment with 90 and 180 mg/kg/day by gavage.

Larson et al. (1995a) also examined the toxicological effects of chloroform administered in the drinking water in rats. Groups of 12 rats were administered chloroform *ad libitum* in drinking water at concentrations of 0, 60, 200, 400, 900, and 1,800 ppm for 4 days. BrdU was administered via an implanted osmotic pump to label cells in S-phase. Cells having incorporated BrdU were visualized in tissue sections immunohistochemically and the LI evaluated as the percentage of S-phase cells. Necropsies and histopathological examinations were performed at death. Average daily doses of chloroform ingested from drinking water were: 0, 6.6, 19.3, 33.2, 68.1, and 57.5 mg/kg/day for 0, 60, 200, 400, 900, and 1,800 ppm concentration levels, respectively. Only mild hepatocyte vacuolation was observed in rats given 900 or 1,800 ppm in water for 4 days; no increase in the hepatic LI was observed at any time point.

In another study by Larson et al. (1995b), female Fisher 344 rats administered 400 mg/kg chloroform by gavage in corn oil for 4 days exhibited hepatic lesions consisting of mild centrolobular vacuolization, scattered necrotic hepatocytes, sinusoidal leucostasis, mild-to-focally severe centrilobular hepatocyte degeneration and necrosis, and diffuse centrolobular swelling. Rats in the 100 and 200 mg/kg groups had only slight centrolobular changes, while those in the 34 mg/kg group did not differ from controls.

Pereira (1994) investigated the effects of chloroform exposure in different vehicles and by different dosing regimes on hepatic cell proliferation in female B6C3F_1 mice. Animals received either 263 mg/kg/day chloroform by gavage in corn oil or 1,800 ppm chloroform in drinking water, 24 hours a day and were sacrificed at 5 or 12 days. When administered by gavage, chloroform exposure resulted in significantly increased relative liver weights at 5 days (53%; $p<0.05$), but not at 12 days. The livers of mice exposed to chloroform for 5 days exhibited toxicity consisting of necrotic hepatocytes, infiltration of macrophages and neutrophils in the central zone, and hydrotropic, swollen, and rounded hepatocytes of a pale ground glass appearance in the midzone. Hepatotoxicity was less severe at 12 days. Cell proliferation was significantly increased at both 5 and 12 days. In contrast, chloroform administered in drinking water for 5 days reduced absolute and relative liver weight while exposure for 12 days had no effect on relative liver weights. The livers of mice exposed to chloroform in drinking water exhibited limited toxicity after 5 days consisting of smaller hepatocytes

with dense nonvacuolated and basophilic cytoplasm; hepatotoxicity after 12 days was limited to vacuolated hepatocytes. Cell proliferation, was significantly reduced ($p<0.05$) at 5 and 12 days as compared to controls.

Liver effects in animals have been reported in numerous oral studies of intermediate duration (Chu et al. 1982b; Eschenbrenner and Miller 1945a; Larson et al. 1995b). Larson et al. (1994b) exposed female mice to 3, 10, 34, 90, 238, and 477 mg/kg/day of chloroform in corn oil via gavage 5 days a week for 3 weeks. Chloroform treatment resulted in significant increases in liver weights of mice at the 90, 238, and 477 mg/kg/day doses. Doses of 34 mg/kg/day resulted in pale cytoplasmic eosinophilia of the centrilobular hepatocytes and mild vacuolation of the centrilobular and mid-zonal hepatocytes relative the periportal hepatocytes and livers from control mice. At the 238 mg/kg/day dose, the livers were characterized by a severe centrilobular hepatocyte necrosis. At 477 mg/kg/day, the central zone of the liver was populated by degenerate vacuolated hepatocytes and regenerating hepatocytes with markedly basophilic cytoplasm and,small round nuclei with clumped chromatin and prominent nucleoli. Significant dose-dependent increases in ALT and SDH were observed at doses of 34 mg/kg/day and greater. Cell proliferation was markedly increased in the liver at the 238 and 477 mg/kg/day doses. Mice dosed with 16, 43, 82, 184, or 329 mg/kg/day of chloroform in the drinking water for 7 days a week for 3 weeks resulted in no histological changes in livers at all doses studied. Liver weights were significantly increased at 82, 184, and 329 mg/kg/day.

Larson et al. (1995a) examined the dose-response relationships for the induction of cytolethality and regenerative cell proliferation in the livers of male Fischer 344 rats given chloroform by gavage. Groups of 12 rats were administered oral doses of 0, 3, 10, 34, 90, and 180 mg/kg/day chloroform in corn oil by gavage for 5 days a week for 3 weeks. BrdU was administered via an implanted osmotic pump to label cells in S-phase. Cells having incorporated BrdU were visualized in tissue sections immunohistochemically and the LI evaluated as the percentage of S-phase cells. Necropsies and histopathological examinations were performed at death. The relative liver weights were increased at doses of 90 mg/kg/day and greater at 3 weeks. After 3 weeks of exposure, livers of rats in the 34 or 90 mg/kg/day dose groups did not differ from controls. In the 180 mg/kg/day dose group, effects were similar to those seen at 4 days after exposure. Dose-dependent increases in both ALT and SDH were observed after 3 weeks in the 180 mg/kg/day dose group only.

Larson et al. (1995a) also examined the toxicological effects of chloroform administered in the drinking water in rats. Groups of 12 rats were administered chloroform *ad libitum* in drinking water at concentrations of 0, 60, 200, 400, 900, and 1,800 ppm for 7 days a week for 3 weeks. BrdU was administered via an implanted osmotic pump to label cells in S-phase. Cells having incorporated BrdU were visualized in tissue sections immunohistochemically and the LI evaluated as the percentage of S-phase cells. Necropsies and histopathological examinations were performed at death. Average daily doses of chloroform ingested from drinking water were: 0, 6, 17.4, 32, 62.3, and 106 mg/kg/day for 3 weeks exposure for 0, 60, 200, 400, 900, and 1,800 ppm concentration levels, respectively. Only mild hepatocyte vacuolation was observed in rats given 1,800 ppm in water for 3 weeks. No increase in the hepatic LI was observed at any time point.

Larson et al. (1995b) further examined the dose-response relationship for chloroform-induced cytotoxicity and cell proliferation in the liver of female Fisher 344 rats via gavage. Animals received chloroform in corn oil at doses of 0, 34, 100, 200, or 400 mg/kg/day for 3 weeks (5 days a week). At completion of dosing, animals were sacrificed, the livers were evaluated microscopically and cell proliferation was quantitated. Exposure to 400 mg/kg chloroform resulted in hepatic lesions consisting of slight to mild diffuse vacuolar change and focal centrilobular degeneration. Rats in the 200 mg/kg groups had only slight centrilobular vacuolation, while those in the 100 and 34 mg/kg dose groups did not differ from controls.

Pereira (1994) provided further evidence of the effect of dosing method (gavage versus drinking water) and vehicle (corn oil versus water) on hepatic cell proliferation in female B6C3F$_1$ mice. Animals received either 263 mg/kg/day chloroform by gavage in corn oil or 1,800 ppm chloroform in drinking water, 24 hours a day and were sacrificed at 33 or 159 days. When administered by corn oil gavage, chloroform exposure resulted in increased relative liver weights at 33 and 159 days (30.1 and 38.2% increases, respectively). The livers of mice exposed to chloroform for 33 or 159 days exhibited limited toxicity consisting of focal areas of necrotic hepatocytes and a limited number of mononuclear cells and swollen, rounded, pale hepatocytes. Cell proliferation was significantly increased at both 33 and 159 days as compared to controls. In contrast, administration of chloroform in drinking water had no effect on absolute or relative liver weights after 33 days, while exposure for 159 days resulted in significantly increased relative liver weights (3 1.4%; $p<0.05$). However, the livers of mice exposed for either 33 or 159 days exhibited no signs of toxicity. In addition, cell proliferation was not significantly affected by exposure to chloroform in drinking water for either 33 or 159 days.

2. HEALTH EFFECTS

Fatty changes, necrosis, increased liver weight, and hyperplasia have been observed in rats exposed to ≥150 mg/kg/day chloroform in a toothpaste vehicle via gavage for 13 weeks (Palmer et al. 1979). An increased incidence of sporadic, mild, reversible, liver changes occurred in rats exposed to chloroform in the drinking water at doses of 0.64-150 mg/kg/day for 90 days, but the incidences were not significantly higher than the incidences in controls (Chu et al. 1982a). The effect- and no-effect-levels in the study are clearly defined. Fatty and hydropic changes, necrosis, and cirrhosis were observed in mice treated by gavage with ≥50 mg/kg/day chloroform in oil for 90 days (Bull et al. 1986; Munson et al. 1982) or 86 mg/kg/day in drinking water for 1 year (Klaunig et al. 1986). In contrast, centrilobular fatty changes observed in mice at 64 mg/kg/day chloroform in drinking water for 90 days appeared to be reversible (Jorgenson and Rushbrook 1980), and no liver effects were found in mice treated with ≥50 mg/kg/day chloroform in aqueous vehicles (Bull et al. 1986). In addition, hepatocellular degeneration was induced in F_1 females in a 2-generation study in which mice were treated by gavage with 41 mg/kg/day chloroform in oil (Gulati et al. 1988). Significantly increased ($p<0.05$) SGPT activity occurred in dogs beginning at 6 weeks of exposure to chloroform in toothpaste in capsules at a dose of 30 mg/kg/day in a 7.5-year study (Heywood et al. 1979). SGPT activity was not increased at 15 mg/kg/day until week 130. Therefore, 15 mg/kg/day was the NOAEL for intermediate-duration exposure. This NOAEL was used to derive an intermediate-duration oral MRL of 0.1 mg/kg/day. More information on this MRL and how it was derived is located in the footnote to Table 2-2, Section 2.5, and in Appendix A of this profile.

The relationships among chloroform-induced cytolethality, regenerative cell proliferation, and tumor induction in male $B6C3F_1$ mice dosed with chloroform by gavage in corn oil has also been studied. Mice received chloroform at doses of 0, 34, 90, 138, or 277 mg/kg for 4 consecutive days or 5 days a week for 3 weeks. To monitor cell proliferation, mice were administered BrU via implanted osmotic pump for the last 3.5 days. Chloroform treatment for 3 weeks also resulted in a small (<10%) but significant increase in relative liver weight of mice at the highest dose level. Macroscopically, pale livers and kidneys were noted at all dose levels after 3 weeks of chloroform exposure; treatment with 138 or 277 mg/kg for 3 weeks resulted in the formation of white subcapsular foci. After 3 weeks of chloroform exposure, the livers of all mice in the 34 mg/kg/day group and 3 of 5 mice in the 90 mg/kg/day group were histologically similar to those of controls. Livers of 2 mice in the 90 mg/kg/day group exhibited centrilobular hepatocyte swelling with loss of eosinophilia. Mice dosed with 138 mg/kg/day experienced marked centrilobular hepatocyte swelling, mild to moderate periportal vacuolation, and scattered centrilobular and periportal degeneration and necrosis. Mice dosed with

277 mg/kg/day experienced marked centrilobular hepatocyte degeneration and necrosis. Cell proliferation was significantly elevated in the 138 and 277 mg/kg dose groups only.

In chronic-duration exposure studies, liver effects have been observed in rats, mice, and dogs after oral exposure to chloroform. Male and female ICI mice were exposed to 17 or 60 mg/kg/day chloroform by gavage using toothpaste as a vehicle for 80 weeks followed by 13-24-week observation period. No significant histopathological findings (noncancerous) were recorded in the kidneys or lung tissues. Moderate or severe fatty degeneration of the liver was slightly more prevalent among treated animals than controls (statistical significance not provided) beginning at the 17 mg/kg/day. Necrosis was observed in female rats treated by gavage with 200 mg/kg/day chloroform in oil for 78 weeks (NCI 1976). Nodular hyperplasia occurred in all groups of male and female mice similarly treated at 138 and 238 mg/kg/day, respectively. Fibrosis of the liver was observed in both sexes of rats exposed to 200 mg/kg/day chloroform in the drinking water for ≤180 weeks (Tumasonis et al. 1985, 1987). Increased SGPT was observed in dogs given chloroform in toothpaste capsules for 7.5 years (Heywood et al. 1979). The lowest oral dose administered to animals in chronic studies was 15 mg/kg/day, which increased SGPT in dogs. This LOAEL was used to derive a chronic oral MRL of 0.01 mg/kg/day. More information on this MRL and how it was derived is located in the footnote to Table 2.2, Section 2.5, and in Appendix A of this profile.

Renal Effects. The kidney is also a major target of chloroform-induced toxicity in humans. Oliguria was observed 1 day after the ingestion of ≈3,755 or 2,410 mg/kg chloroform (Piersol et al. 1933; Schroeder 1965). Increased blood urea nitrogen (BUN) and creatinine levels also indicated renal injury. Albuminuria and casts were detected in the urine. Histopathological examination at autopsy revealed epithelial swelling and hyaline and fatty degeneration in the convoluted tubules of kidneys in one fatal case of oral exposure to chloroform (Piersol et al. 1933). 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. No indications of renal effects were observed in humans who ingested estimated doses of 0.34-0.96 mg/kg/day chloroform in mouthwash for 5 years (De Salva et al. 1975).

The renal toxicity of chloroform in animals has been reported in many studies of acute duration. Larson et al. (1993) studied the effects of dose and time after chloroform administration on the renal

toxicology of chloroform in male rats. Rats were given 34, 180, or 477 mg/kg once in corn oil by gavage and sacrificed 24 hours after administration. In a related time-course study (which focused on histologic changes in tissues over time), rats received 180 mg/kg chloroform in corn oil by gavage and were sacrificed at 0.5, 1, 2, 4, and 8 days after treatment; others received 477 mg/kg in corn oil by gavage and were sacrificed either 1 or 2 days after administration. Histologically, chloroform caused extensive renal damage and, to a much lesser extent, hepatic injury, in a dose-related manner. One day after treatment with a single dose of chloroform of 34 mg/kg or greater, the kidneys of male rats developed tubular necrosis that was restricted to the proximal convoluted tubules. The severity of these lesions occurred in a dose-dependent manner. Rats given 34 mg/kg had scattered necrotic tubules affecting less than 10% of the midcortical nephrons. In the 180 mg/kg group, 25% of the proximal convoluted tubules were necrotic. Nearly all segments of the proximal tubules had necrosis in the rats receiving 477 mg/kg chloroform. Despite extensive renal injury, increases in BUN or in urinary protein or glucose were not observed. In the time-course study, the kidneys, after 12 hours of treatment, had a diffuse granularity of cytoplasm of the epithelium lining of the proximal convoluted tubules in the 180 mg/kg group. Damage was severe after 1 day, and after 2 days, 100% of the proximal tubules were lined by necrotic epithelium. After 8 days, the kidneys had returned to normal appearance. No increases in BUN or urinary protein or glucose were noted at any time after treatment.

Larson et al. (1995a) also examined the toxicological effects of chloroform administered in the drinking water in rats. Groups of 12 rats were administered chloroform *ad libitum* in drinking water at concentrations of 0, 60, 200, 400, 900, and 1,800 ppm for 4 days. BrdU was administered via an implanted osmotic pump to label cells in S-phase. Cells having incorporated BrdU were visualized in tissue sections immunohistochemically and the LI evaluated as the percentage of S-phase cells. Necropsies and histopathological examinations were performed at death. Average daily doses of chloroform ingested from drinking water were: 0, 6.6, 19.3, 33.2, 68.1, and 57.5 mg/kg/day for 4 days exposure for the 0, 60, 200, 400, 900, and 1,800 ppm concentration levels, respectively. When chloroform was administered in the drinking water, no microscopic alterations were seen in the kidneys after 4 days of treatment. The overall renal LI was not increased at any dose.

The same study (Larson et al. 1995a) examined the dose-response relationships for the induction of cytolethality and regenerative cell proliferation in the kidneys of male Fischer 344 rats given chloroform by gavage. Groups of 12 rats were administered oral doses of 0, 3, 10, 34, 90, and

180 mg/kg/day chloroform in corn oil by gavage for 4 days. BrdU was administered via an implanted osmotic pump to label cells in S-phase. Cells having incorporated BrdU were visualized in tissue sections immunohistochemically and the LI evaluated as the percentage of S-phase cells. Necropsies and histopathological examinations were performed at death. Rats treated with 34, 90, or 180 mg/kg/day by gavage for 4 days had mild-to-moderate degeneration of renal proximal tubules. After 4 days of dosing with 34 mg/kg/day, the proximal convoluted tubule epithelial cells had increased numbers and prominence of apical cytoplasmic vacuoles. Likewise, rats given 90 mglkglday for 4 days displayed swelling and vacuolation of 25-50% of the proximal tubules. Progressive degeneration of the proximal tubules was observed in rats exposed to 180 mg/kg/day. At 4 days, swollen and vacuolated cytoplasm in approximately 10-20% of proximal tubule epithelium was observed. LI were increased in the kidney cortex only in the rats treated with 180 mg/kg/day for 4 days.

The dose-response relationship for chloroform-induced cytotoxicity and cell proliferation in the kidneys of female Fisher 344 rats has also been elucidated. Animals received 34, 100, 200, or 400 mg/kg chloroform by gavage in corn oil for 4 days. At completion of dosing, kidneys were prepared for microscopic evaluation, and cell proliferation was quantitated. Rats in the high dose group had 50-75% of proximal tubules lined with necrotic or attenuated regenerating epithelium, as well as distal nephrons containing hyaline casts. Rats in the 200 mg/kg group had kidneys with 25-50% of proximal tubules lined with degenerated, necrotic or regenerating epithelium. Kidneys from rats in the 2 lowest dose groups were similar to those of controls (Larson et al. 1995b).

Acute toxic nephrosis was observed in female rats exposed to 516 mg/kg/day chloroform by gavage in oil during Gd 6-15, with maternal lesions characterized by tubular swelling, hydropic or fatty degeneration and necrosis (Thompson et al. 1974). Increased kidney weight was observed in female rats after a single gavage dose of 546 mg/kg chloroform (Chu et al. 1982b). Similarly, rats exposed to 400 mg/kg/day by gavage during gestation had increased kidney weight (Ruddick et al. 1983). No increase in kidney weight was found in the rats treated with 200 mg/kg/day during gestation. Renal necrosis in convoluted tubules was observed in male mice after a single dose of 199 mg/kg chloroform in toothpaste or 65.6 mg/kg chloroform in oil (Moore et al. 1982).

The relationships among chloroform-induced cytolethality, regenerative cell proliferation, and tumor induction in male $B6C3F_1$ mice dosed with chloroform by gavage in corn oil of acute duration has

also been reported. Mice received chloroform at doses of 0, 34, 90, 138, or 277 mg/kg/day by gavage for 4 consecutive days. To monitor cell proliferation, mice were administered BrdU via implanted osmotic pump for the last 3.5 days. After four days of exposure, 2 of 4 mice dosed with 34 mg/kg/day and all mice dosed with 90 mg/kg/day or more experienced extensive (>75% of tissue) acute necrosis of the proximal convoluted tubule, characterized by a thin layer of eosinophilic necrotic cellular debris lining the tubular basement membrane. Distal tubules and collecting ducts were filled with hyaline casts. The remaining 2 mice in the 34 mg/kg/day dose group experienced scattered necrosis in proximal convoluted tubules. In the kidneys, significant increases in cell proliferation were noted in all dose groups given chloroform for 4 days. The authors concluded that the results of this study confirmed the sensitivity of the male mouse, relative to the female mouse, to the nephrotoxic effects of chloroform (Larson et al. 1994d).

In intermediate-duration exposures, several studies suggest that mice appeared to be more sensitive than rats to the nephrotoxic effects of chloroform. Rats exposed to 193 mg/kg/day for 28 days (Chu et al. 1982b) or to 160 mg/kg/day chloroform for 90 days (Jorgenson and Rushbrook 1980) in drinking water had no kidney effects. Increased relative kidney weight was observed in rats exposed by gavage to 150 mg/kg/day for 13 weeks, but not in rats exposed to 30 mg/kg/day (Palmer et al. 1979). Chronic inflammatory changes were observed in the kidneys of mice exposed to 50 mg/kg/day chlorofoorm (dissolved in an emulsion prepared with emulphor in water) by gavage (Munson et al. 1982); however, no changes were observed in mice exposed to 41 mg/kg/day by gavage (Gulati et al. 1988) or in mice exposed to 435 mg/kg/day chloroform in drinking water (Jorgenson and Rushbrook 1980). Nonetheless, exposure to 86 mg/kg/day in drinking water for 1 year caused tubular necrosis in mice (Klaunig et al. 1986).

Larson et al. (1995b) examined the dose-response relationship for chloroform-induced cytotoxicity and cell proliferation in the kidneys of female Fisher 344 rats using a wide range of doses. Animals received 34, 100, 200, or 400 mg/kg chloroform by gavage in corn oil for 3 weeks (5 days a week). At completion of dosing, the kidneys were prepared for microscopic evaluation, and cell proliferation was quantitated. Rats in the 100, 200, and 400 mg/kg dose groups had 50-75% of proximal tubules lined with regenerating epithelium; many of the tubules were dilated and contained mineralized concretions. Kidneys from rats in the lowest dose group were similar to those of controls.

2. HEALTH EFFECTS

Lipsky et al. (1993) studied groups of male Fischer 344 rats gavaged with either 90 or 180 mg/kg/day of chloroform in corn oil or water for 5 days a week for 4 weeks. Rats exposed to chloroform by gavage in corn oil displayed acute cell injury and necrosis, primarily in the epithelial cells lining the S2 segment of the proximal tubule, with some apparent damage/necrosis occurring in the Sl segment as well. This injury was present in all rats exposed to the 180 mg/kg/day dose and in less than half of the animals exposed to the 90 mg/kg/day dose. There was also a dose-dependent increase in the total BrdU labeling of nuclei in renal cells of the chloroform-treated oil-gavaged animals compared to controls. The largest increase in DNA BrdU labeling was in the cells of the S2 segment. The 90 mg/kg/day dose of chloroform also produced increase in DNA labeling in the S3 segment, but not for the 180 mg/kg/day dose of chloroform. Animals exposed to chloroform in water showed minimal histopathologic alterations in the kidneys. Mild injury and necrosis was seen in cells of the S2 segment in 1 of 6 animals in the 180 mg/kg/day group, while none were seen in the 90 mg/kg/day dose group. Little to no change in DNA labeling of renal cells was seen in the water-gavaged rats.

Larson et al. (1995a) examined the dose-response relationships for the induction of cytolethality and regenerative cell proliferation in the kidneys of male Fischer 344 rats given chloroform by gavage. Groups of 12 rats were administered oral doses of 0, 3, 10, 34, 90, and 180 mg/kg/day chloroform for 5 days a week for 3 weeks. BrdU was administered via an implanted osmotic pump to label cells in S-phase. Cells having incorporated BrdU were visualized in tissue sections immunohistochemically and the LI evaluated as the percentage of S-phase cells. Necropsies and histopathological examinations were performed at death. Relative kidney weights were increased after 3 weeks in the 180 mg/kg/day dose group only. Rats treated with 34, 90, or 180 mg/kg/day by gavage for 4 days had mild-to-moderate degeneration of renal proximal tubules. These alterations were absent or slight after 3 weeks of treatment, except at the highest dose level. After 4 days of dosing with 34 mg/kg/day, the proximal convoluted tubule epithelial cells had increased numbers and prominence of apical cytoplasmic vacuoles, but these changes were not observed at 3 weeks. Likewise, rats given 90 mg/kg/day for 4 days displayed swelling and vacuolation of 25-50% of the proximal tubules, at 3 weeks only 1 of 3 rats had vacuolated and degenerated epithelium. Progressive degeneration of the proximal tubules was observed in rats exposed to 180 mg/kg/day. At 4 days, swollen and vacuolated cytoplasm in approximately 10-20% of proximal tubule epithelium was observed, while at 3 weeks the percentage was 25-50%. At 3 weeks, scattered tubules also had mineral concretions that appeared subepithelial. LI was increased in the kidney cortex only in the rats treated with 180 mg/kg/day for 4 days.

Larson et al. (1995a) also examined the toxicological effects of chloroform administered in the drinking water in rats. Groups of 12 rats were administered chloroform *ad libitum* in drinking water at concentrations of 0, 60, 200, 400, 900, and 1,800 ppm for 7 days a week for 3 weeks. BrdU was administered via an implanted osmotic pump to label cells in S-phase. Cells having incorporated BrdU were visualized in tissue sections immunohistochemically and the LI evaluated as the percentage of S-phase cells. Necropsies and histopathological examinations were performed at death. Average daily doses of chloroform ingested via drinking water were 0, 6, 17.4, 32, 62.3, and 106 mg/kg/day for 3 weeks exposure for 0, 60, 200, 400, 900, and 1,800 ppm concentration levels, respectively. As a general observation, rats treated for 3 weeks with 200 ppm chloroform and greater had slightly increased numbers of focal areas of regenerating renal proximal tubular epithelium and cell proliferation than were noted in controls, but no clear dose-response relationship was evident. The overall renal LI was not increased at any dose or time point.

Larson et al. (1994b) exposed female mice to 3, 10, 34, 90, 238, and 477 mg/kg/day of chloroform in corn oil via gavage for 5 days a week for 3 weeks. Mice were also dosed with 16, 43, 82, 184, or 329 mg/kg/day of chloroform in the drinking water for 7 day a week for 3 weeks. In both studies, no increases in cell proliferation were noted and no significant changes in renal histopathology were reported.

In another study by Larson et al. (1994d) the possible relationships among chloroform-induced cytolethality, regenerative cell proliferation, and tumor induction were identified in male $B6C3F_1$ mice dosed with chloroform by gavage in corn oil. Mice received chloroform at doses of 0, 34, 90, 138, or 277 mg/kg/day for 5 days a week for 3 weeks. To monitor cell proliferation, mice were administered BrdU via implanted osmotic pump for the last 3.5 days. Renal lesions were similar to, but less severe than, lesions seen in mice exposed for 4 days and were characterized by extensive tubular regeneration. The kidneys of mice dosed with 34-138 mg/kg/day chloroform exhibited dose-dependent increases in regenerating proximal convoluted tubules. The kidneys of mice dosed with 277 mg/kg/day chloroform exhibited severe nephropathy characterized by degeneration, necrosis, and regeneration of the proximal tubules. The renal interstitium was swollen due to fibroplasia, edema, and inflammatory cell infiltration. After 3 weeks of exposure, renal cell proliferation was still elevated relative to controls at doses of ≥90 mg/kg/day, but LI values declined from levels seen after 4 days of exposure.

2. HEALTH EFFECTS

In chronic oral studies, no definite renal effects were observed in rats exposed to ≤200 mg/kg/day or mice exposed to <477 mg/kg/day TWA (Heindel et al. 1995; Jorgenson et al. 1985; NCI 1976; Roe et al. 1979). In dogs, however, fat deposition in renal glomeruli was observed at a dose of 30 mg/kg!day chloroform for 7.5 years, but not at 15 mg/kg/day (Heywood et al. 1979).

Dermal Effects. No studies were located regarding dermal effects in humans after oral exposure to chloroform.

Alopecia was observed in pregnant rats exposed to 126 mg/kg/day chloroform in oil (Thompson et al. 1974). Rough coats were observed in mice exposed to 100 mg/kg/day chloroform in oil for 14 days (Gulati et al. 1988).

Ocular Effects. Only one reference was located that discussed the ocular effects of chloroform after oral ingestion. Li et al. (1994) examined the effects of chloroform administered in drinking water to guinea pigs with cedar pollen-induced allergic conjunctivitis, prepared by passive cutaneous anaphylaxis. Groups of 5 male Hartley guinea pigs were given drinking water with chloroform concentrations of 0.01, 0.1, 1, 10, 100, or 1,000 ppm 48 hours before applying an antigen eye drop (starting on the 8th day after antiserum administration). One control group was not administered chloroform and another control group was not administered the antiserum (chloroform alone) for every dose level. The light absorption rate of Evans blue extracted from conjunctiva was used as an index of the relative intensity of allergic conjunctivitis. In a separate experiment, using the dose level which caused the most intense aggravating effect in the above testing, groups of 3 male guinea pigs were given 1 ppm chloroform in drinking water for 48 hours and the residual effect on the allergic conjunctivitis was examined. Animals were examined immediately after, and 1, 2, 4, 7, and 14 days after exposure; antigen eye drops were applied 10 days after the antiserum administration. Water intake was monitored and blood chloroform concentrations were measured. At 0.1 ppm chloroform, significant aggravation of allergic conjunctivitis was observed. Allergic conjunctivitis was most intensely aggravated at I ppm chloroform. At higher doses (10 and 100 ppm) the aggravation was still noticeable, yet less significant. At 1,000 ppm chloroform, the aggravating effect was not present. Rlood chloroform concentrations increased as the concentration in drinking water increased from 0.01 to 1,000 ppm.

2. HEALTH EFFECTS

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

Several studies were located regarding body weight changes in animals after oral exposure to chloroform; however, the effect of chloroform on body weight is variable and depends somewhat on the dose and dosing method. Body weight was unaffected in male Wistar rats receiving single doses of chloroform ranging from 0.1 to 0.5 mL/kg (Nakajima et al. 1995), in female $B6C3F_1$ mice exposed to 263 mg/kg/day chloroform by gavage in corn oil for 5 or 12 days (Pereira 1994), and in male rats exposed once to 477 mg/kg day of chloroform (Larson et al. 1993), or for 4 days in male mice dosed at 277 mg/kg/day by gavage (Larson et al. 1994d). When female mice were exposed to 1,800 ppm chloroform in drinking water (24 hours a day) for 5 or 12 days, body weight initially declined; however, this was attributed to decreased water consumption (Pereira 1994). A dose-related decrease in body weight gain was observed in rats exposed to 100 mg/kg/day and in rabbits exposed to 50 mg/kg/day chloroform by gavage in oil during gestation (Ruddick et al. 1983; Thompson et al. 1974). In addition, decreased body weight was observed in male mice after acute exposure to 250 mg/kg/day chloroform by gavage in oil (Gulati et al. 1988; Munson et al. 1982). Others have reported similar reductions in body weight after oral dosing with chloroform (Davis and Berndt 1992, 1994b, 1995a, 1995b; Reddy et al. 1992).

In studies of intermediate duration, dose-related decreases in body weight or body weight gain were observed in rats exposed to ≥81 mg/kg/day in water (Jorgenson and Rushbrook 1980) or in oil (Larson et al. 1994b, 1995b; NCI 1976) and in mice (Bull et al. 1986; Klaunig et al. 1986; Roe et al. 1979). Similar effects were found in rats exposed to ≥60 mg/kg/day regardless of the vehicle (Jorgenson et al. 1985; Larson 1995a; NCI 1976; Palmer et al. 1979; Tumasonis et al. 1985) and mice exposed to 263 mg/kg/day in water (Jorgenson et al. 1985) in studies of chronic exposure; taste aversion may have been a complicating factor in these studies. In contrast, no effect on body weight was observed in mice treated with 477 mg/kg/day by gavage in oil (NCI 1976) or dogs treated with 30 mg/kg/day chloroform (Heywood et al. 1979) in studies of chronic duration. Food and/or water consumption were decreased in chloroform-exposed animals in some studies (Chu et al. 1982a; Jorgenson and Rushbrook 1980), but others reported fluctuating food intake unrelated to chloroform exposure (Palmer et al. 1979) or no significantly depressed food consumption at the lowest LOAEL level for body weight effects (Thompson et al. 1974). The effects chloroform has on changes in body weight and water consumption when administered orally in different vehicles and varying doses have also been

reported in female mice (Pereira 1994). In this study, female $B6C3F_1$ mice received 263 mg/kg/day chloroform by gavage in corn oil or 1,800 ppm/day chloroform in their drinking water, and animals were sacrificed at 5, 12, 33, or 159 days. Chloroform administered by gavage did not affect body weight; however, when administered in drinking water, body weights in exposed animals initially declined, but increased by day 33 to control levels. This was attributed to changes in drinking-water consumption, which was suppressed during the first 5 days but was greater than that of controls from days 6-12.

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to chloroform. Information regarding immunological effects in animals after oral exposure to chloroform is limited to three studies. Reduced lymphocyte counts were observed in female rats after a single gavage dose of 1,071 mg/kg chloroform (Chu et al. 1982b); no effects were observed in the 765 mg/kg group. Humoral immunity, defined as antibody-forming cells (AFC)/spleen x 100,000, was depressed in both sexes of mice after oral dosing with 50 mg/kg/day chloroform for 14 days (Munson et al. 1982). In contrast, hemagglutination titer was not significantly influenced, and no changes in cell-mediated immunity were recorded. Similar results were obtained in a 90-day experiment (Munson et al. 1982). Depressed humoral immunity was observed in mice exposed to 50 mg/kg/day chloroform. Cellmediated immunity (delayed-type hypersensitivity) was affected in the high-dose (250 mg/kg/day) group of females. The chloroform-induced changes were more marked in the 14-day study than in the 90-day study. Although the data are limited, there are indications that the immune system is a target of chloroform-induced toxicity after oral exposure. The data also indicate that humoral immunity may be more severely affected than cell-mediated immunity. These conclusions, however, should be viewed with caution due to the small number of studies.

Li et al. (1994) examined the effects of chloroform administered in drinking water to guinea pigs with cedar pollen-induced allergic conjunctivitis, prepared by passive cutaneous anaphylaxis. Groups of 5 male Hartley guinea pigs were given drinking water with chloroform concentrations of 0.01, 0.1, 1, 10, 100, or 1,000 ppm 48 hours before applying an antigen eye drop (starting on the 8th day after antiserum administration). At 0.1 ppm chloroform, significant aggravation of allergic conjunctivitis was observed. Allergic conjunctivitis was most intensely aggravated at 1 ppm chloroform. At higher

doses (10 and 100 ppm) the aggravation was still noticeable, yet less significant. At 1,000 ppm chloroform, the aggravating effect was not present.

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

2.2.2.4 Neurological Effects

The data regarding neurological effects in humans after oral exposure to chloroform were obtained from clinical case reports. Deep coma occurred immediately after exposure to 2,410 or 3,755 mg/kg in all cases of intentional or accidental ingestion of chloroform (Piersol et al. 1933; Schroeder 1965; Storms 1973). All reflexes were abolished, and pupil size varied. All patients survived the first coma and became fully conscious; 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 two weeks (Storms 1973).

The central nervous system in animals is a target of chloroform toxicity after oral exposure to chloroform. High single doses of chloroform caused ataxia, incoordination, and anesthesia in mice (Balster and Borzelleca 1982; Bowman et al. 1978). Sprague-Dawley rats administered a single 200 mg/kg dose of chloroform gavage experienced significant decreases in midbrain 5-hydroxyindolacetic acid (5-HIAA) levels and significant increases in hypothalamic dopamine concentrations 2 hours after dosing ($p<0.05$) (Kanada et al. 1994). The calculated ED_{50} (dose is effective on 50% of animals) for motor performance was 484 mg/kg chloroform (Balster and Borzelleca 1982). The effects disappeared within 90 minutes postexposure. A minimal narcotic dose for 50% of the treated mice was calculated to be 350 mg/kg (Jones et al. 1958). Hunched posture and inactivity were observed in male mice exposed by gavage to 250 mg/kg chloroform in oil for 14 days (Gulati et al. 1988). No effects were observed after exposure to 100 mg/kg day. 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). Lower concentrations of chloroform-induced taste aversion to a saccharin solution in mice exposed by gavage for 10 days to 30 mg/kg/day in oil, but not in mice exposed to 10 mg/kg/day (Landauer et al. 1982). No signs of behavioral toxicity were observed in mice exposed to 31.l mg/kg/day chloroform for 14, 60, or 90 days, or in mice exposed to 100 mg/kg for 30 days (Balster and Borzelleca 1982). Operant behavior in mice was affected after exposure to

100 mg/kg/day for 60 days (Balster and Borzelleca 1982). The most severe effects were observed early in the experiment; partial tolerance was observed later. No histopathological changes were observed in the brains of rats after chronic exposure to 200 mg/kg/day, in the brains of mice after chronic exposure to 477 mg/kg/day (NCI 1976) or in the brains of mice after chronic exposure to 60 mg/kg/day (Roe et al. 1979).

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

2.2.2.5 Reproductive Effects

No definitive studies were located regarding reproductive effects in humans after oral exposure to chloroform. However, in a study by Bove et al. (1995), the effects of drinking-water consumption on birth outcomes were evaluated in women giving birth in northern New Jersey during the period of Jan 1, 1985 to Dec 31, 1988. A total of 80,938 live births and 594 fetal deaths were studied. Exposure to total trihalomethane (TTHM) levels >0.l ppm resulted in a 70.4 g reduction in mean birth weight among term babies, increased odds ratio (OR) for low birth weight among term births (1.42), an increased OR for reduced size at gestational age birth (1.50) and an increased OR for oral cleft defects (3.17). In addition, exposure to TTHM of >0.08 ppm resulted in an increased OR for central nervous system defects (2.59) and neural tube defects (2.96). The results of this study should be viewed with caution since the outcomes data were not correlated directly with chloroform concentrations, but rather with TTHM concentrations; hence, the effects observed may be due to exposure to other THMs. The authors of this study also acknowledged the presence of other non-THM contaminants, and that some or all of these contaminants may have contributed to the observed effects as well.

In rats, increased resorptions were observed at a dose of 316 mg/kg/day chloroform during gestation but not at 300 mg/kg/day; increased resorptions were also observed in rabbits exposed to 100 mg/kg/day during gestation (Thompson et al. 1974). Furthermore, abortions (not otherwise specified) were observed in rabbits exposed to 63 mg/kg/day chloroform during gestation (Thompson et al. 1974). No histopathological changes were observed in the testes of rats exposed to 160 mg/kg/day chloroform in drinking water for intermediate durations (Jorgenson and Rushbrook 1980). Gonadal atrophy was observed in both sexes of rats treated by gavage with 410 mg/kg/day

chloroform in toothpaste (but not with 150 mg/kg/day) (Palmer et al. 1979). In a 2-generation reproductive study in mice, exposure to 41 mg/kg/day of chloroform by gavage in oil did not affect the fertility in either generation (Gulati et al. 1988). No remarkable histopathological differences regarding the reproductive system were observed in dogs receiving up to 30 mg/kg/day of chloroform delivered in toothpaste capsules for 7.5 years (Heywood et al. 1979). No histopathological changes were observed in the reproductive organs of male and female rats and mice chronically exposed to 200 and 477 mg/kg/day chloroform via gavage (NCI 1976).

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

2.2.2.6 Developmental Effects

One study (Kramer et al. 1992) was located regarding developmental effects in humans after oral exposure to chloroform via the drinking water. The study was conducted to determine whether water supplies containing relatively high levels of chloroform and other THMs within the state of Iowa are associated with low birth weight, prematurity, or intrauterine growth retardation (the most sensitive end point). Subjects selected include 159 low-birth-weight infants, 342 premature infants, and 187 grow-thretarded infants; however, case definitions were not mutually exclusive. Infants studied were divided into three groups: those who lived in areas where the water supply had undetectable amounts of chloroform, those who lived in areas where the water supply had l-9 μg/L chloroform, and those who lived in areas where the water supply had more than 10 μg/L. The estimated relative risk of low birth weight associated with drinking-water sources having chloroform levels of greater than or equal to 10 μg/L was 30% higher than the risk for sources with undetectable levels of chloroform. Prematurity was not associated with chloroform/THM exposure. The estimated relative risk of intrauterine growth retardation associated with drinking-water supplies with chloroform concentration of >10 μg/L was 80% more than the risk for those sources with undetectable levels of chloroform. Sources with intermediate chloroform levels (l-9 μg/L) had an elevated risk of 30%. The authors concluded that there is an increased risk of intrauterine growth retardation associated with higher concentrations of waterborne chloroform and dichlorobromomethane; however, it also should be noted that other organic halides that can co-occur in chlorinated drinking water (haloacetic acids and haloacetonitriles) produce developmental effects in animals.

No teratological effects or skeletal anomalies in rats or rabbits after oral exposure to chloroform were reported in developmental studies (Ruddick et al. 1983; Thompson et al. 1974). Decreased fetal weight was observed in the offspring of rats exposed by gavage to 400 mg/kg/day chloroform during gestation, but not in those exposed to 200 mg/kg/day (Ruddick et al. 1983). In a preliminary dosefinding study, decreased fetal weight and increased resorptions were observed in rats exposed to 316 mg/kg/day chloroform during gestation (Thompson et al. 1974). In the principal study, reduced birth weight of the offspring was reported in the 126 mg/kg/day group; no effects were observed in the 50 mg/kg/day exposure group. No behavioral effects were observed in the offspring of the F_0 generation mice treated for 6-10 weeks with 31.l mg/kg chloroform (Burkhalter and Balster 1979).

In a 2-generation reproductive study, increased epididymal weights and degeneration of epididymal ductal epithelium were observed in mice in the F_1 generation dosed with 41 mg/kg/day in oil (Gulati et al. 1988). The production and viability of sperm was not affected, however. Swiss mice given drinking water containing a mixture of contaminants including 7 ppm chloroform experienced no significant developmental effects. In the same study, Sprague-Dawley rat pups of the F_l generation had lower body weights from birth through mating; however, this was likely an artifact of decreased water intake. No other developmental effects were noted (Heindel et al. 1995). These data are limited by the possible interactions caused by the concurrent exposure to other water contaminants.

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

2.2.2.7 Genotoxic Effects

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

Unscheduled DNA synthesis (UDS) in hepatocytes was not increased in rats exposed to chloroform at gavage doses ≥400 mg/kg in oil (Mirsalis et al. 1982). Exposure to 200 mg/kg/day chloroform in oil by gavage for 4 days increased sister chromatid exchange frequency in bone marrow cells of mice (Morimoto and Koizumi 1983). Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

Epidemiology studies suggest an association between cancer in humans and the consumption of chlorinated drinking water, but the results are not conclusive at this time (Alavanja et al. 1978; Cantor et al. 1978; Ijsselmuiden et al. 1992; McGeehin et al. 1993; Young et al. 1981; Zierler et al. 1988). Such an association implicates chloroform because chloroform is a known animal carcinogen (see below) and is the predominant THM in chlorinated drinking water (see Chapter 5); however, it is important to note that some of the many chemicals produced in the process of water chlorination are highly mutagenic and/or carcinogenic. Although attempts were made to control for various demographic variables in all of these studies (e.g., social class, ethnic group, marital status, occupation, urban or rural, etc.), many confounding effects remained unaccounted for, most notably the likelihood that numerous chemicals other than chloroform were present in the drinking water, as stated above. Furthermore, the studies differed regarding the type of cancer associated with consumption of chlorinated water. Bladder cancer was reported to have the strongest association with chlorinated water in several studies (Cantor et al. 1978; McGeehin et al. 1993; Zierler et al. 1988), but only colon cancer had an elevated OR (3.6) in another study (Young et al. 1981). In addition, Ijsselmuiden et al. (1992) found the use of municipal water to be associated with pancreatic cancer. All these studies superficially suggest that low-level oral chloroform consumption may increase the risk of some cancers in humans; however, it is equally important to note that most of these studies had confounding factors that make it difficult to definitively state that chloroform is the chemical chiefly responsible for the induction of these specific types of cancer. Confounding factors, such as the presence of other THMs (i.e., brominated THMs), haloacetic acids, haloacetonitriles, halogenated aldehydes, ketones and furanones, and chlorine content (both free and total), all of which may vary widely from one chlorinated drinking-water source to another, may have a large influence on the incidence of these cancers. In addition, many of these studies did not account for migration and historical exposures to any THMs, the wide ranges of potential exposure doses, occupational exposures to other chemicals, and the lack of a direct measurement of chloroform (or other THM) consumption in the drinking-water source (Cantor et al. 1978; Ijsselmuiden et al. 1992; Young et al. 1981; Zierler et al. 1988). Overall, the human data are insufficient to support any conclusion regarding the carcinogenic potential of orally consumed chloroform in humans.

Differing results on the carcinogenic capabilities of chloroform have been demonstrated in laboratory animals. Chloroform is carcinogenic in laboratory animals after oral exposure in some studies of

intermediate durations. An increased incidence of hepatomas was observed in mice exposed by gavage for 30 days to 594 mg/kg/day chloroform in oil, but not in mice exposed to 297 mg/kg/day (Eschenbrenner and Miller 1945a). An 8-week exposure to 1,800 mg/kg/day chloroform in oil by gavage did not induce lung tumors in mice (Stoner et al. 1986). Chloroform in corn oil acted as a promoter rather than an initiator of preneoplastic foci in a rat liver bioassay (Deml and Oesterle 1985). In addition, no increase in tumors was found in mice exposed to 257 mg/kg/day chloroform in drinking water for 52 weeks (Klaunig et al. 1986). Interestingly, Reddy et al. (1992) dosed male rats with 14, 25, 52, and 98 mg/kg/day of chloroform in the drinking water for 12 weeks. The study conclusively showed that chloroform, at the doses administered and routes studied in the rat, reduced the number of preneoplastic enzyme-altered foci (gamma-glutamyltranspeptidase-positive and GSH S-transferase-positive) in the liver of male rats after induction of foci with diethylnitrosamine in a dose-related fashion. The exact mechanism behind this effect was not determined.

Chloroform was found to be carcinogenic in several chronic animal studies of oral exposure. Renal tumors (tubular cell adenoma and carcinoma) were observed in male Osborne-Mendel rats after a 78-week exposure to 90 mg/kg/day chloroform by gavage in corn oil (NCI 1976). Dunnick and Melnick (1993) demonstrated the incidence of liver and kidney tumors in rats and mice dosed by gavage in corn oil for 5 days a week for 78 weeks. In rats, kidney tubular cell neoplasms did not occur in controls but were observed at 90 mg/kg/day (4 of 50) and at 180 mg/kg/day (12 of 50) in males, and at 200 mg/kg/day (2 of 48) in females. In male mice, hepatocellular neoplasms were rarely seen in controls (1 of 18), but were frequently observed in the 138 mg/kg group (18 of 50) and 277 mg/kg group (44 of 45). In female mice, no hepatocellular neoplasms were recorded in controls but were observed in the 238 mg/kg group (36 of 45) and 477 mg/kg group (39 of 41). The incidence of hepatic neoplastic nodules was increased in female Wistar rats chronically exposed to 200 mg/kg/day chloroform in drinking water (Tumasonis et al. 1987). An increased incidence of tubular cell adenoma and carcinoma was observed in the kidneys of Osborne-Mendel rats chronically exposed to 160 mg/kg/day chloroform in drinking water but not in those exposed to 81 mg/kg/day (Jorgenson et al. 1985). The 160 mg/kg/day dose in this study also resulted in decreased water consumption (taste aversion). In contrast, no increase in the incidence of tumors was observed in Sprague-Dawley rats exposed by gavage to 60 and 165 mg/kg/day chloroform in toothpaste for 80 and 52 weeks, respectively (Palmer et al. 1979). Hepatocellular carcinoma was observed in all groups of male $B6C3F_1$ mice exposed to gavage doses ≥138 mg/kg/day chloroform in oil for 78 weeks (NCI 1976). An increased incidence of kidney tumors was observed in ICI mice chronically exposed to

60 mg/kg/day chloroform by gavage, but not in those exposed to 17 mg/kg/day (Roe et al. 1979). Under the same experimental conditions, chloroform exposure had no effect on the frequency of tumors in C57BL, CBA, and CF-1 mice. Moreover, no increase in tumor incidence was observed in $B6C3F_1$ mice exposed to 263 mg/kg/day chloroform in drinking water for 2 years (Jorgenson et al. 1985). Cancer was not observed in dogs exposed to 30 mg/kg/day chloroform in toothpaste capsules for 7.5 years (Heywood et al. 1979). From these data it would appear that the method of dosing (e.g., gavage versus drinking water) and the vehicle utilized may influence outcomes in chronic trials. The CELs (cancer effect levels) are recorded in Table 2-2 and plotted in Figure 2-2. EPA (IRIS 1995) selected the study by Jorgenson et al. (1985) as the basis for the q_1* for oral exposure to chloroform because administration via drinking water better approximates oral exposure in humans than does administration in corn oil by gavage as used in the NCI (1976) study. Based on the incidence of renal tumors in male Osborne-Mendel rats, the q_1 * was calculated to be $6.1x10^{-3}$ $(mg/kg/day)^{-1}$. The oral doses associated with individual lifetime upper-bound risks of 10^{-4} to 10^{-7} are $1.6x10^{-2}$ to $1.6x10^{-5}$ mg/kg/day, respectively, and are plotted in Figure 2-2.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to chloroform.

No deaths resulted from dermal exposure of rabbits exposed to doses of up to 3,980 mg/kg chloroform for 24 hours (Torkelson et al. 1976).

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, or ocular effects in humans or animals after dermal exposure to chloroform.

Hepatic Effects. No studies were located regarding hepatic effects in humans after dermal exposure to chloroform.

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No hepatic effects were observed in rabbits when 3,980 mg/kg chloroform was applied to the abdominal skin for 24 hours (Torkelson et al. 1976). The NOAEL for hepatic effects is recorded in Table 2-3.

Renal Effects. No studies were located regarding renal effects in humans after dermal exposure to chloroform.

Renal degenerative tubular changes were observed in rabbits when 1,000 mg/kg chloroform was applied to the abdominal skin for 24 hours (Table 2-3) (Torkelson et al. 1976).

Dermal Effects. Completely destroyed stratum corneum was observed in the skin of 2 young volunteers exposed to chloroform for 15 minutes on 6 consecutive days (Malten et al. 1968). Milder changes were observed in two older individuals. Chloroform was applied in a glass cylinder (exact exposure was not specified).

A clinical study of 21 females and 21 males used to determine the efficacy of using aspirin dissolved in chloroform which was then applied topically to patients infected with herpes zoster and post--therapeutic neuralgia with painful skin lesions has been reported. When an aspirin/chloroform combination (approximately 43.3 mg/mL) was applied, the only reported side-effect was an occasional burning sensation on the skin as the chloroform evaporated from the skin surface; however, the possible impact on other major body organs (liver, kidney, etc.) was not investigated (King 1993).

Application of 0.01 mL chloroform for 24 hours to the skin of rabbits caused only slight irritation (Smyth et al. 1962). Skin necrosis was observed in rabbits dermally exposed to 1,000 mg/kg chloroform for 24 hours (Torkelson et al. 1976). These LOAEL values are recorded in Table 2-3.

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

Dermal exposure to 1,000 mg/kg chloroform for 24 hours caused weight loss in rabbits (Table 2-3) (Torkelson et al. 1976).

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

Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL	LOAEL				Reference
				Less Serious		Serious		
ACUTE EXPOSURE								
Systemic								
Rabbit (New Zealand)	24 hr	Dermal		0.01mL M	(slight skin irritation)			Smyth et al. 1962
Rabbit (NS)	24 hr	Hepatic	3980 mg/kg					Torkelson et al. 1976
		Renal		1000 mg/kg	(degenerative tubular changes)			
		Dermal				1000 mg/kg	(necrosis)	
		Bd Wt		1000 mg/kg	(unspecified weight loss)			

Bd Wt = body weight; hr = hour(s); LOAEL = lowest-observed-adverse-effect-level; M = male; NOAEL = no-observed-adverse-effect-level; NS = not specified

2. HEALTH EFFECTS

No credible studies were located regarding the following health effects in humans or animals after dermal exposure to chloroform:

2.2.3.3 Immunological and Lymphoreticular Effects

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genatoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

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

2.3 TOXICOKINETICS

Overview. Sufficient information exists on the absorption, distribution, metabolism, and excretion of chloroform, with most information on the pharmacokinetics being derived from animal data. Generally, chloroform is absorbed easily into the blood from the lungs after inhalation exposures. Following oral exposure, peak blood levels are achieved within 5-6 minutes, depending on the dosing vehicle and dosing frequency used. The chemical properties of chloroform also permit percutaneous absorption without difficulty. After absorption, chloroform has been reported to distribute to adipose tissues, brain, liver, kidneys, blood, adrenals, and embryonic neural tissues. Higher levels of chloroform can be found in the renal cortex of male animals than in female animals, a finding apparently mediated by the presence of testosterone. Approximately 50% of a dose of chloroform is eventually metabolized to carbon dioxide in humans; however, an intermediate toxic metabolite, phosgene, is formed in the process in the liver. Chloroform undergoes metabolism primarily in the liver and may undergo covalent binding to both lipid and microsomal protein. Chloroform is excreted from the body either unchanged by pulmonary desorption or in the form of carbon dioxide, with small amounts of either detectable in the urine and feces.

2. HEALTH EFFECTS

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Chloroform absorption depends on the concentration in inhaled air, the duration of exposure, the blood/air partition coefficient, the solubility in various tissues, and the state of physical activity which influences the ventilation rate and cardiac output. Pulmonary absorption of chloroform is also influenced by total body weight and total fat content, with uptake and storage in adipose tissue increasing with excess body weight and obesity.

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 two hours in normal humans at resting ventilation and cardiac output (Smith et al. 1973).

The amount of chloroform absorbed and exhaled from the body in alveolar air from male and female swimmers in indoor swimming pools in Italy was measured by Aggazzotti et al. (1993). Alveolar air samples were collected from both swimmers and observers present in indoor chlorinated swimming pools. Of all the nonexposed subjects, 47% had chloroform concentrations below the detection limit of the assay, and the remainder of this control group had low concentrations (75.39 nmol/m^3) of chloroform present in their alveolar air. Median alveolar chloroform concentrations for persons exposed to the indoor swimming pools (swimmers and observers), were significantly higher than those of nonexposed subjects (median=695.02 nmol/m^3). No differences were found between males and females in any exposure group.

Cammann and Huebner (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 ug/m3. 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-l.65 μg/L) and agonistic swimmers

(1.14-5.23 μg/L). Based upon the differences seen in the two swimming groups, the authors concluded that increased physical activity leads to increased absorption and/or ingestion of chloroform.

In a similar study, Levesque 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 seven 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 100-950 and 104-1,093 ppb, respectively. 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. The authors concluded from this data that the average proportion of body burden due to inhalation after 35 and 55 minutes exercise was 76 and 78%, respectively.

Nashelsky et al. (1995) described one non-fatal 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 2 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.

No studies were located regarding absorption in animals after inhalation exposure to chloroform. Evidence that chloroform is absorbed after inhalation exposure is provided in toxicity studies (see Section 2.2.1), but the rate and extent cannot be determined from the toxicity data.

2.3.1.2 Oral Exposure

In one case report, a 33-year-old female (weight not reported) injected herself intravenously with 0.5 mL of chloroform and became unconscious. She awoke approximately 12 hours later and drank another 120 mL of chloroform. Plasma chloroform levels were determined 18 hours after ingestion by gas chromatography (GC) and showed a blood chloroform level of 0.66 mg/dL. Subsequent serum

samples were analyzed for chloroform content and were reported to have been less than this level, steadily declining over time (Rao et al. 1993).

Absorption of an oral dose of ^{13}C-labeled chloroform (0.5 grams in a gelatin capsule) was rapid in volunteers, reaching peak blood levels in 1 hour (Fry et al. 1972). Almost 100% of the dose was absorbed from the gastrointestinal tract.

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 a 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; the peak blood levels were reached 1 hour after oral administration of 60 mg/kg chloroform in olive oil.

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 concentration of chloroform in blood was 39.3 μg/mL when administered in water and 5.9 μg/mL when administered in corn oil. 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. In a similar study, Pereira (1994) investigated the uptake and protein binding of chloroform in the liver and kidney in female $B6C3F_1$ 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 2 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 recently reported elsewhere (Larson et al. 1994b, 1995a)

2.3.1.3 Dermal Exposure

A limited number of experimental studies were located regarding dermal absorption of chloroform in humans. Levesque 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 seven days. On each exposure day the subjects exercised for a 55-minute period. On day 6 of the experiment, subjects wore scuba gear so as 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 this data it would appear that the average proportion of body burden due to dermal exposure after 35 and 55 minutes exercise was 24 and 22%, respectively.

Cammann and Huebner (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 $\mu g/m^3$. 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 authors concluded that increased physical activity leads to increased absorption and/or ingestion. With the exception of the inclusion of attendants, the 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

Dick et al. (1995) examined the absorption of chloroform through human skin *in vivo* using volunteers and *in vitro* using fresh, excised abdominal skin. In the *in vivo* study, the ventral forearm skin of four male volunteers was dosed with a solution of chloroform in either water or ethanol. The solution remained on the skin for eight hours. When administered in water, the total absorbed dose was 8.2%. In contrast, the total absorbed dose was only 1.68% when chloroform was administered in ethanol. In

the *in vitro* study, two doses were applied to the skin and remained there for four hours. At study termination, the percentages of the low and high doses of chloroform absorbed were 5.6 and 7.1%, respectively.

According to dermal absorption studies with solvents other than chloroform, the absorption of such solvents in guinea pigs is more rapid than the 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) investigated the fate of topically applied chloroform in male hairless rats. For exposures under 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. It appeared that there was an incremental build-up of chloroform in the skin over the first four minutes. When compared to uptake measured by bath concentration differences, approximately 88% of lost chloroform was not accounted for in the stratum corneum and was assumed to be systemically absorbed.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

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). Recently, 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).

2. HEALTH EFFECTS

The chloroform levels in 7 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) describe 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 2 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 were 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). In 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.

2.3.2.2 Oral Exposure

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

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 l%, 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).

2.3.2.3 Dermal Exposure

A limited number of studies were located regarding distribution in humans or animals after dermal exposure to chloroform.

Dick et al. (1995) examined the absorption of chloroform through human skin in *vivo* using volunteers and *in vitro* using fresh, excised abdominal skin. In the *in vivo* study, the ventral forearm skin of four male volunteers was dosed with a solution of chloroform in either water or ethanol. The solution remained on the skin for eight hours. When administered in water, urinary excretion was 0.42%, while excretion from the lungs over the first 48 hours postexposure averaged 7.8%. Tape-stripping data indicated that only 0.01% of the dose remained in the skin after 3 days. When chloroform was administered in ethanol, urinary excretion was 0.07% while excretion from the lungs over the first 48 hours postexposure averaged 0.83%. Tape-stripping data indicated that the percentage of the dose remaining in the skin after three days was non detectable. In the *in vitro* study, two doses were applied to the skin and remained there for four hours. At study termination, the majority of the

absorbed dose was found in the perfusate (7.0%), with only minor amounts remaining in the skin (0.14%).

Islam et al. (1995) investigated the fate of topically applied chloroform in male hairless rats. For exposures under 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 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 comeum; by five 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 four minutes. When compared to uptake measured by bath concentration differences, approximately 88% of the chloroform dose was not accounted for in the stratum comeum and was assumed to be systemically absorbed.

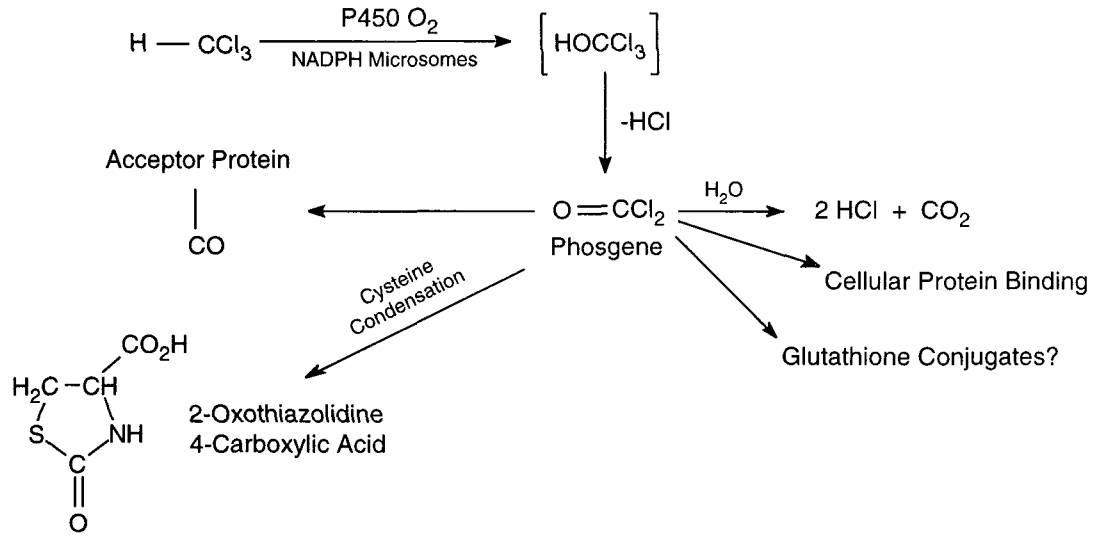
2.3.3 Metabolism

The metabolism of chloroform is well understood. Approximately 50% of an oral dose of 0.5 grams of 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. On the basis of pharmacokinetic results obtained in rats and mice exposed to chloroform by inhalation, and of enzymatic studies in human tissues *in vitro, in vivo* metabolic rate constants ($V_{max}C$ = 15.7 mg/hour/kg, Km = 0.448 mg/L) were defined for humans (Corley et al. 1990). The metabolic activation of chloroform to its toxic intermediate, phosgene, was slower in humans than in rodents.

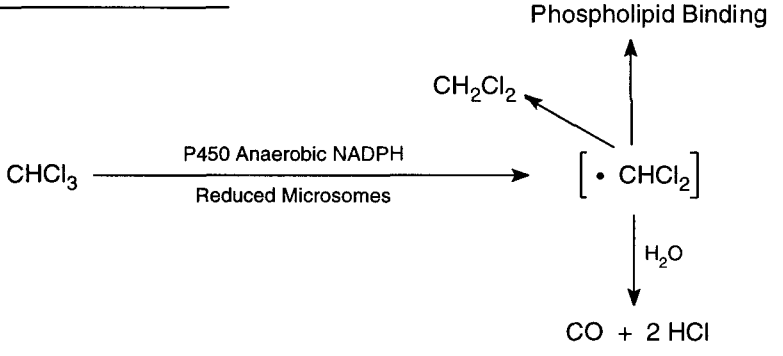
Metabolic pathways of chloroform biotransformation are shown in Figure 2-3. Metabolism studies indicated that chloroform was, in part, exhaled from the lungs or was converted by oxidative dehydrochlorination of its carbon-hydrogen bond to form phosgene (Pohl et al. 1981; Stevens and Anders 1981). This reaction was mediated by cytochrome P-450 and was observed in the liver and kidneys (Ade et al. 1994; Branchflower et al. 1984; Smith et al. 1984). In renal cortex microsomes of

Figure 2-3. Metabolic Pathways of Chloroform Biotransformation

MAJOR AEROBIC PATHWAY



MINOR ANAEROBIC PATHWAY



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 P-450 (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 P-450 in rat liver microsomes under anaerobic conditions (De Groot and No11 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 carbonhydrogen bond is provided by experiments using the deuterated derivative of chloroform (Branchflower et al. 1984; McCarty et al. 1979; Pohl et al. 1980a). Deuterated chloroform was onehalf 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.

A recent *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). It was demonstrated that radical chloroform metabolites bind to macromolecules (proteins, lipids) and the process can be inhibited by reduced GSH.

2. HEALTH EFFECTS

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).

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 I S-fold but did not affect hepatic microsomal protein of cytochrome P-450 content. In addition, intraperitoneal administration of chloroform resulted in greater blood concentrations, peak values, and area of the curves (AUCs), as compared to oral administration. AUCs in rats administered chloroform orally ranged from 0.34 to 6.45 versus 0.58 to 8.78 in rats administered chloroform intraperitoneally. The 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 authors also concluded that intraperitoneal exposure produced data which 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 dose-dependant. 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).

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) 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 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 3 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.

2.3.4 Elimination and Excretion

2.3.4.1 Inhalation Exposure

Chloroform was detected in the exhaled air of volunteers exposed to a normal environment, to heavy automobile traffic, or to 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.

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; 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-l1 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-l 1 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.

2.3.4.2 Oral Exposure

Following a single, oral exposure, most of the 0.5 grams 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 eight 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.

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 do. 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.

2.3.4.3 Dermal Exposure

One study was located regarding excretion in humans after dermal exposure to chloroform. Dick et al. (1995) examined the fate of chloroform applied to human skin *in vivo* using volunteers and *in vitro* using fresh, excised abdominal skin. In the *in vivo* study, the ventral forearm skin of four male volunteers was dosed with a solution of chloroform in either water or ethanol. The solution remained on the skin for eight hours. When administered in water, urinary excretion was 0.42%, while excretion from the lungs over the first 48 hours postexposure averaged 7.8%. Tape-stripping data indicated that only 0.01% of the dose remained in the skin after three days. When chloroform was administered in ethanol, urinary excretion was 0.07% while excretion from the lungs over the first 48 hours postexposure averaged 0.83%. Tape-stripping data indicated that the percentage of the dose remaining in the skin after three days was non detectable. In the *in vitro* study, two doses were

applied to the skin and remained there for four hours. At study termination, the majority of the absorbed dose was found in the perfusate (7%), with only minor amounts remaining in the skin (0.14%).

No animal studies were located regarding the excretion of chloroform after dermal exposure to chloroform.

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

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

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

The PBPK model for a chemical substance is developed in four interconnected steps: model representation, model parameterization, model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular chemical substance require estimates of the

chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true. complexities of biological systems. This simplification, however, is desirable if the uptake and disposition of the chemical substance(s) is adequately described because data are often unavailable for many biological processes and using a simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is therefore of great importance and thus model validation must be critically considered.

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

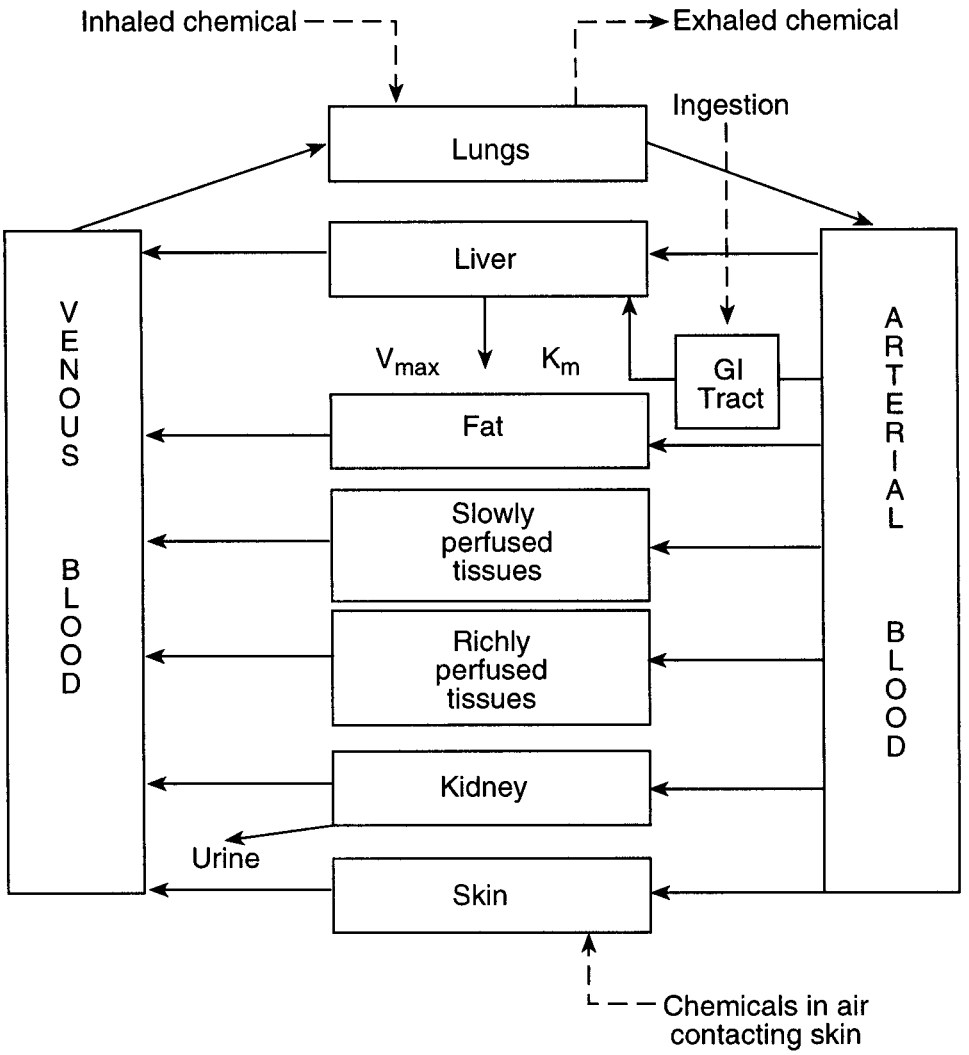
If PBPK models for chloroform exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

2.3.5.1 Summary of PBPK/PD Models

Several rodent and human models have been used to predict the absorption (oral, inhalation, and dermal) from water and air, distribution, metabolism, and excretion of chloroform.

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 authors suggest 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

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



Source: adapted from Krishnan et al. 1992

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

larger species. Assuming that 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 end point (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 shower. The model also predicted a steady-state *stratum corneum* permeability of chloroform in human skin in the range of 0.16-3.6 cm/hour with the most likely value being 0.2 cm/hour. The authors suggest 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.

2.3.5.2 Chloroform PBPK Model Comparison

Five chloroform PBPK models that describe the disposition of chloroform in animals and humans have been identified from the recent open literature (early 1980s-1994). Based on the information presented in these five models, there appears to be sufficient evidence to suggest that PBPK models for chloroform are fairly refined and have a strong potential for use in human risk assessments. The PBPK model developed by Corley et al. (1990) has provided a basic model for the fate of chloroform in humans and laboratory animals. Using this model as a template, other more sophisticated and refined models have been developed that can be used in human risk assessment work. 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) and Chinery and Gleason (1993) address both the inhalation and dermal exposure routes in humans the Chinery and Gleason model uses a 3-compartment skin component which may more accurately reflect the flux of chloroform through the skin after dermal only or dermal plus inhalation exposure scenarios, while the McKone model uses a single compartment within the skin to describe chloroform flux. Further discussion of each model and its application in human risk assessments is presented below.

2.3.5.3 Discussion of Chloroform Models

The Corley 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 (Chinery and Gleason 1993; McKone 1993) are based on the Corley model. The Corley model has been used for cancer risk assessment (Reitz et al. 1990).

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 consequent to binding to macromolecules (MMB) to be mouse > rat > human. Linking the pharmacokinetic parameters of this model to the pharmacodynamic

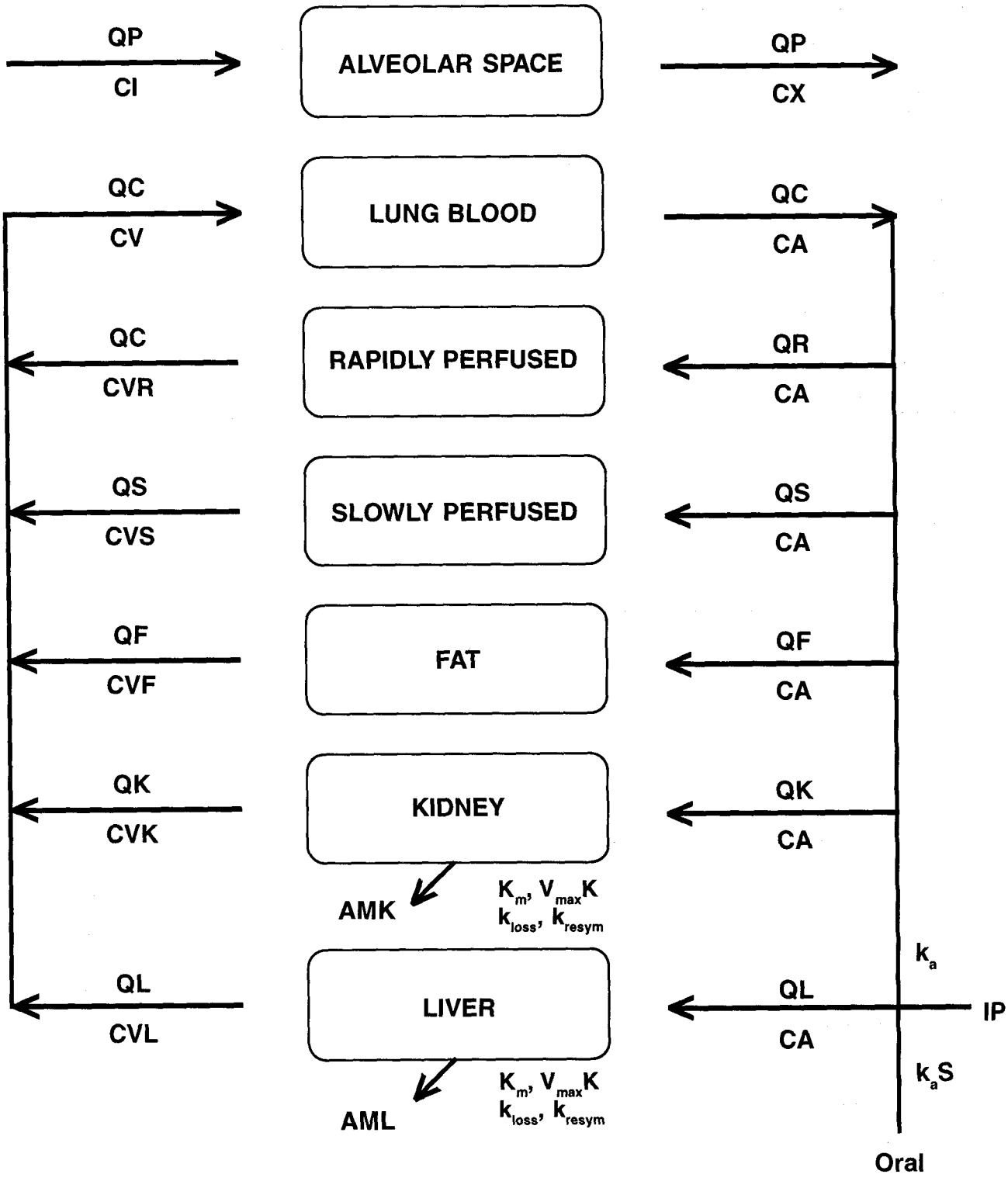
cancer model of Reitz et al. (1990) provides a biologically based risk assessment model for chloroform.

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 2-5 with oral, inhalation, and intraperitoneal routes represented. The dermal route of exposure is not represented in this model; however, others have modified the Corley model to include this route of exposure (see below). Liver and kidney are represented as separate compartments since both are target organs for chloroform.

The physiologic, biochemical constants and partition coefficients required for the model are shown in Table 2-4. 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 gasuptake experiments. Human metabolic rate constants were obtained from *in vitro* human microsomal fractions of liver and kidney samples using $^{14C}CHCL_3$ as the substrate. Binding of chloroform metabolites (phosgene) to MMBs was assumed to occur in bioactivating tissues (liver and kidney) in a non-enzymatic, nonspecific, and dose-independent fashion. Macromolecular binding constants for the liver and kidney were estimated from *in vivo* MMB data obtained from rats and mice exposed to $^{14C}CHCL_3$ via inhalation.

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 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 phenobarbitalinduced 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.

Figure 2-5. Parameters Used in the Corley PBPK Model



Physiological model used to describe the pharmacokinetics of chloroform in rats, mice, and humans during inhalation, oral, and intraperitoneal exposures.

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

Table 2-4. Parameters Used in the Corley PBPK Model

Parameters	Mouse	Rat	Human
	Weights (kg):		
Body	0.02858	0.230	70.0
	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
	Flows (L/hr/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	29.0	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	21.1	17.0
Slowly perfused/air	13.0	13.9	12.0
	Metabolic and macromolecular binding constants		
V_{max} C (mg/hr/kg)	22.8	6.8	15.7
K_m (mg/L)	0.352	0.543	0.448
K_{loss} (l/mg)	$5.72x10^{-4}$	0	0
K_{resyn} (hr^{-1})	0.125	0	0
A (kidney/liver)	0.153	0.052	0.033
fMMB (hr^{-1}), liver	0.003	0.00104	0.00202
fMMB (hr^{-1}), kidney	0.010	0.0086	0.00931
	Gavage Absorption Rate Constants		
k_{aS} (hr^{-1}), corn oil	0.6	0.6	0.6
k_{aS} (hr^{-1}), water	5.0	5.0	5.0
	Intraperitoneal Injection Absorption Rate Constant		
k_a (hr^{-1})	1.0	1.0	1.0

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.

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.

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 was 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, see Table 2-4).

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.

Interroute Extrapolation. The Corley model used three routes of administration, intraperitoneal, oral and inhalation, in rats and mice to describe the disposition of chloroform. This data was 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 Reitz Model

Risk Assessment. The Reitz model is based on the assumption 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 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, hence the kidney compartment toxicity was not addressed. The kidney compartment was combined with the rapidly perfused tissue group. The Reitz model used two types of dose measurement, referred to as dose surrogates. One type of dose surrogate used was covalent binding to MMBs (average daily macromolecular binding, AVEMMB), a rate independent parameter. 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: that liver cells have a finite capability for repairing damage caused by $CHCl_3$ metabolites; that liver cells differ from cell to cell in their capabilities to repair this damage; and that induction of cytotoxicity in liver cells does not occur instantaneously.

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. See Table 2-4 for these parameters. 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.

Interroute Extrapolation. Inhalation and oral routes of administration were examined in the Reitz model; however, interroute extrapolations were not specifically addressed in the Reitz model.

The Gearhart Model

Risk Assessment. The Gearhart 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 B6C3F_1 mice were exposed for 6 hours to chloroform vapor concentrations of l00-5,500 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 the Corley 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=l.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 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 firstorder 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 model.

High-low Dose Extrapolation. No high-low dose extrapolation was specifically addressed by the Gearhart model.

Interroute Extrapolation. No interroute extrapolation was specifically addressed by the Gearhart model.

The Chinery-Gleason Model

Risk Assessment. The Chinery-Gleason model has the greatest potential for use in 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) PBPK 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 an experimentally derived exhaled breath concentrations of chloroform following exposure in a shower stall (Jo et al. 1990a).

Target Tissues. Based on the data set of Jo et al. (1990a), the Chinery-Gleason model predicted the stratum comeum permeability coefficient for chloroform to be 0.2 cm/hour (range, 0.6 and 2.2) and the estimated ratio of the dermally and inhaled absorbed doses to be 0.75 (range, 0.6 and 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 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 the this model.

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

Interroute Extrapolation. The Chinery-Gleason 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 Model

Risk Assessment. The McKone model has 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) PBPK model addressed potential exposure to chloroform by the inhalation and dermal routes. McKone 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. (1990a) 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. 1990a) were compared with the model predictions.

Validation of the Model. The McKone model used one data set to evaluate the model results (Jo et al. 1990a). The McKone model results were also compared to other existing chloroform models, with an in-depth discussion of similarities an 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 model is appropriate for simulating chloroform breath levels in persons exposed in showers by both exposure routes. A major difference between the McKone model and the Chinery-Gleason model is that the McKone model assumes the skin to be a one compartment organ, whereas the Chinery-Gleason model assumed three compartments within the skin. The McKone 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 model. No extrapolation between species was addressed in this model.

High-low Dose Extrapolation. For tap-water concentrations below 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 greater than 100 mg/dL for inhalation-only showers and 60 mg/L or normal showers, however, the relationship was no longer linear and modifications to this model may be required.

Interroute Extrapolation. The dermal and inhalation routes were addressed in this model. The McKone model did not specifically address interroute extrapolations for chloroform.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

Absorption. In humans and laboratory animals, chloroform is generally absorbed quickly. Primarily because of its high blood/air partition coefficient, it passes with some ease through most tissue and cellular barriers in the body. Chloroform can be absorbed by inhalation and ingestion, and by dermal routes of exposure. Inhalation studies were performed by Corley et al. (1990) on groups of mice exposed to various concentrations of chloroform for 6 hours and sacrificed 48 hours after the last exposure. Chloroform absorption by the lungs varied by concentration and was generally 34-46%. An earlier study by Von Oettingen (1964) found that when dogs were exposed to 15,000 ppm chloroform, the concentration of chloroform in the blood rose quickly and leveled off, apparently establishing a steady-state concentration in the blood at 80-100 minutes after inhalation exposures began. The average steady-state concentration in the blood was 0.4 mg/mL. Less information is available on the absorption of chloroform by inhalation in humans. Humans exposed to 10,000 ppm of chloroform during surgical anesthesia showed a rapid absorption of chloroform detected in arterial blood samples, with peak concentrations occurring within 2 hours after initiation of anesthesia. The average arterial blood concentration of chloroform was reported to be about 0.1 mg/mL (Smith et al. 1973). Dick et al. (1995) examined the absorption of chloroform through human skin *in vivo* using volunteers and *in vitro* using fresh, excised abdominal skin. In the *in vivo* study, the ventral forearm skin of four male volunteers was dosed with a solution of chloroform in either water or ethanol. The solution remained on the skin for eight hours. When administered in water, the total absorbed dose was 8.2%. In contrast, the total absorbed dose was only 1.68% when chloroform was administered in ethanol. In the *in vitro* study, two doses were applied to the skin and remained there for four hours.

At study termination, the percentages of the low and high doses of chloroform absorbed were 5.6 and 7.1%, respectively.

Rats and mice exposed to 60 mg/kg ^{14}C-chloroform orally demonstrated that absorption was practically complete within 48 hours for mice and within 96 hours in rats. Peak blood levels occurred within 1 hour after the oral dose (Brown et al. 1974a). Humans dosed orally with 0.5 grams of ^{13}C-chloroform delivered as a capsule containing olive oil showed near complete absorption of chloroform within 8 hours after administration. Peak blood levels generally occurred at approximately 1 hour after dosing, with ^{13}C-chloroform concentrations in blood ranging from 1 to 5 μg/mL (Fry et al. 1972).

Chloroform can also permeate the stratum corneum of rabbit skin (Torkelson et al. 1976) and mouse skin (Tsuruta 1975). Percutaneous absorption of chloroform across mouse skin was calculated to be approximately 38 μg/min/cm^2, indicating that the dermal absorption of chloroform occurs fairly rapidly in mice. No reliable studies report the percutaneous absorption of chloroform in humans; however, a few clinical reports indicate that chloroform is used as a vehicle for drug delivery (King 1993). Islam et al. (1995) investigated the fate of topically applied chloroform in male hairless rats. For exposures under 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. It appeared that there was an incremental build-up of chloroform in the skin over the first four minutes. When compared to uptake measured by bath concentration differences, approximately 88% of lost chloroform was not accounted for in the stratum corneum and was assumed to be systemically absorbed.

Distribution. Radiolabeled chloroform in mice, once absorbed, is widely distributed to most organs and tissues, specifically the liver, kidney, lungs, spleen, body fat, muscle, and nervous tissue, as reported by Cohen and Hood (1969) and Bergman (1979). Significant accumulations were noted 48 hours after inhalation exposure in the central nervous system, particularly in the cerebellar cortex, spinal nerves, and meninges. When administered orally (Brown et al. 1974a), rats and squirrel monkeys showed significant accumulations of ^{14}C-chloroform in the brain, lung, muscle, and kidney in both species, with an unusual accumulation of chloroform in the gall bladder of the monkey. When administered orally to mice, similar accumulations of chloroform occurred in the liver, kidney, lung,

muscle, blood, intestines, and gall bladder (Taylor et al. 1974). Little current information on the distribution of chloroform in humans was available for review.

Chloroform (or phosgene) tends to accumulate to a significantly higher degree in the kidneys of male mice than in those of female mice given equivalent doses, which leads to a higher degree of chloroform nephrotoxicity in male mice. The sex differences seen with the renal cortical accumulation of chloroform or phosgene can be halted if chloroform is administered to castrated males; the sex difference can be reversed if chloroform is administered to females pretreated with testosterone prior to dosing with chloroform. This difference in chloroform accumulation is obviously dependent on the presence of testosterone and is very consistent with a body of evidence that indicates chloroform is more nephrotoxic to male mice than to female mice (Ilett et al. 1973; Pohl et al. 1984; Smith et al. 1973). Although this sex-related toxic effect is known to occur in mice, it is not known at present if a similar effect occurs in humans.

Excretion. Chloroform is largely excreted either in the parent form or as the end metabolite (carbon dioxide, CO_2) in the bodies of both laboratory animals and humans. Corley et al. (1990) demonstrated that mice exposed to 10 or 89 ppm of chloroform by inhalation excreted 99% of the chloroform body burden as CO_2 in exhaled air. As the chloroform concentrations in the air rose however, the amount of chloroform metabolized to CO_2 decreased and the amount of unchanged chloroform rose in the exhaled air, indicating that chloroform metabolism in mice is a saturable process. Rats exposed in a similar manner to 93, 356, and 1,041 ppm chloroform excreted 2, 20, and 42.5%, respectively, of the total body burden of chloroform as unchanged parent compound, indicating that chloroform is metabolized to CO_2 in rats but to a lesser degree than in mice.

In humans, Fry et al. (1972) administered 500 mg of chloroform orally in olive oil in capsular form and found that 17-67% of the total dose of chloroform was exhaled as unchanged parent compound, and that the extent of pulmonary elimination of chloroform was governed inversely by the amount of adipose tissue on the individual ingesting the chloroform. The study also found that most of the chloroform tended to be exhaled between 40 minutes and 2 hours after dosing, which coincided with peak blood levels of chloroform produced at approximately 1 hour after dosing.

2. HEALTH EFFECTS

Chloroform in humans tends to be eliminated in a biphasic manner. After ingesting 500 mg of chloroform orally, an initial (a) half-life in the blood of 9-21 minutes was reported, with the second (p) half-life ranging from 86 to 96 hours.

2.4.2 Mechanisms of Toxicity

Chloroform is widely distributed to many tissues of the body in laboratory animals and, presumably, in humans; however, many studies have demonstrated that chloroform does not tend to accumulate in the body for extended periods. Chloroform may accumulate to some degree in the body fat stores; however, it quickly partitions out the fat and is excreted by the normal routes and mechanisms. The liver (primary) and kidneys (secondary) are considered to be the target organs for chloroform toxicity in both humans and laboratory animals. Thus, humans (and animals) with existing hepatic or renal disease who are exposed to chloroform, particularly by the oral or inhalation routes, are more likely to be at risk to the toxic effects of chloroform. Reproductive/developmental effects due to chloroform's presence in the drinking water of both humans and laboratory animals has been reported, thereby placing women of childbearing age at a potentially higher risk of reproductive organ anomalies than those women past menopausal age.

Chloroform is largely metabolized in many tissues (particularly the liver and kidney) to CO_2, in humans and animals (Brown et al. 1974a; Corley et al. 1990; Fry et al. 1972). Chloroform metabolism is catalyzed by cytochrome P-450, initiating an oxidative cleavage of the C-H bond producing trichloromethanol. Trichloromethanol is unstable and is rapidly transformed to phosgene ($COCl_2$). Phosgene may react with water to form CO_2, which can be exhaled by the lung or excreted in the urine as carbonate or bicarbonate, and hydrochloric acid. Phosgene can also react with other molecules such as cysteine, deplete hepatic GSH (Docks and Krishna 1976; Pohl et al. 1981) and form adducts with microsomal proteins (Corley et al. 1990).

Chloroform toxicity can be attributed to the presence of both the parent compound and the formation of phosgene in most instances of toxicosis. High doses of inhaled chloroform have been reported to cause death (due to respiratory depression), ataxia, narcosis, and central nervous system depression, and are due to the direct effects of the parent compound. Lower doses of chloroform in the air, feed, or water, or administered by gavage, with variable exposure times, may induce toxicity due to the presence of the parent compound or to production of phosgene during metabolism. It appears that the

metabolite is responsible for hepatocellular damage, resulting in the ultimate elevation of hepatic enzymes (SGPT, SGOT, GGT, etc.) and cell damage/necrosis. The accumulation of chloroform in the renal cortex of mice with the subsequent metabolism to phosgene most likely contributes to the renal toxicity of chloroform seen in male mice. Tubular necrosis, calcification, nephritis, increased kidney weight, alterations in Na/K excretion, and other cellular anomalies were observed in response to one or both of these toxicants. Although the sex-related nephrotoxic effect is known to occur in mice, it is not known at present if a similar effect occurs in humans or other laboratory animals.

2.4.3 Animal-to-Human Extrapolations

Many laboratory animal models have been used to describe the toxicity and pharmacology of chloroform. By far, the most commonly used laboratory animal species are the rat and mouse models. Generally, the pharmacokinetic and toxicokinetic data gathered from rats and mice compare favorably with the limited information available from human studies. PBPK models have been developed using pharmacokinetic and toxicokinetic data for use in risk assessment work for the human. The models are discussed in depth in Section 2.3.5. As mentioned previously, male 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 as well, since experiments in rabbits and guinea pigs found no sex-related differences in renal toxicity.

2.5 RELEVANCE TO PUBLIC HEALTH

Overview. Data are available regarding health effects in humans and animals after inhalation, oral, and dermal exposure to chloroform; however, data regarding dermal exposure are quite limited. Chloroform was used as a general anesthetic, pain reliever, and antispasmodic for more than a century before its toxic effects were fully recognized. High levels of chloroform (23-400 ppm) (Challen et al. 1958; Phoon et al. 1983) in the air are found specifically in highly industrialized areas. Exposure of the general population to chloroform can also occur via the drinking water as a result of the chlorination process. Occupational exposure is another source of inhalation and/or dermal exposure for humans.

Most of the presented information regarding chloroform toxicity following inhalation exposure in humans was obtained from clinical case reports of patients undergoing anesthesia. In some instances,

the results in these studies may have been confounded by unreported data, such as the intake of other drugs or the use of artificial respiration during anesthesia.

The target organs of chloroform toxicity in humans and animals are the central nervous system, liver, and kidneys. There is a great deal of similarity between chloroform-induced effects following inhalation and oral exposure. No studies were located regarding reproductive effects in humans after exposure to chloroform alone; however, Bove et al. (1995) studied the effects of drinking-water consumption on birth outcomes and found that exposure to TTHM at levels >0.l ppm resulted in reduced birth weight and size as well as an increased risk of oral cleft, central nervous system, and neural tube defects. Since the authors did not specifically monitor chloroform levels, the effects seen may be due to exposure to other THMs. In addition, non-THM contaminants in the drinking water may have contributed to the observed effects as well. Only one study was located regarding the developmental effects of chloroform in humans. Animal studies indicate that chloroform can cross the placenta and cause fetotoxic and teratogenic effects. Chloroform exposure has also caused increased resorptions in animals. Epidemiology studies suggest a possible risk of colon and bladder cancer in humans that is associated with chloroform in drinking water. In animals, chloroform was carcinogenic after oral exposure.

Minimal Risk Levels for Chloroform.

Inhalation MRLs.

- An MRL of 0.1 ppm has been derived for acute-duration inhalation exposure (14 days or less) to chloroform.

The MRL was based on a hepatic NOAEL of 3 ppm chloroform administered for 6 hours a day for 7 consecutive days to mice (Larson et al. 1994c). Female mice exposed to 100 or 300 ppm exhibited centrilobular hepatocyte necrosis and severe diffuse vacuolar degeneration of midzonal and periportal hepatocytes, while exposure to 10 or 30 ppm resulted in mild-to-moderate vacuolar changes in centrilobular hepatocytes. Decreased eosinophilia of the centrilobular and midzonal hepatocyte cytoplasm relative to periportal hepatocytes was observed at 30 ppm. Livers of mice in the 1 and 3 ppm groups did not differ significantly from control animals and were considered to be NOAELs for liver effects. The NOAEL of 3 ppm was converted to the Human Equivalent Concentration (HEC) as described in Equation 4-10 in Interim Methods for Development of Inhalation Reference

Concentrations (EPA 1990b). This calculation resulted in a $NOAEL_{[HEC]}$, of 3 ppm. An uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability) was applied to the $NOAEL_{[HEC]}$, value, which resulted in an MRL of 0.1 ppm.

Reports regarding chloroform hepatotoxicity in animals are numerous (Larson et al. 1993, 1994a, 1994b, 1994c). Liver damage has been reported in several other studies, and was usually indicated by liver biochemical/enzyme alterations in rats (Lundberg et al. 1986) and mice (Gehring 1968; Murray et al. 1979) after acute inhalation exposure. Fatty changes (Culliford and Hewitt 1957; Kylin et al. 1963) and liver necrosis (Deringer et al. 1953) were observed histologically in mice after acute inhalation exposure. Histological findings indicative of liver toxicity were also observed in other laboratory animals following inhalation exposure of intermediate duration, but the findings were not dose-related (Torkelson et al. 1976). The 17.3 mg/kg dose was also a NOAEL for kidney effects, but tubular necrosis occurred at 65.6 mg/kg/day.

- An MRL of 0.05 ppm has been derived for intermediate-duration inhalation exposure (15 days to 364 days) to chloroform.

The MRL was based on a LOAEL of 14 ppm in workers exposed to concentrations of chloroform of up to 400 ppm for less than 6 months (Phoon et al. 1983). Vomiting and toxic hepatitis were noted to occur at an inhaled chloroform concentration of 14 ppm. The LOAEL of 14 ppm was divided by an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability) and a modifying factor of 3 (insufficient diagnostic data to determine the seriousness of hepatotoxic effects) to arrive at the MRL of 0.05 ppm. Alterations in liver functions have been reported in several studies in both humans and animals, and is discussed in more detail in the chronic-duration inhalation MRL section immediately below.

- An MRL of 0.02 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to chloroform.

The MRL was based on a LOAEL of 2 ppm in workers exposed to concentrations of chloroform ranging from 2 to 205 ppm for l-4 years (Bomski et al. 1967). The LOAEL of 2 ppm was divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability) to arrive at the MRL of 0.02 ppm. Hepatomegaly was found in 25% of chloroform-exposed workers. Toxic hepatitis was found in 5.6% of the liver enlargement cases. Hepatosteatosis (fatty liver) was detected in 20.6%

of liver-enlargement cases. Chloroform-exposed workers had a higher frequency of jaundice over the years than the control group. Alterations in liver functions have been reported in several studies in both humans and animals. In humans, impaired liver function was indicated by increased sulfobromophthalein retention in some patients exposed to chloroform via anesthesia (Smith et al. 1973), in addition to acute toxic hepatitis developing after childbirth in several women exposed to chloroform via anesthesia (Lunt 1953; Royston 1924; Townsend 1939). In contrast, no clinical evidence of liver toxicity was found in another study among chloroform workers exposed to ≤237 ppm (Challen et al. 1958).

Oral MRLs.

- An MRL of 0.3 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to chloroform.

The MRL was based on a NOAEL of 26 mg/kg/day in the drinking water for 4 days for hepatic effects in mice (Larson et al. 1994b). The NOAEL of 26.4 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to arrive at the MRL of 0.3 mg/kg/day. A study performed by Moore et al. (1982) found renal effects in CFLP Swiss mice dosed at 65.5 mg/kg/day by gavage in oil. Another study by Larson et al. (1993) found both hepatic (elevated SDH, ALT and AST, hepatocyte necrosis) and renal (proximal tubule necrosis) lesions in Fischer 344 rats and hepatic lesions only in $B6C3F_1$ mice induced by chloroform administered at 34 mg/kg/day once by gavage in oil. Lesions in the Larson et al. (1993) study were ranked as less serious LOAELs.

- An MRL of 0.1 mg/kg/day has been derived for intermediate-duration oral exposure (15-364 days) to chloroform.

This MRL is based on a NOAEL of 15 mg/kg/day for hepatic effects in dogs dosed with chloroform in a capsule 1 time a day, 6 days a week for 6 weeks (Heywood et al. 1979). The NOAEL of 15 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to arrive at the MRL of 0.1 mg/kg/day. Clinical chemistry parameters showed significantly increase SGPT in the 30 mg/kg/day group beginning at 6 weeks. SGPT activity was not increased in the 15 mg/kg/day group until week 130. Liver effects in animals have been reported in numerous oral studies of intermediate duration. Fatty changes, necrosis,

increased liver weight, and hyperplasia have been observed in rats exposed to ≥150 mg/kg/day chloroform in drinking water for 90 days (Palmer et al. 1979). An increased incidence of sporadic, mild, reversible liver changes occurred in mice exposed to chloroform in drinking water at doses of 0.3-l14 mg/kg/day for 90 days, but the incidences were not significantly higher than the incidences in controls (Chu et al. 1982a). Fatty and hydropic changes, necrosis, and cirrhosis were observed in mice treated by gavage with ≥50 mg/kg/day chloroform in oil for 90 days (Bull et al. 1986; Munson et al. 1982) or at 86 mg/kg/day in drinking water for 1 year (Klaunig et al. 1986). In contrast, centrilobular fatty changes observed in mice at 64 mg/kg/day chloroform in drinking water for 90 days appeared to be reversible (Jorgenson and Rushbrook 1980), and no liver effects were found in mice treated with ≥50 mg/kg/day in aqueous vehicles (Bull et al. 1986). In addition, hepatocellular degeneration was induced in F_1 females in a 2-generation study in which mice were treated by gavage with 41 mg/kg/day chloroform in oil (Gulati et al. 1988).

- An MRL of 0.01 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to chloroform.

This MRL is based on a LOAEL of 15 mg/kg/day for hepatic effects in dogs dosed with chloroform 6 days a week for 7.5 years (Heywood et al. 1979). The LOAEL of 15 mg/kg/day was divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans and 10 for human variability) to arrive at the MRL of 0.01 mg/kg/day. SGPT activity was not increased in the 15 mg/kg/day group until week 130, providing the LOAEL on which this MRL was based. Numerous chronic oral studies examined hepatic and renal end points as well as neurological and cancer effects. Serious effects occurred at higher doses; 15 mg/kg/day was the lowest dose used in available animals studies. A NOAEL of 2.46 mg/kg/day for liver and kidney effects (SGPT, SGOT, BUN and SAP) was found in humans who used a dentifrice containing 0.34% or a mouthwash containing 0.43% chloroform for l-5 years (DeSalva et al. 1975).

The reader is advised to exercise caution in the extrapolation of toxicity data from animals to humans. Species-related differences in sensitivity must be accounted for. Some studies utilized to derive MRLs or otherwise extrapolate data, is dated; however, they do represent the body of knowledge regarding chloroform toxicity. In addition, many of the human studies quoted involved clinical case reports in which chloroform was utilized either as an anesthetic or as an agent of suicide. Such doses are clearly

excessive and would not be encountered by the general population. These and other issues are addressed in Section 2.10.

Death. Chloroform levels of ≈40,000 ppm cause death in patients under chloroform anesthesia (Featherstone 1947; Whitaker and Jones 1965). Death is usually due to severe respiratory depression/ failure or disturbances in cardiac rhythm. Accidental or intentional ingestion of large doses of chloroform may lead to death (Piersol et al. 1933). 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, or severe hepatic injury (Piersol et al. 1933; Schroeder 1965).

The levels of chloroform exposure that cause death in animals are usually lower than those administered to patients to induce anesthesia; however, the duration of exposure in animals is generally longer. Following acute exposure to high concentrations of chloroform, all male mice died; however, most females survived the exposure for several months (Deringer et al. 1953). Survival was associated with lower testosterone levels, as suggested by the higher mortality rate in noncastrated adult males. This conclusion is supported by similar observations of higher survival rates in female rats, compared to male rats, after intermediate-duration exposure to chloroform (Torkelson et al. 1976). In regard to LC_{50} values in rats, survival rates were highest among females and lowest among young adult males. The correlation between mortality rates and male hormone levels is evident. Deaths were apparently potentiated by starvation, dehydration, and exhaustion (Ekstrom et al. 1986, 1988; Royston 1924; Townsend 1939). Increased mortality was also observed in rats and mice after oral exposure of intermediate and chronic duration (Balster and Borzelleca 1982; Chu et al. 1982a; Jorgenson et al. 1985; Klaunig et al. 1986; NCI 1976; Palmer et al. 1979; Roe et al. 1979). Deaths were caused by toxic liver and kidney effects, and tumors. Deaths after dermal exposures in either humans or laboratory animals have not been reported.

Chloroform concentrations in air and drinking water in the general environment or near hazardous waste sites are not likely to be high enough to cause death in humans after acute exposure. Whether chronic exposure to low levels of chloroform in the environment, drinking water, or hazardous wastes could shorten the life span of humans is not currently known. Currently available epidemiologic findings about the chronic exposure to chloroform are inconsistent at best which, in large part, may be due to study design issues.

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Systemic Effects.

Respiratory Effects. The respiratory failure observed in patients under chloroform anesthesia was probably due to a direct effect of chloroform on the respiratory center of the central nervous 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). Upper respiratory tract obstruction can occur in patients after inhalation exposure to chloroform via anesthesia (Featherstone 1947) and after chloroform ingestion (Schroeder 1965). Few autopsy reports were located in the literature. Hemorrhage into the lungs, without any signs of consolidation, was reported in a case study involving death after inhalation exposure (Royston 1924); however, congested lungs with pneumonic consolidation were observed in a man who died after drinking chloroform (Piersol et al. 1933).

In addition to lower respiratory tract effects, chloroform has been demonstrated to induce changes in the nasal region of rats and mice after inhalation and oral exposure. Increased sizes of goblet cells and nasal epithelium, degeneration of the nasal epithelium and Bowman's glands, changes in the proliferation rates of cells, new bone formation, and changes in biochemical parameters (especially cytochrome P-450-2El) have been reported (Larson et al. 1995b, 1996; Mery et al. 1994), indicating that chloroform can adversely affect the upper as well as the lower respiratory tract at low concentrations. The incidence of respiratory lesions after oral (gavage) administration also indicates a systemic mechanism of action for chloroform-induced toxicity.

Interstitial pneumonitis was observed in male rats and rabbits after inhalation exposure to 85 or 50 ppm chloroform, respectively, for 6 months (Torkelson et al. 1976). In most oral studies, no exposure-related histopathological changes were observed in the lungs of exposed animals (Gulati et al. 1988; Jorgenson and Rushbrook 1980; NCI 1976; Palmer et al. 1979; Roe et al. 1979).

Respiratory effects are more likely to occur after inhalation exposure to high concentrations of chloroform. 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 and can cause collapse of the respiratory bronchiole due to the sudden increase in inhalation tension. Immediate death after chloroform inhalation may be due principally to this effect

in the lungs (Fagan et al. 1977). It is unlikely that exposure levels of chloroform in the general environment or at hazardous waste sites would be high enough to cause these severe respiratory effects.

Cardiovascular Effects. Chloroform induces cardiac arrhythmia in patients exposed to chloroform via anesthesia (Smith et al. 1973; Whitaker and Jones 1965). Similarly, heart effects were observed upon electrocardiography of an individual who accidentally ingested chloroform (Schroeder 1965). Hypotension was observed in 12-27% of patients exposed to chloroform via anesthesia (Smith et al. 1973; Whitaker and Jones 1965) and also was observed in a patient who ingested chloroform (Storms 1973).

No studies were located regarding cardiovascular effects in animals after inhalation exposure to chloroform. No histopathological changes were observed in the heart of rats, mice (NCI 1976), or dogs (Heywood et al. 1979) chronically exposed to chloroform; however, cardiovascular function was not assessed in these studies. It has been demonstrated in an *in vitro* study on heart-lung preparations of guinea pigs that chloroform may cause a permanent contractile failure of the heart (Doring 1975). The effect is due to structural damage of the transverse tubular system and is accompanied by increased storage of adenosine triphosphate (ATP) and phosphocreatine. The *in vitro* induction of changes showed that contractile failure is a direct effect on the cardiovascular system rather than an indirect cardiovascular effect on the central nervous system. This mechanism may operate in humans exposed to high vapor concentrations such as those used in anesthesia or in humans exposed to high oral doses from accidental or intentional ingestion. It is unlikely, however, that concentrations of chloroform in the environment would be high enough to cause overt cardiovascular effects.

Cytotoxicity of chloroform (1,000 ppm) in male Sprague-Dawley rat cardiac myocytes has been examined *in vitro*. Cell viability was measured using the criterion of Trypan blue exclusion as well as counting the number of rod and spherical cells in the media. Creatinine phosphokinase (CPK) leakage was measured as an indirect measurement of heart cell function. Myocytes treated with chloroform showed statistically significant decreases in cell viability and significant decreases in rod-shaped cells compared to controls. Significant increases in enzyme leakage of CPK from myocytes were noted (El-Shenawy and Abdel-Rahman 1993b). The effects of various concentrations of chloroform on the *in vitro* transfer of dyes between cardiac myocytes from Sprague-Dawley rats has also been examined (Toraason et al. 1992). The cells were exposed to one of 11 concentrations of chloroform in dimethyl

sulfoxide (DMSO), and Lucifer yellow CH dye injected the cells, and the rate of transfer of dye from the injected myocyte to the non-injected myocyte recorded. As the cells were exposed to increasing concentrations of chloroform, the number of cells that transferred the dye decreased to zero. Heart cells also tended to beat slower or stop beating completely when exposed to chloroform but resumed normal spontaneous beating when chloroform was washed out. This *in vitro* data suggest that incorporation of halocarbons in the membrane may block intercellular communication through modification of the immediate environment of gap junctions. The data from these two studies indicate that chloroform exposure may induce reversible toxicity in the heart.

Gastrointestinal Effects. Nausea and vomiting were not only frequently observed side effects in patients exposed to chloroform via anesthesia (Hakim et al. 1992; Royston 1924; Smith et al. 1973; Townsend 1939; Whitaker and Jones 1965), but also occurred in humans exposed to lower chloroform concentrations (22-237 ppm) in occupational settings (Challen et al. 1958; Phoon et al. 1983). Vomiting, gastric distress, and pain were observed in individuals who intentionally or accidentally ingested high doses of chloroform (Piersol et al. 1933; Schroeder 1965; Storms 1973).

No studies were located regarding gastrointestinal effects in animals after inhalation exposure to chloroform. Vomiting in dogs (Heywood et al. 1979) and gastric erosions in rats (Thompson et al. 1974) were observed in oral studies of intermediate duration. These results suggest that severe gastrointestinal irritation in humans and animals is due to direct damage of the gastrointestinal mucosa caused by ingesting high concentrations of chloroform (Piersol et al. 1933; Schroeder 1965; Thompson et al. 1974). Nausea and vomiting experienced by occupationally exposed individuals is likely due to neurotoxicity. Since toxic hepatitis may occur at occupational levels as low as 2 ppm (Bomski et al. 1967), it is possible that levels of chloroform in the air at hazardous waste sites may be high enough to cause some liver effects with secondary gastrointestinal effects, if exposure is prolonged.

Hematological Effects. Information regarding hematological effects in humans exposed to chloroform is limited. Increased prothrombin time was observed in some patients, following exposure to chloroform via anesthesia (Smith et al. 1973). This effect, however, reflects chloroform hepatotoxicity, because prothrombin is formed in the liver. Decreased erythrocytes and hemoglobin were observed in a patient who was chronically exposed to chloroform in a cough medicine (Wallace 1950).

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No hematological effects were observed in rats, rabbits, guinea pigs, or dogs after inhalation exposure to chloroform for intermediate durations (Torkelson et al. 1976). Studies report conflicting results regarding hematological effects in animals after oral exposure to chloroform.

No conclusion about hematological effects in humans after exposure to chloroform can be made on the basis of one case study in humans. From the experimental data in animals, it is evident that all hematological effects observed in rats were due to oral exposure of acute, intermediate, or chronic duration. It is possible that the hematological effects observed in rats are transient. Human exposure to chloroform in the environment, drinking water, or at hazardous waste sites is likely to cause few or no hematological effects.

Musculoskeletal Effects. Little data is available that examines the effects of chloroform toxicity on the musculoskeletal system; however, it appears that chloroform has few significant toxic effects on this system.

Hepatic Effects. The liver is a primary target organ of chloroform toxicity in humans and animals after inhalation and oral exposure, with some evidence that suggests that the damage may be reversible (Wallace 1950). Impaired liver function was indicated by increased sulfobromophthalein retention in some patients exposed to chloroform via anesthesia (Smith et al. 1973). Acute toxic hepatitis developed after childbirth in several women exposed to chloroform via anesthesia (Lunt 1953; Royston 1924; Townsend 1939). Upon autopsy, centrilobular necrosis was observed in the women who died; however, the hepatotoxicity was associated with exhaustion from prolonged delivery, starvation, and dehydration, indicating improper handling of the delivery procedure by an obstetrician. Toxic hepatic disease, characterized by hepatomegaly and abnormal liver sonograms as late as seven days after an unknown amount of oral chloroform, has been reported (Hakim et al. 1992). Elevated liver enzymes and changes in GGT, alpha-feto protein and retinol binding protein were reported in a female who injected herself intravenously and also consumed chloroform orally during a 12-hour period (Rao et al. 1993). During occupational exposure to concentrations ranging from 14 to 400 ppm, chloroform hepatotoxicity was characterized by jaundice (Phoon et al. 1983), hepatomegaly, enhanced SGPT and SGOT activities, and hypergammaglobulinemia following exposure to concentrations ranging from 2 to 205 ppm (Bomski et al. 1967). In contrast, no clinical evidence of liver toxicity was found in another study among chloroform workers exposed to ≤237 ppm (Challen et al. 1958). Case reports of intentional and accidental ingestion of high doses (≥2,410 mg/kg) of chloroform indicate severe liver

injury (Piersol et al. 1933; Schroeder 1965; Storms 1973). The diagnosis was supported by clinical and biochemical results: fatty degeneration and extensive centrilobular necrosis were observed in one patient who died (Piersol et al. 1933). Liver damage was induced by chronic use of a cough medicine containing chloroform (Wallace 1950), but not by chronic exposure to chloroform in mouthwash (De Salva et al. 1975). An intermediate-duration inhalation MRL of 0.05 ppm was derived from the LOAEL of 14 ppm from the data presented by Phoon et al. (1983); a chronic-duration inhalation MRL of 0.02 ppm was derived from the LOAEL of 2 ppm from the data presented by Bomski et al. (1967).

Reports regarding chloroform hepatotoxicity in animals are numerous (Larson et al. 1993, 1994a, 1994b, 1994c, 1995b, 1996; Nakajima et al. 1995; Pereira 1994; Wang et al. 1994, 1995). An acute-duration inhalation MRL of 0.1 ppm was based on a NOAEL for hepatic effects in mice exposed to 3 ppm chloroform for 6 hours a day for 7 days (Larson et al. 1994c).

Liver damage was indicated by biochemical changes in rats (Lundberg et al. 1986; Nakajima et al. 1995; Wang et al. 1994, 1995) and mice (Gehring 1968; Murray et al. 1979) after acute inhalation exposure. Fatty changes (Culliford and Hewitt 1957; Kylin et al. 1963) and liver necrosis (Deringer et al. 1953; Larson et al. 1995b, 1996; Pereira 1994) were observed histologically in mice and rats after acute inhalation exposure. Histological findings indicative of liver toxicity were also observed in rabbits and guinea pigs following inhalation exposure of intermediate duration, but the findings were not dose-related (Torkelson et al. 1976). Liver effects have been observed in many species (rats, mice, and dogs) that were tested by the oral route by various methods of administration (gavage or drinking water) and durations (acute, intermediate, or chronic). Observed effects include increased liver weight, increased serum levels of transaminases indicative of liver necrosis, and histological evidence of swelling, fatty changes, hydropic changes, vacuolation, necrosis, hyperplasia, cirrhosis, macrophage and neutrophil infiltration, and toxic hepatitis (Bull et al. 1986; Chu et al. 1982b; Heindel et al. 1995; Heywood et al. 1979; Jones et al. 1958; Jorgenson and Rushbrook 1980; Klaunig et al. 1986; Larson et al. 1993, 1994b, 1995b, 1996; Nakajima et al. 1995; NCI 1976; Pereira 1994; Tumasonis 1985, 1987). Two acute oral studies define a LOAEL and a NOAEL for liver effects in mice. Fatty infiltration was observed in mice given a single gavage dose of 35 mg/kg/day chloroform in oil (Jones et al. 1958). No toxic effects on the livers of mice occurred after a single dose of 17.3 or 59.2 mg/kg chloroform in oil, but increased SGPT occurred at 199 mg/kg (Moore et al. 1982). The 17.3 mg/kg dose was also a NOAEL for kidney effects, but tubular necrosis occurred at 65.6 mg/kg/day. In a 7.5-year study in which dogs were administered chloroform in toothpaste, SGPT activity was

significantly increased at 30 mg/kg/day beginning at 6 weeks (Heywood et al. 1979). SGPT activity was not increased at 15 mg/kg/day until 130 weeks. Therefore, 15 mg/kg/day was a NOAEL for intermediate-duration exposure and a LOAEL for chronic-duration exposure. The 15 mg/kg/day dose was used to derive MRL values of 0.1 and 0.01 mg/kg/day for intermediate- and chronic-duration oral exposure, respectively.

Data regarding chloroform-induced hepatotoxicity were also supported by results obtained after acute intraperitoneal exposure in rats (Bai et al. 1992; Ebel et al. 1987; El-Shenawy and Abdel-Rahman 1993a; Lundberg et al. 1986; Wang et al. 1994), mice (Klaassen and Plaa 1966), dogs (Klaassen and Plaa 1967), and gerbils (Ebel et al. 1987). No hepatic effects were observed in rabbits when chloroform was applied to their skin for 24 hours (Torkelson et al. 1976). The toxicity of chloroform on laboratory animal hepatocytes *in vitro* has been reported (Azri-Meehan et al. 1992, 1994; Bai and Stacey 1993; El-Shenawy and Abdel-Rahman 1993a; Suzuki et al. 1994).

As discussed in Section 2.3.3, the mechanism of chloroform-induced liver toxicity may involve metabolism to the reactive intermediate, phosgene, which binds to lipids and proteins of the endoplasmic reticulum, lipid peroxidation, or depletion of GSH by reactive intermediates. Because liver toxicity has been observed in humans exposed to chloroform levels as low as 2 ppm in the workplace and in several animal species after inhalation and oral exposure, it is possible that liver effects could occur in humans exposed to environmental levels, to levels in drinking water, or to levels found at hazardous waste sites.

Endocrine Effects. No reports of chloroform toxicity to endocrine organs have been reported.

Renal Effects. Clinical reports indicate that the renal damage observed in women exposed to chloroform via anesthesia during prolonged parturition most likely occurs when chloroform anesthesia is associated with anoxia. Competitive swimmers who swim in indoor pools have been reported to have elevated β-2microglobin, suggesting some degree of renal damage (Aiking et al. 1994). Case studies of individuals who intentionally or accidentally ingested high doses of chloroform report biochemical changes indicative of kidney damage, as well as fatty degeneration at autopsy (Piersol et al. 1933; Schroeder 1965). Albuminuria and casts were also reported in a case of chronic use of a cough medicine containing chloroform (Wallace 1950); however, no renal effects were observed in individuals chronically exposed to chloroform in a mouthwash (De Salva et al. 1975).

Renal effects of chloroform after inhalation have also been examined in animals (Larson et al. 1994b, 1994c, 1996). Kidney effects in animals after inhalation exposure to chloroform include tubular necrosis, tubular calcification, increased kidney weight, cloudy swelling, mineralization of the cortex, and interstitial nephritis. Animal studies regarding renal toxicity after oral exposure are numerous. Effects include acute toxic nephrosis, tubular dilation, necrosis and regeneration, chronic inflammation, mineralized concretions, hyaline cast formation, and fatty degeneration. The effects of dose and vehicle have been examined (Heindel et al. 1995; Larson et al. 1993, 1995b; Lipsky et al. 1993).

Mice seem to be more sensitive to chloroform-induced renal toxicity than other experimental animals. Certain strains of male mice are susceptible to chloroform-induced nephrotoxicity, while female mice appear to be somewhat resistant (Culliford and Hewitt 1957; Eschenbrenner and Miller 1945b; Larson et al. 1996). Castrated mice were no longer susceptible to the effect, and testosterone treatment increased the severity of kidney damage in females, suggesting the role of hormones in chloroforminduced nephrotoxicity. It has been demonstrated that sensitivity to kidney damage is related to the capacity of the kidney to metabolize chloroform to phosgene (Pohl et al. 1984). The activation of chloroform to its reactive metabolites appeared to be cytochrome P-450-dependent: the covalent binding to microsomal protein required nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen, and could be inhibited by carbon monoxide (Hook and Smith 1985; Smith and Hook 1983, 1984; Smith et al. 1984). Furthermore, administration of chloroform to male mice caused a depletion of renal GSH, indicating that GSH can react with reactive intermediates, thereby reducing the extent of the reaction with tissue MMBs and kidney damage.

The renal toxicity of chloroform in rats after intraperitoneal dosing has also been reported (Kroll et al. 1994a, 1994b).

It is likely that kidney effects may occur in humans after inhalation or oral exposure to high levels of chloroform; however, it is not known whether such effects would occur at the levels of chloroform found in the environment, in drinking water, or at hazardous waste sites.

Dermal Effects. No reports are available on the toxicity of chloroform to skin after inhalation and oral exposures in humans. Stratum comeum damage was reported after a topical exposure of chloroform of 15 minutes duration for 6 consecutive days (Malten et al. 1968). Chloroform was used as a vehicle for the topical application of aspirin for the treatment of painful herpes zoster lesions in

male and female humans. The only reported side-effect was an occasional burning sensation to the skin as the chloroform evaporated after application (King 1993).

Few reports exist on the dermal effects of chloroform in animals after inhalation or oral exposures. Alopecia has been observed in pregnant rats (Thompson et al. 1974) and in mice (Gulati et al. 1988). Skin irritation and necrosis and been reported in rabbits after topical application of chloroform (Smyth et al. 1962; Torkelson et al. 1976).

Ocular Effects. No studies were located regarding the ocular effects of chloroform in humans or animals.

Body Weight Effects. Decreased body weight has been observed frequently in animals after inhalation or oral exposure to chloroform, although the degree of body weight changes are somewhat variable and may be linked to taste aversion (in oral studies) (Chu et al. 1982b; Larson et al. 1995b, 1996; Munson et al. 1982; Newell and Dilley 1978; Torkelson et al. 1976; Tumasonis et al. 1985, 1987). The degree of decreased weight gain was often dose-related and was caused by chloroform toxicity. Decreased weight gain generally occurred at exposure levels similar to or lower than those that induced liver and kidney effects in animals. The possibility of effects on body weight in humans exposed to ambient or elevated levels of chloroform cannot be dismissed.

Immunological and Lymphoreticular Effects. No studies were located regarding immunological effects in humans after inhalation, oral, or dermal exposure to chloroform.

Information about immunological effects in animals is limited. After repeated inhalation exposure to chloroform, mortality was increased in mice challenged with streptococcus infection, suggesting increased susceptibility (Aranyi et al. 1986). However, the bacterial activity of alveolar macrophages was not suppressed in this study. After acute oral exposure, reduced lymphocyte counts were observed in rats (Chu et al. 1982b). Furthermore, humoral immunity was depressed in mice exposed to 50 mg/kg/day chloroform for acute or intermediate durations (Munson et al. 1982). In contrast, cell-mediated immunity was influenced only at high chloroform concentrations administered orally for intermediate durations; however, the chloroform-induced immunological changes appeared to be more severe following acute exposure. *In vitro* treatment of serum with chloroform resulted in a loss of complement activity (Stefanovic et al. 1987). Immunological effects may result from the ability of

chloroform to dissociate antigen-antibody complexes, since it can cause dissociation of certain enzymeinhibitor complexes (Berger et al. 1983). Every day, humans are exposed to very low levels of chloroform in the environment, mainly via inhalation and oral exposure (Hajimiragha et al. 1986; Peoples et al. 1979; Wallace et al. 1987a, 1989). There is a risk of chloroform exposure at or near hazardous waste sites. Although no evidence that chloroform can cause immunological effects in humans was located in the literature, the possibility remains that these effects may result from exposure to chloroform.

Neurological Effects. Neurological effects in humans after acute inhalation exposure to chloroform are well documented because chloroform has been used as an anesthetic for surgery. Inhaled chloroform acts as a depressant on the central nervous system. Chronic inhalation exposure to chloroform resulted in exhaustion, lack of concentration, depression, and irritability in occupationally exposed people (Challen et al. 1958). In a case study, chloroform inhalation for 12 years resulted in psychotic episodes, hallucinations, and convulsions (Heilbrunn et al. 1945). Central nervous system toxicity was observed in humans after oral exposure to chloroform, which suggests that the effects of inhalation and oral exposure are similar. In case reports of patients who intentionally or accidentally ingested several ounces of chloroform, deep coma with abolished reflexes occurred within a few minutes (Piersol et al. 1933; Schroeder 1965; Storms 1973).

Inhalation exposure to high chloroform concentrations induced narcosis (Lehmann and Flury 1943; Sax 1979) and reversible impairment of memory retrieval in animals. High, single, oral doses of chloroform caused ataxia, incoordination, anesthesia, and brain hemorrhage in mice (Balster and Borzelleca 1982; Bowman et al. 1978). Behavioral effects were observed at lower oral doses.

Chloroform concentrations from 1.5 to 6 mmol chloroform were used to determine how chloroform may modify glutamate receptor agonist responses in mouse brain cortical wedges. The two agonists examined were N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA). Responses were determined by measuring electrical responses within the cortical slices. Three mmol of chloroform selectively inhibited AMPA but did not affect NMDA responses. Higher concentrations of chloroform failed to inhibit the AMPA or NMDA content in the wedges (Carla and Moroni 1992). Male Sprague-Dawley rats administered a single 200 mg/kg dose of chloroform experienced a significant decrease in midbrain 5-HIAA levels and a significant increase in hypothalamic dopamine concentrations (Kanada et al. 1994).

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The clinical effects of chloroform toxicity on the central nervous system are well documented. However, the molecular mechanism of action is not well understood. It has been postulated that anesthetics induce their action at a cell-membrane level due to lipid solubility. The lipid-disordering effect of chloroform and other anesthetics on membrane lipids was increased by gangliosides (Harris and Groh 1985), which may explain why the outer leaflet of the lipid bilayer of neuronal membranes, which has a large ganglioside content, is unusually sensitive to anesthetic agents. Anesthetics may affect calcium-dependent potassium conductance in the central nervous system (Caldwell and Harris 1985). The blockage of potassium conductance by chloroform and other anesthetics resulted in depolarization of squid axon (Haydon et al. 1988).

Based upon existing data, the potential for neurological and behavioral effects in humans exposed to chloroform at levels found in the environment, in drinking water, or at hazardous waste sites is very minimal.

Reproductive Effects. It has not been definitively determined whether chloroform exposure induces reproductive effects in humans. No studies were located regarding reproductive effects in humans after inhalation or dermal exposure to chloroform. Only one study was located regarding reproductive effects in humans after oral exposure to chloroform. Bove et al. (1995) studied the effects of drinking-water consumption on birth outcomes and found that exposure to TTHM at levels >0.l ppm resulted in reduced birth weight and size as well as an increased risk of oral cleft, central nervous system, and neural tube defects. These results should be viewed with caution since the authors did not specifically monitor chloroform levels. The effects seen may be due to exposure to other THMs or non-THM contaminants in the drinking water.

Studies indicate that exposure to chloroform causes reproductive effects in animals. Dose-related increases of embryonal resorptions were observed in rats and mice after inhalation or oral exposure to chloroform during gestation. A significant increase in the incidence of abnormal sperm was observed in mice after acute inhalation exposure (Land et al. 1979, 1981). Gonadal atrophy was observed in male and female rats treated by gavage (Palmer et al. 1979). Fertility was not affected in either generation of mice exposed orally to chloroform in a 2-generation study (Gulati et al. 1988). In contrast, a 2-generation study in Sprague-Dawley rats and Swiss mice demonstrated significant decreases in combined live pup weights and in the proportion of male pups born live (rats), a significant reduction in sperm concentrations and sperm head counts, and increased numbers of unclear

or no estrous cycles (mice). However, the animals in this study were administered drinking water containing a mixture of 25 contaminants; thus, other toxicants may have elicited these effects. Oral exposure to chloroform did not induce histopathological changes in the reproductive organs of rats exposed for intermediate durations (Jorgenson and Rushbrook 1980) or in rats and mice (NCI 1976) and dogs (Heywood et al. 1979) exposed for chronic durations.

Developmental Effects. One study regarding developmental effects in humans after oral exposure to chloroform has been reported (Kramer et al. 1992). The estimated relative risk of low birth weight associated with drinking-water sources having chloroform levels of ≥10 μg/L was 30% higher than sources with undetectable levels of chloroform. Prematurity was not associated with chloroform/THM exposure. The estimated relative risk of intrauterine growth retardation associated with drinking-water supplies with chloroform concentrations of >l0 μg/L was 80% higher than the risk for sources with undetectable levels of chloroform. Sources with intermediate chloroform levels (l-9 μg/L) had an elevated risk of 30%. There seems to be reasonable evidence to suggest that some correlation with an increased risk of intrauterine growth retardation associated with higher concentrations of waterborne chloroform and dichlorobromomethane does exist.

Inhalation exposure to chloroform during gestation induced fetotoxicity and teratogenicity in rats (Schwetz et al. 1974) and mice (Murray et al. 1979). Decreased fetal crown-rump length, decreased ossifications, imperforate anus (rats), and cleft palate (mice) were observed in the offspring of exposed dams. In contrast, fetotoxicity (decreased fetal weight), but not teratogenicity, was observed in rats after oral exposure to chloroform (Ruddick et al. 1983; Thompson et al. 1974). Increased resorptions were observed in rats and rabbits (Thompson et al. 1974). In a 2-generation oral study, degeneration of the epididymal ductal epithelium was observed in mice of the F_1 generation (Gulati et al. 1988). Due to its chemical nature, chloroform can cross the placenta easily, as demonstrated by its detection in the placenta and fetuses of mice a short time after inhalation exposure (Danielsson et al. 1986). Chloroform may accumulate in the amniotic fluid and fetal tissues. Various developmental effects may result from exposure, depending on the period of *in utero* exposure. Although no studies have conclusively reported developmental effects in humans, chloroform (or in tandem with other organic halomethanes) may have the potential to cause developmental effects in humans. Whether such effects could occur from exposure to levels in the environment, in drinking water, or at hazardous waste sites is not known.

Genotoxic Effects. *In vivo* and *in vitro* studies of the genotoxic effects of chloroform are summarized in Tables 2-5 and 2-6. Information regarding genotoxic effects after *in vivo* exposure to chloroform is limited. Mice exposed to chloroform by gavage had an increase in sister chromatid exchange frequency in bone marrow cells (Morimoto and Koizumi 1983). No sperm-head abnormalities were noted in mice after receiving 5 daily intraperitoneal injections of chloroform in concentrations up to 0.25 mg/kg/day in corn oil (Topham 1980). Oral exposure to chloroform did not increase UDS in rat hepatocytes (Mirsalis et al. 1982). Chloroform exposure caused mitotic arrest in grasshopper embryos (Liang et al. 1983) and a nonsignificant increase in the recessive lethals in *Drosophila melanoguster* (Gocke et al. 1981). In general, most of the assays for chloroform genotoxicity are negative. Therefore, it seems that chloroform is a weak mutagen and that its potential to interact with DNA is low.

In *in vitro* experiments, chloroform did not cause reverse mutations in *Salmonella typhimurium* (Gocke et al. 1981; San Augustin and Lim-Sylianco 1978; Simmon et al. 1977; Uehleke et al. 1977; Van Abbe et al. 1982; Varma et al. 1988) or in *Escherichia coli* (Kirkland et al. 1981) with or without metabolic activation. Inconclusive results were obtained in *Saccharomyces cerevisiae* and *Schistozosaccharomyces pombe* (Callen et al. 1980; De Serres et al. 1981). Chloroform, however, induced Aneuploidia in *Aspergillus niduluns* (Crebelli et al. 1988). Chloroform caused forward mutations in L5 178Y mouse lymphoma cells after metabolic activation (Mitchell et al. 1988), but did not cause mutations at 8-azaguanine locus in Chinese hamster lung fibroblasts (Sturrock 1977) or sister chromatid exchange in Chinese hamster ovary cells (White et al. 1979). A study performed in mice examined the ability of chloroform to induce UDS in hepatocytes *in vitro* from 15-week-old female $B6C3F_1$ mice. Chloroform concentrations ranged from 0.01 to 10 mmol. Mice were sacrificed at 2 and 12 hours postdosing to determine if and when UDS began to occur. Dimethylnitrosamine, a known inducer of UDS, was used as a positive control and did induce UDS in these hepatic cells. No induction of DNA repair was observed at any concentration of chloroform at either the 2-hour or 12-hour posttreatment groups. All concentrations of chloroform added to the cell cultures of mouse hepatocytes proved to be toxic. The study showed that chloroform is not directly genotoxic in hepatocytes of female mice, either *in vivo* or *in vitro,* despite the fact that it is the target organ of chloroform carcinogenesis (Larson et al. 1994a). In human lymphocytes, chloroform did not induce UDS (Peroccio and Prodi 1981) and did not increase the frequency of sister chromatid exchange and chromosome aberrations (Kirkland et al. 1981). In contrast, increases in sister chromatid exchange were reported after metabolic activation in another study (Morimoto and Koizumi 1983).

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

Species (test system)	End point	Results	Reference
Mammalian cells:			
Rat hepatocytes	Unscheduled DNA synthesis	-	Mirsalis et al. 1982
Mouse bone marrow	Sister chromatid exchange	-	Morimoto and Koizumi 1983
Mouse	Sperm-head abnormalities	-	Topham 1980
Mouse	Sperm abnormalities	+	Land et al. 1981
Grasshopper embryo	Mitotic arrest	+	Liang et al. 1983
Drosophila melanogaster	Recessive lethals	-	Gocke et al. 1981
Host-mediated assays:			
Salmonella typhimurium TA1535 (mouse host-mediated assay)	Reverse mutation	-	San Agustin and Lim-Sylianco 1978
S. typhimurium TA1537 (mouse host-mediated assay) (males only)	Reverse mutation	+	San Agustin and Lim-Sylianco 1978

- = negative result; + = positive result; DNA = Deoxyribonucleic acid

Table 2-6. Genotoxicity of Chloroform *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organism:				
Salmonella typhimurium TA98	Reverse mutation	-	-	Gocke et al. 1981
S. typhimurium TA100	Reverse mutation	-	-	Gocke et al. 1981
S. typhimurium TA1535	Reverse mutation	-	-	Gocke et al. 1981
S. typhimurium TA1535	Reverse mutation	-	-	Uehleke et al. 1977
S. typhimurium TA1538	Reverse mutation	-	-	Uehleke et al. 1977
S. typhimurium TA98	Reverse mutation	-	-	Simmon et al. 1977
S. typhimurium TA100	Reverse mutation	-	-	Simmon et al. 1977
S. typhimurium TA1535	Reverse mutation	-	-	Simmon et al. 1977
S. typhimurium TA1537	Reverse mutation	-	-	Simmon et al. 1977
S. typhimurium TA98	Reverse mutation	-	-	Van Abbe et al. 1982
S. typhimurium TA100	Reverse mutation	-	-	Van Abbe et al. 1982
S. typhimurium TA1535	Reverse mutation	-	-	Van Abbe et al. 1982
S. typhimurium TA1537	Reverse mutation	-	-	Van Abbe et al. 1982
S. typhimurium TA1538	Reverse mutation	-	-	Van Abbe et al. 1982
S. typhimurium TA98	Reverse mutation	-	(+)	Varma et al. 1988
S. typhimurium TA100	Reverse mutation	+	(+)	Varma et al. 1988
S. typhimurium TA1535	Reverse mutation	-	(+)	Varma et al. 1988
S. typhimurium TA1537	Reverse mutation	-	(+)	Varma et al. 1988
S. typhimurium TA98	Reverse mutation	Not tested	-	San Augustin and Lim-Sylianco 1978
S. typhimurium TA1535	Reverse mutation	Not tested	-	San Augustin and Lim-Sylianco 1978
S. typhimurium TA1537	Reverse mutation	Not tested	-	San Augustin and Lim-Sylianco 1978

Table 2-6. Genotoxicity of Chloroform *In Vitro* (continued)

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Escherichia coli	Reverse mutation	-	-	Kirkland et al. 1981
Aspergillus nidulans	Aneuploidia	+	Not tested	Crebelli et al. 1988
Saccharomyces cerevisiae	Reverse mutation	-	(+)	De Serres et al. 1981
Schizosaccharomyces pombe	Recombinations	-	(+)	Callen et al. 1980
Eukaryotic organisms: Mammalian cells:				
L5178Y mouse lymphoma cells	Forward mutation	+	-	Mitchell et al. 1988
Chinese hamster lung fibroblasts	Mutation at 8-azaquonine		-	Sturrock 1977
Chinese hamster ovary cells	Sister chromatid exchange		-	White et al. 1979
Human lymphocytes	Unscheduled DNA synthesis	-	-	Perocco and Prodi 1981
Human lymphocytes	Sister chromatid exchange	-	+	Morimoto and Koizumi 1983
Human lymphocytes	Sister chromatid exchange	-	-	Kirkland et al. 1981
Human lymphocytes	Chromosome aberrations		-	Kirkland et al. 1981

- = negative result; + = positive result; (+) = weakly positive; DNA = Deoxyribonucleic acid

2. HEALTH EFFECTS

Cancer. No studies were available regarding cancer in humans or animals after inhalation exposure to chloroform. Epidemiology studies suggest an association between chronic exposure to chlorinated drinking-water sources and increased incidences of colon cancer (Young et al. 1981), pancreatic cancer (Ijsselmuiden et al. 1982) and bladder cancer (Cantor et al. 1978; McGeehin et al. 1993; Zierler et al. 1988). However, numerous other potential toxicants known to exist in chlorinated drinking water may easily account for these effects.

The carcinogenic potential of chloroform has been tested in animal studies. A dose-related increase in the incidence of hepatomas was observed in mice exposed to chloroform for intermediate durations (Eschenbrenner and Miller 1945a). Chronic-duration exposure induced an increased incidence of renal adenoma and carcinoma in rats exposed to chloroform in drinking water (Jorgenson et al. 1985). Increased incidence of neoplastic nodules in the liver was observed in female Wistar rats ingesting chloroform in drinking water (Tumasonis et al. 1987). In addition, hepatocellular carcinoma was observed in $B6C3F_1$ mice given chloroform in oil by gavage (NCI 1976), and kidney tumors were observed in male ICI mice exposed by gavage to chloroform in toothpaste (Roe et al. 1979). The incidence of liver and kidney tumors in male and female rats given chloroform in a chronic-duration study has been reported (Dunnick and Melnick 1993; NCI 1976). While no hepatocellular or large intestine neoplasms were noted in either sex of rat, kidney tubular cell neoplasms were observed at 90 mg/kg/day and 180 mg/kg/day in male rats and at 200 mg/kg/day in female rats. In a another study by the same authors, using male and female mice dosed with similar amounts of chloroform, no kidney tubular cell neoplasms or large intestine neoplasms were reported in either sex of mice, while hepatocellular neoplasms were recorded in both sexes. In a similar study, Jorgenson et al. (1985) examined the carcinogenic effects of chloroform administered chronically (104 weeks) in drinking water to male Osborne-Mendel rats and female B6C3Fl mice. While no treatment related enhancement of tumor formation was observed in mice, kidney tubular cell neoplasms were observed in male rats exposed to 160 mg/kg/day chloroform.

The data concerning mouse liver tumors are conflicting. In contrast to the increased incidence of liver tumors observed in $B6C3F_l$ mice exposed by gavage to chloroform in oil (NCI 1976), no increased incidence of liver tumors was observed in female $B6C3F_1$ mice exposed to chloroform in drinking water (Jorgenson et al. 1985). This result is consistent with the absence of liver tumor effects in four other strains of mice exposed by gavage to chloroform in toothpaste (Roe et al. 1979). In a pharmacokinetic study, chloroform was absorbed more slowly and to a lesser extent from corn oil than

water (Withey et al. 1983), suggesting that pharmacokinetic effects are not responsible for the differences in liver tumor responses. Nevertheless, data from historical controls indicate that corn oil alone is not responsible for the increased incidence of liver tumors (Jorgenson et al. 1985).

The corn oil vehicle effect on the induction of mouse liver neoplasms may be due to an interaction between the vehicle and chloroform (Bull et al. 1986; Jorgenson et al. 1985), possibly resulting in altered pharmacokinetics. Larson et al. (1994b) demonstrated that female $B6C3F_1$ mice developed increased hepatocyte toxicity after gavage dosing in oil with chloroform concentrations of 238 and 477 mg/kg/day for 4 days or 3 weeks (as determined by BrdU-labelling of hepatocytes). However, there was no increase in LI in the liver of the same strain of female mice administered up to 1,800 ppm in the drinking water for 4 days or 3 weeks. The actual doses for the mice administered at 1,800 ppm were 105 and 329 mg/kg/day, respectively. Other studies have shown similar intake dose difference due to vehicle effect (Jorgenson et al. 1985; NCI 1976). The difference in results are most likely due to the method of dosing and the vehicle used, both having effects on the pharmacokinetics of chloroform and hence the degree of hepatotoxicity (and perhaps the renal toxicity in males) that chloroform may induce in these mice. Gavaged animals typically receive a large dose of chloroform all at one time over a period of several days, while the animals in the drinking-water studies consume somewhat equal amounts of chloroform; however, it is consumed in small sips (Larson et al. 1994b). It seems clear that the design of the gavage studies inherently results in repeated and relatively massive doses of chloroform to the liver (and other susceptible cells) over a short period of time that likely overwhelm the liver defense mechanisms for chloroform detoxification, resulting in hepatotoxicity, cell death, or both. Conversely, drinking-water studies expose the liver to continuous, low doses of chloroform, resulting in detoxification, elimination, and few apparent signs of hepatocellular damage. Clearly, further studies that describe the differences in pharmacokinetics between dosing method (gavage as opposed to drinking water) and vehicle effects (oil as opposed to water) need to be performed to correctly estimate human risk to orally consumed chloroform.

The possible association between cell proliferation as a result of cytotoxicity and chemical carcinogenesis has been the subject of considerable debate (Melnick et al. 1996). Chemically induced cell proliferation does play an important role in the carcinogenic process; however, the relationship between induced cell proliferation and tumorigenesis is not a direct cause-and-effect relationship (Farber 1995). Based on short-term exposures studies (4, 7, or 21 days), Larson et al. (1994b) suggest that chloroform causes cancer by inducing cytotoxicity, followed by cell regeneration. However, a

review by Chiu et al. (1996), correlating chloroform-induced short-term and long-term cytotoxicity studies with cancer in target tissues, suggests that the mode of action of chloroform carcinogenesis can not be concluded with the currently available data.

There is a qualitative correlation between short-term toxicological end points of cytotoxicity and the occurrence of neoplasia in the liver of $B6C3F_1$ mice exposed to chloroform either by corn oil gavage or in the drinking water. However, the association between cytotoxicity and cancer is lacking in other test systems. For example, short-term cytotoxicity (4 days) was observed in the kidneys of $B6C3F_1$ mice (Larson et al. 1994d) without an observable increase in neoplasia in male $B6C3F_1$ mice exposed to chloroform in corn oil by gavage for 78 weeks at similar doses (NCI 1976). These data indicate that increased cell proliferation may not always be sufficient to cause increased tumor incidence.

Some studies suggest that the carcinogenic response in male rat kidneys (Jorgensen and Rushbrook 1980; Jorgensen et al. 1985) may not be mediated by a mechanism involving necrosis and regenerative cell proliferation. This observation is supported by the fact that chloroform exposure, by either drinking water or corn oil gavage, induced kidney neoplasia in male Osborne-Mendel rats without any reported short-term and long-term cytotoxicity. There was no treatment-reIated biochemical and microscopic/gross histopathological changes in the kidneys of the rats at 30, 60, or 90 days after receiving chloroform in the drinking water (Jorgensen and Rushbrook 1980). Neither necrosis nor tubular cell hyperplasia was found in the kidneys of male Osborne-Mendel rats treated with chloroform by gavage or drinking water in the 2-year bioassays (Jorgensen et al. 198.5; NCI 1986).

In the liver bioassay for GGTase positive foci, chloroform had neither an initiating effect nor a promoting effect when administered in drinking water (Herren-Freund and Pereira 1987), but had a promoting effect of these loci initiated by diethylnitrosamine if given in a corn oil vehicle (Deml and Oesterle 1985); both studies were performed in rats. Moreover, chloroform enhanced the growth of experimentally inoculated tumors in mice (Cape1 et al. 1979). In contrast, chloroform had an inhibiting effect on the growth of tumors induced by known carcinogens (1,2-dimethylhydrazine and ethylnitrosurea) in rats (Daniel et al. 1989; Herren-Freund and Pereira 1987).

In epidemiologic studies, chloroform is not identified as the sole or primary cause of excess cancer rates, but it is one of many organic contaminants found in chlorinated drinking water, many of which are considered to have carcinogenic potential. These studies are often flawed by a lack of measured

chloroform concentrations in drinking water; lack of data concerning concentrations of other organics, limited information concerning personal drinking-water consumption, long latency periods, and effects of migration, making it difficult to quantify exposure. Although human data suggest a possible increased risk of cancer from exposure to chloroform in chlorinated drinking water, the data are too weak to draw a conclusion about the carcinogenic potential of chloroform in humans. Based on animal studies, chloroform has been classified as a probable human carcinogen by EPA (IRIS 1995), as a possible human carcinogen by IARC (1987), and as a substance that may reasonably be anticipated to be carcinogenic in humans (NTP 1989).

2.6 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to chloroform are discussed in Section 2.6.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health

impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are often not substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by chloroform are discussed in Section 2.6.2.

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 pre-existing disease that results in an increase in absorbed dose, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, Populations That Are Unusually Susceptible.

2.6.1 Biomarkers Used to Identify or Quantify Exposure to Chloroform

Chloroform concentrations measured in tissue and/or air samples can not be currently be used as specific biomarkers for chloroform exposure; however, they may indicate exposure to chloroform or other halogenated compounds that have undergone metabolism to chloroform. Methods for measuring chloroform in biological fluids, tissues, and exhaled breath are available; however, there is relatively little quantitative information relating monitored chloroform levels in tissues or fluids to exposure. The presence of chloroform or its metabolites in biological fluids and tissues may result from the metabolism of other chlorinated hydrocarbons; thus, elevated tissue levels of chloroform or its metabolites may reflect exposure to other compounds. 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). In another group of 18 workers with toxic hepatitis, blood samples revealed chloroform in some but not all workers, and workroom air contained 14.4-50.4 ppm on various days. 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.

Environmental exposure to chloroform in humans probably represents a combination of inhalation exposure (from the air polluted with volatile halogenated hydrocarbons) and oral exposure (from chlorinated water sources), in addition to a dermal absorption route for chloroform from contaminated water sources (from showering, bathing, or swimming). The chloroform levels detected in human blood varied according to geographical areas. Chloroform levels ranged from 13 to 49 μg/L in serum samples taken from 10 individuals in Florida (Peoples et al. 1979). The level of environmental exposure was not reported. The mean blood chloroform concentration was 1.5 μg/L in blood samples taken from 250 individuals in Louisiana; exposure levels were not reported (Antoine et al. 1986).

Chloroform was found in breath samples from large cohorts of people from New Jersey, North Carolina, and North Dakota (Wallace et al. 1987a). The levels of chloroform in breathing zone (personal) air were consistently higher than outdoor concentrations and correlated with chloroform concentrations in the exhaled breath samples. Some activities such as visiting the dry cleaners (an industry associated with high chloroform levels) or showering were associated with increased chloroform breath levels (Jo et al. 1990a, 1990b; Wallace et al. 1989). Chloroform was detected in 7 of 42 samples of human milk collected in 4 geographical areas in the United States (Pellizzari et al. 1982).

Tissue levels of chloroform obtained at autopsy reflected environmental exposure levels in other studies. The levels ranged from 20 to 49 μg/kg of chloroform from adipose tissue extracted into hexane from samples taken from 10 individuals in Florida (Peoples et al. 1979). In 30 autopsy cases in Germany, the adipose tissue contained a mean of 23.4 μg/kg wet tissue; 24.8 μg/kg perinephric fat; 10.8 μg/kg liver tissue; 9.9 μg/kg lung tissue; and 10 μg/kg muscle tissue (Alles et al. 1988). The maximum chloroform content increased with age and was not dependent on the volume of fat in the tissues.

No correlation has been made between the exact environmental levels of chloroform and the amount of chloroform in the exhaled breath or in the blood. Furthermore, chloroform also can be detected in the breath after exposure to carbon tetrachloride and other chlorinated hydrocarbons (Butler 1961). Therefore, chloroform levels cannot be used as reliable biomarkers of exposure to this chemical.

2.6.2 Biomarkers Used to Characterize Effects Caused by Chloroform

The primary targets of chloroform toxicity are the central nervous system, liver, and kidney. The signs and symptoms of central nervous system 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 sulfobromophthalein 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. No specific biomarkers used to characterize effects caused specifically by chloroform were located.

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

2.7 INTERACTIONS WITH OTHER CHEMICALS

The interactions of chloroform with other chemicals are an issue of great importance; as with many chemicals, exposure to chloroform alone seldom occurs. This is especially true when considering exposure to chlorinated water, which usually contains other trihalomethanes and may contain other potential toxicants.

Clinical reports of patients who underwent chloroform anesthesia indicated that premeditation with morphine caused serious respiratory depression when chloroform was co-administered. Thiopentone (thiopental Na, an ultra-short-acting barbiturate anesthetic) was associated with increased incidences of hypotension in chloroform-anesthetized patients (Whitaker and Jones 1965).

Several animal studies indicate that chloroform interacts with other chemicals within the organism. 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 carbon tetrachloride in rats (Harris et al. 1982) and by co-exposure to ethanol in mice (Kutob and Plaa 1962). Furthermore, ethanol pretreatment in rats enhanced chloroform-induced hepatotoxicity (Wang et al. 1994) and increased the in vitro metabolism of chloroform (Sato et al. 1981).

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.

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. The effects of monochloroacetate (MCA) on chloroform toxicity has also been investigated, with the combination (MCA + chloroform) shown to have toxic effects on the liver and kidneys of rats (Davis and Bemdt 1992). The effect of chloroform and other organic halides (i.e., dichlorobromomethane) on intrauterine growth retardation has also been explored (Kramer et al. 1992).

Ikatsu and Nakajima (1992) studied the effect of low-dose inhalation of chloroform with or without co-exposure to carbon tetrachloride on hepatotoxicity when rats were or were not previously exposed to ethanol. Groups of control or ethanol-pretreated rats inhaled 0, 50, or 100 ppm chloroform alone; 0, 25, or 50 ppm chloroform with 5 ppm carbon tetrachloride; or 0, 10, 25, or 50 ppm chloroform with 10 ppm carbon tetrachloride. Exposures to either 50 or 100 ppm of chloroform alone did not significantly change SGOT, SGPT, liver, or serum malondialdehyde (MDA) concentrations. In the rats pretreated with ethanol, SGOT and SGPT levels were significantly elevated above control animals at 100 ppm chloroform and SGOT levels were increased at 50 ppm chloroform as well. There was no

change in either serum or liver concentrations of MDA in either exposure group. Liver triglycerides and GSH levels were significantly elevated above those of control animals for both exposure levels for animals not pretreated with ethanol; however, overall liver weights were elevated at only 100 ppm chloroform. In rats pretreated with ethanol, there was no significant change in liver triglyceride concentrations at either dose; however, liver GSH and liver weights were significantly elevated above control at both exposure concentrations. In chloroform and carbon tetrachloride treated rats not pretreated with ethanol, elevations in SGOT (10 ppm $CC1_4$ plus 10 and 50 ppm $CHC1_3$), SGPT (5 ppm $CC1_4$ plus 50 ppm $CHC1_3$, 10 ppm $CC1_4$ plus 25 ppm $CHC1_3$, and plasma MDA (10 ppm $CC1_4$ plus 10, 25, and 50 ppm $CHC1_3$ were observed. In chloroform and carbon tetrachloride-treated rats pretreated with ethanol, elevations in SGOT (all doses), SGPT (all doses except 5 ppm $CC1_4$ + 25 ppm $CHCl_3$), liver MDA (all doses), and plasma MDA (5 and 10 ppm $CC1_4$ + 50 ppm $CHC1_3$, 10 ppm $CC1_4$ + 25 ppm $CHC1_3$,) were observed. In chloroform and carbon tetrachloride treated rats not pretreated with ethanol, elevations in liver triglyceride (all doses) and GSH (5 ppm $CC1_4$ + 50 ppm $CHC1_3$, 10 ppm $CC1_4$ + 10 and 50 ppm $CHC1_3$) were observed. In chloroform and carbon tetrachloride treated rats pretreated with ethanol, elevations in liver triglyceride (all chloroform doses at 10 ppm CCl_4) and GSH (all doses except 5 ppm $CC1_4$ + 25 ppm $CHC1_3$ were observed. The results suggest that chloroform enhances carbon tetrachloride-induced hepatotoxicity.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to chloroform than will most persons exposed to the same level of chloroform in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters may result in reduced detoxification or excretion of chloroform, or compromised function of target organs affected by chloroform. Populations who are at greater risk due to their unusually high exposure to chloroform are discussed in Section 5.6, Populations With Potentially High Exposure.

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 abuse alcohol (Wang et al. 1994; Kutob and Plaa 1962). Also, exhaustion and starvation may potentiate chloroform hepatotoxicity, as indicated in some human clinical reports (Royston 1924; Townsend 1939) and in animal studies (Ekstrom et al. 1988; McMartin

et al. 1981). Animal studies indicate that male mice and rats may be more susceptible to the lethal and renal effects of chloroform than female mice and rats (Deringer et al. 19.53; Torkelson et al. 1976). The greater susceptibility of adult male animals is associated with testosterone levels in the animals (Deringer et al. 1953). Evidence also exists for age-related effects; young male mice were less susceptible to the lethal effects of chloroform compared to adult males (Deringer et al. 1953). Kimura et al. (1971) noted similar differences between young and old adult rats, but also found that chloroform was significantly more toxic in 14-day-old rats than in adult rats. Whether or not these subpopulations in humans would be more susceptible than their respective counterparts is not presently known.

2.9. METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to chloroform. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to chloroform. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to chloroform:

Ellenhom, MJ and Barceloux, DG, (eds.) (1988). Medical Toxicology: Diagnosis and Treatment of Human Poisoning. Elsevier Publishing, New York, NY., pp. 972-974.

Dreisback, RH, (ed.) (1987). Handbook of Poisoning. Appleton and Lange, Norwalk, CT.

Haddad, LM and Winchester, JF, teds.) (1990). Clinical Management of Poisoning and Drug Overdose. 2nd edition, WB Saunders, Philadelphia, PA.

Aaron, CK and Howland, MA (eds.) (1994). Goldfrank's Toxicologic Emergencies. Appleton and Lange, Norwalk, CT.

2.9.1 Reducing Peak Absorption Following Exposure

Human exposure to chloroform may occur by inhalation, ingestion, or by dermal contact. General recommendations for reducing absorption of chloroform include removing the exposed individual from the contaminated area and removing the contaminated clothing. If the eyes and skin were exposed, they should be flushed with water. In order to reduce absorption of ingested chloroform, emesis may

be considered unless the patient is comatose, is convulsing, or has lost the gag reflex. Controversy exists concerning use of emesis because of the rapid onset of central nervous system depression, the risk of aspiration of stomach contents into the lungs, and the relative ineffectiveness of this method. In comatose patients with absent gag reflexes, an endotracheal intubation may be performed in advance to reduce the risk of aspiration pneumonia. Gastric lavage may also be used.

2.9.2 Reducing Body Burden

Chloroform is not stored to any appreciable extent in the human body and is mostly metabolized to phosgene and eventually CO_2 (see Section 2.3); however, some chloroform may be stored in fat depots in the body. The half-life of chloroform in humans has been calculated to be 7.9 hours following inhalation exposure (Gordon et al. 1988). Furthermore, an oral-exposure study found most of the chloroform dose being eliminated within 8 hours postexposure (Fry et al. 1972). Hepatic and pulmonary first-pass effect was reported in humans (Chiou 1975).

Despite a relatively fast clearance of chloroform from the body, toxic effects may develop in exposed individuals. No method is commonly practiced to enhance the elimination of the absorbed dose of chloroform. Although there is evidence that ethanol pretreatment of rats can increase the in vitro metabolism of chloroform (Sato et al. 1981), such treatment would not be recommended (Kutob and Plaa 1962) because it would increase the toxicity of chloroform and it is a very poor practice generally.

2.9.3 Interfering with the Mechanism of Action for Toxic Effects

Target organs of chloroform toxicity are the central nervous system, liver, and kidneys (see Section 2.2). Respiratory, cardiovascular, and gastrointestinal toxic effects have also been reported. Studies in animals also indicated that chloroform exposure may induce reproductive and developmental effects and cause cancer. Several studies investigated the possible mechanism for chloroform-induced toxicity (see Section 2.5). Proposed mechanisms of chloroform toxicity and potential mitigations based on these mechanisms are discussed below. The potential mitigation techniques mentioned are all experimental.

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One of the possible mechanisms of chloroform toxicity is thought to be linked to its high lipid solubility and its ability to bind covalently to lipids (Testai et al. 1987). For example, neurotoxic and respiratory effects of chloroform may be due to the interaction of chloroform with gangliosides in neuronal membranes (Harris and Groh 1985) and phospholipids in the surfactant monolayer of the lower respiratory tract (Enhorning et al. 1986), respectively. Another proposed reaction of chloroform and lipids would result in the formation of conjugated dienes which are indicative of lipid peroxidation (De Groot and Noll 1989). Some authors reported that conjugated dienes may play a key role in the hepatotoxicity induced by haloalkanes (Comporti 1985; Recknagel et al. 1982). Others, however, argue that lipid peroxidation alone is not responsible for all changes found in the liver following chloroform exposure (Brown et al. 1974b; Lavigne and Marchand 1974). Instead, it was proposed that the mechanism of chloroform-induced liver and kidney toxicity involved metabolism to the reactive intermediate, phosgene, which binds to proteins of the endoplasmatic reticulum (Pohl et al. 1980a, 1980b). While this is true in the rat, it has not been established in other species, including humans.

The toxicity of chloroform is increased by inducers of cytochrome P-450 such as phenobarbital (Scholler 1970). The involvement of cytochrome P-450 is further supported by the finding that disulfiram (Scholler 1970) and methoxsalen (Letteron et al. 1987), both inhibitors of microsomal enzymes, decreased the liver injury caused by chloroform in rats and mice, respectively. In addition, pretreatment with diethyldithiocarbamate and carbon disulfide protected mice against chloroform hepatotoxicity as indicated by biochemical and histopathological results (Gopinath and Ford 1975; Masuda and Nakayama 1982, 1983). Similarly, pretreatment of mice with methoxsalen (Letteron et al. 1987) and piperonyl butoxide (Kluwe and Hook 1981) reduced the chloroform-induced nephrotoxicity. Further research to determine which isozymes of P-450 are involved in metabolism to the more harmful metabolite, phosgene, as well as which isozymes are involved in enhancing the elimination of chloroform, could lead to the development of strategies designed to selectively inhibit specific P-450 isozymes, and thus reduce the toxic effects of chloroform.

Administration of chloroform to laboratory animals resulted in the depletion of renal GSH, indicating that GSH reacts with reactive intermediates, thus reducing the kidney damage otherwise caused by the reaction of these intermediates with tissue MMBs (Hook and Smith 1985; Smith and Hook 1983, 1984; Smith et al. 1984). Similarly, chloroform treatment resulted in the depletion of hepatic GSH and alkylation of MMBs (Docks and Krishna 1976). Other studies demonstrated that sulfhydryl compounds such as L-cysteine (Bailie et al. 1984) and reduced GSH (Kluwe and Hook 1981) may

provide protection against nephrotoxicity induced by chloroform. The sulfhydryl compound N-acetylcysteine is an effective antidote for poisoning by acetaminophen, which, like chloroform, depletes GSH and produces toxicity by reactive intermediates.

All mitigations of the chloroform-induced toxicity cited above are experimental. Further studies would be needed for implications of any of these methods to humans.

2.10 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA 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 the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of chloroform.

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

2.10.1 Existing Information on Health Effects of Chloroform

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to chloroform are summarized in Figure 2-6. The purpose of this figure is to illustrate the existing information concerning the health effects of chloroform. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR

defines a data gap more broadly as any substance-specific information missing from the scientific literature.

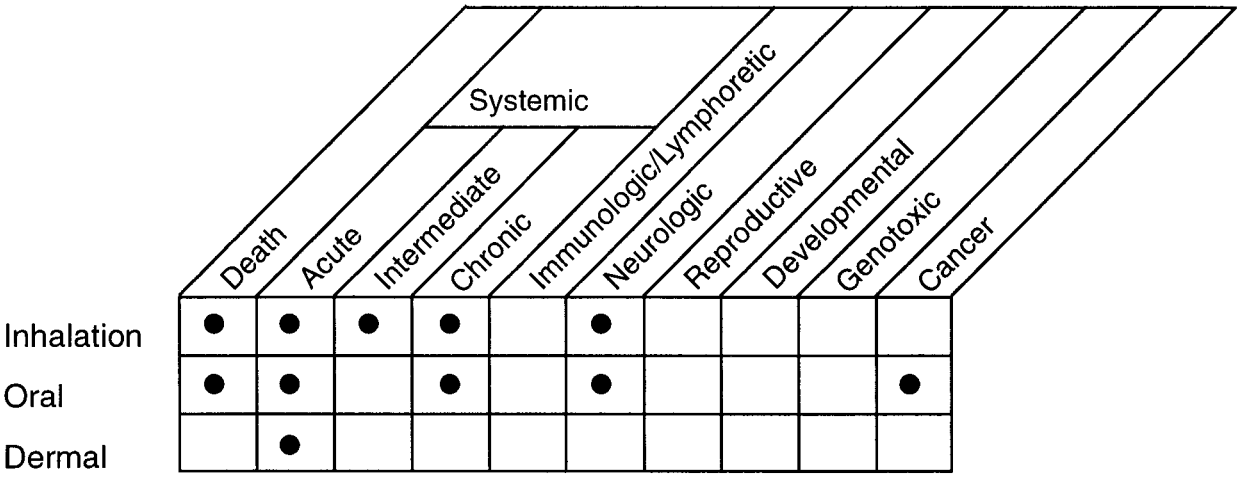
As seen from Figure 2-6, information is available regarding death, systemic effects, and neurological effects in humans after inhalation and oral exposure to chloroform. In addition, information is available regarding carcinogenic effects in humans after oral exposure to chlorinated drinking water. Limited information is available regarding dermal effects in humans after exposure to chloroform.

Inhalation and oral studies in animals provide data on death, systemic effects after acute- and intermediate-duration exposure, immunological effects, neurological effects, developmental effects, reproductive effects, and genotoxic effects. Information is available regarding systemic effects and carcinogenic effects in animals after oral exposure to chloroform. The carcinogenic effects after oral exposure is inconsistent and not totally conclusive. In addition, data regarding death and acute systemic effects in animals after dermal exposure to chloroform were located in the available literature.

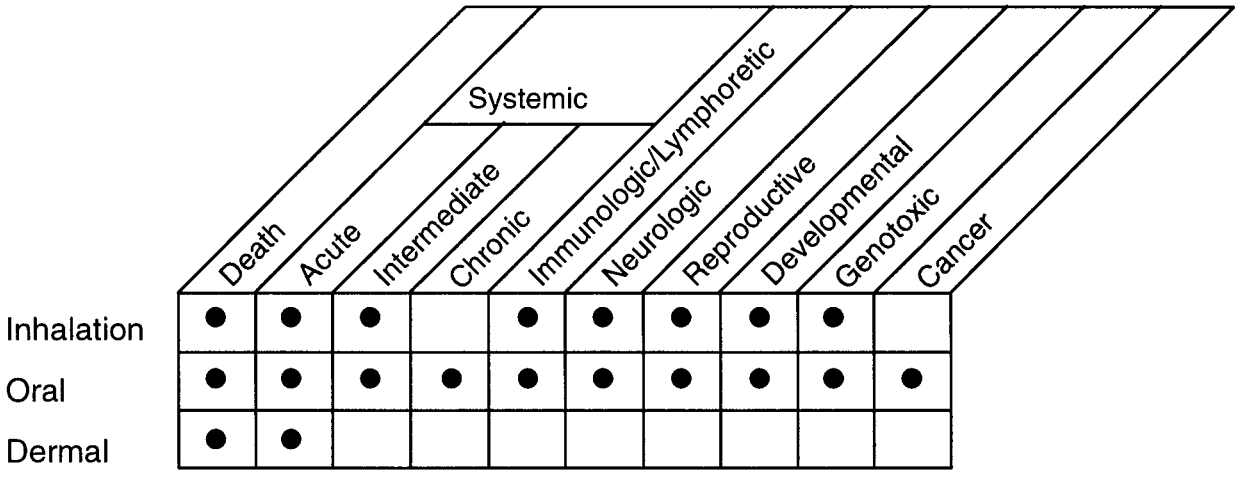
2.10.2 Identification of Data Needs

Acute-Duration Exposure. Clinical reports indicate that the central nervous system, cardiovascular system, stomach, liver, and kidneys in humans are target organs of chloroform toxicity after inhalation and oral exposure to chloroform (Schroeder 1965; Smith et al. 1973; Whitaker and Jones 1965). These findings are supported by results obtained from acute inhalation and oral-exposure studies in animals in which target organs identical to those observed in human studies (central nervous

Figure 2-6. Existing Information on Health Effects of Chloroform



Human



Animal

● Existing Studies

system, liver, and kidney) were identified (Culliford and Hewitt 1957; Jones et al. 1958; Lehmann and Flury 1943; Lundberg et al. 1986; Moore et al. 1982). The data are sufficient to derive an MRL for acute oral exposure. An acute inhalation MRL was derived based on a NOAEL for hepatic effects in mice (Larson et al. 1994c). Lethality studies were conducted in rats and mice after acute inhalation exposure (Deringer et al. 1953; Gehring 1968; Lundberg et al. 1986; Smyth et al. 1962). Similarly, lethal doses were identified after single oral exposure in rats and mice (Bowman et al. 1978; Chu et al. 1982b; Jones et al. 19.58; Kimura et al. 1971; Smyth et al. 1962). Information regarding dermal effects in humans and animals after exposure to chloroform is limited. Degenerative changes in the kidney tubules of rabbits were reported in one dermal study (Torkelson et al. 1976). Toxicokinetic data regarding dermal exposure are very limited; however, there is evidence that chloroform can be absorbed through the skin (Tsuruta 1975). Due to its lipophilic quality after dermal exposure, chloroform is likely to be distributed in the organism in patterns similar to those for inhalation and oral exposure. Information regarding acute dermal exposure in rodents would be useful to identify target organs and threshold levels of chloroform toxicity. Several *in vitro* skin models are available that would be adequate for describing the absorption of chloroform through the skin and the effects that differing concentrations of chloroform would have on skin histology.

Intermediate-Duration Exposure. An occupational study suggests that the liver is a target organ of chloroform toxicity after inhalation exposure of intermediate duration (Phoon et al. 1983). No data were located regarding intermediate-duration oral and dermal exposure in humans. Several studies were located regarding chloroform toxicity in animals after oral exposure (including 3 90-day studies in rats, 3 90-day studies in mice, and a ≥6-week oral study in dogs) (Bull et al. 1986; Chu et al. 1982a, 1982b; Heywood et al. 1979; Jorgenson and Rushbrook 1980; Klaunig et al. 1986; Munson et al. 1982; Palmer et al. 1979); fewer data were located regarding inhalation exposure (Torkelson et al. 1976), and no data were located regarding dermal exposure. In animals, the target organs for chloroform toxicity were identified as the central nervous system, liver, and kidneys.

An intermediate-duration oral MRL was derived based on liver effects in dogs (Heywood et al. 1979). An intermediate-duration inhalation MRL was derived based on toxic hepatitis which occurred in humans (Phoon et al. 1983). Pharmacokinetic data regarding dermal exposure to chloroform are limited, but it is known that chloroform can be absorbed through the skin. Intermediate-duration dermal studies in animals would provide information about chloroform toxicity via this exposure route.

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The information would be useful for populations living at or near hazardous waste sites, who may be exposed to chloroform for intermediate durations.

Chronic-Duration Exposure and Cancer. Information regarding chronic-exposure inhalation exposure to chloroform in humans is limited to occupational studies (Bomski et al. 1967; Challen et al. 1958). The liver and central nervous system are target organs of chloroform toxicity. Regarding chronic-duration oral exposure in humans, limited information is available from a case study reporting hematological, hepatic, and renal effects in an individual who used a cough medicine containing chloroform for 10 years (Wallace 1950) and from a follow-up study of individuals who used a mouthwash containing chloroform for 15 years (De Salva et al. 1975). Animal data indicate that the central nervous system, liver, and kidneys are target organs of chloroform toxicity after chronic oral exposure (Heywood et al. 1979; Jorgenson et al. 1985; NCI 1976; Roe et al. 1979; Tumasonis et al. 1985, 1987). The data are sufficient to derive a chronic oral MRL. No studies were located regarding chloroform toxicity in humans and animals after dermal exposure to chloroform and in animals after inhalation exposure to chloroform. Considering the similar pattern of chloroform toxicity after inhalation and oral exposures for acute and intermediate durations, similar target organs in animals after chronic inhalation exposure to chloroform may be predicted. Nonetheless, studies designed to assess the chronic toxicity of chloroform in animals after inhalation and dermal exposure would be useful to establish dose-response relationships. This information is important to humans occupationally exposed or exposed to contaminated air, water, or soil at or near hazardous waste sites.

Epidemiology studies suggest a possible association between chloroform in drinking water and cancer risk. Increased incidences of colon and bladder cancer were identified in separate populations exposed to chlorinated water. However, as mentioned before, other toxic compounds have also been identified in chlorinated drinking water, making the role of chloroform in cancer induction questionable. Studies in rats and mice indicate that oral exposure to chloroform causes cancer (Jorgenson et al. 1985; NCI 1976; Roe et al. 1979; Tumasonis et al. 1985, 1987); however, some of these studies utilized gavage dosing instead of drinking water. No data were located regarding carcinogenicity in humans and animals following inhalation and dermal exposure to chloroform. Nonetheless, pharmacokinetic data indicate similar toxicokinetics of chloroform after inhalation and oral exposure; therefore, similar targets for carcinogenic effects may be predicted. Data were located suggesting different effects of chloroform depending on the vehicle and method of oral administration. Chloroform in corn oil administered by gavage caused an increased incidence of liver tumors (NCI 1976) while

administration of the same dose in drinking water did not (Jorgenson et al. 1985). It was demonstrated, however, that chloroform uptake is much slower from the oil vehicle (Withey et al. 1983). Therefore, the higher cancer incidence cannot be explained merely by the levels of chloroform in tissues. Furthermore, chloroform acted as a promoter rather than an initiator of preneoplastic foci in a rat liver bioassay (Deml and Oesterle 1985). In contrast, some studies indicate that chloroform inhibits the growth of tumors induced by known carcinogens (Daniel et al. 1989; Herren-Freund and Pereira 1987). Animal studies also suggest an epigenetic mechanism for the carcinogenicity of chloroform. Because of these differences, further studies on the possible mechanism of chloroform carcinogenicity would be useful.

Genotoxicity. Chloroform has been tested for genotoxicity in several *in vitro* and *in vivo* experiments. Its potency to induce mutations seems to be weak. No induction of reverse mutations was observed in prokaryotic systems (Gocke et al. 1981; Kirkland et al. 1981; San Augustin and Lim-Sylianco 1978; Simmon et al. 1977; Uehleke et al. 1977; Van Abbe et al. 1982; Vat-ma et al. 1988). Mixed results were obtained in the induction of mutations in human lymphocytes and Chinese hamster cells *in vitro* (Kirkland et al. 1981; Mitchell et al. 1988; Peroccio and Prodi 1981; White et al. 1979). Nonetheless, an increase in sperm anomalies and sister chromatid exchanges in the bone marrow of rodents was observed after *in vivo* exposure (Land et al. 1979, 1981; Morimoto and Koizumi 1983). Cytogenetic analysis of peripheral lymphocytes from exposed individuals would provide useful information about the ability of chloroform to induce mutations in-humans if a suitable population can be identified.

Reproductive Toxicity. No information was located regarding reproductive effects in humans exposed to chloroform via any route or in animals exposed by the dermal route. Increased resorptions were observed in rats and mice after inhalation exposure to chloroform during gestation (Murray et al. 1979; Schwetz et al. 1974) and in rats and rabbits after oral exposure (Thompson et al. 1974). In addition to effects in dams, abnormal sperm were found in mice after inhalation exposure (Land et al. 1979, 1981). Furthermore, exposure-related gonadal atrophy was observed in both sexes of rats following oral exposure to chloroform (Palmer et al. 1979). The results suggest that reproductive organs are a target of chloroform toxicity in animals; however, some inhalation and oral studies in animals do not report any effects. More studies assessing the reproductive function in animals would be useful for the purpose of extrapolating the data to human exposure.

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Developmental Toxicity. Only one study was located regarding developmental effects in humans exposed to chloroform via an oral route. Animal data indicate that chloroform can cross the placenta. Fetotoxicity effects (decreased birth weight, decreased fetal crown-rump length, increased resorptions) and teratogenicity (acaudate fetuses with imperforate anus, cleft palates) were observed in rats and mice after inhalation exposure to chloroform (Murray et al. 1979; Schwetz et al. 1974). Oral exposure to chloroform-induced fetotoxicity in rats and rabbits (Ruddick et al. 1983; Thompson et al. 1974). Degeneration of the epididymal ductal epithelium (not affecting the fertility) was observed in mice in the F_1 generation in a 2-generation oral reproductive study (Gulati et al. 1988). No information is available regarding the developmental toxicity of chloroform after dermal exposure. More data regarding developmental toxicity both in humans and in experimental animals (especially after oral and dermal exposure) would be useful to identify the possible risk for humans.

Immunotoxicity. No data were located regarding immunological effects in humans after inhalation, oral, or dermal exposure to chloroform. The data obtained from animal studies are limited to one inhalation study in mice and three oral studies in rats and mice (Aranyi et al. 1986; Chu et al. 1982b; Munson et al. 1982). Depressed humoral and cell-mediated immunity were detected; however, the chloroform-induced changes were more serious in the acute exposure study than in the intermediateduration study, indicating that the changes may be transient. Studies regarding skin sensitization with chloroform were not performed. A battery of immune function tests has not been performed in humans or in animals, but would provide helpful information to support or refute the limited evidence for chloroform immunotoxicity.

Neurotoxicity. The central nervous system 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). Lower chloroform doses produced neurological effects during occupational exposure (Challen et al. 1958). Similarly, neurotoxicity is reported in animal studies involving inhalation and oral exposure to chloroform (Bowman et al. 1978; Jones et al. 1958; Lehmann and Flury 1943). A battery of neurobehavioral tests was conducted in mice after oral exposure to chloroform (Balster and Borzelleca 1982). No data were located regarding chloroform neurotoxicity in humans or animals after dermal exposure to chloroform. Animal studies involving dermal exposure to chloroform would be useful for risk assessment of occupational exposure. Continued research on the

toxicity of inhaled and dermally absorbed chloroform in humans when exposed to water sources containing elevated concentrations of chloroform during showering would also be useful. More information regarding the mechanism of chloroform-induced neurotoxicity and structural alterations produced in the central nervous system would be helpful.

Epidemiological 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 central nervous system and liver effects in exposed workers (Bomski et al. 1967; Challen et al. 1958; Phoon et al. 1983). 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 colon, rectal, and bladder cancer in humans (Alavanja et al. 1978; Cantor et al. 1978; Young et al. 1981). All of these studies were limited by a lack of attention to important details (e.g., migration, exposure to other carcinogens). Better designed and better conducted epidemiology studies of occupational exposure would be helpful. The information can be useful to populations living near hazardous waste sites where chloroform is present. In addition, further refining of the PBPK/PD models would further advance our understanding of chloroform tissue dosimetry in humans and animals.

Biomarkers of Exposure and Effect.

Exposure. Methods for detecting chloroform in exhaled breath, blood, 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 to better quantitate chloroform exposure would enhance the database.

Effect. 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 central nervous system, the 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 (Smith et al. 1973). The data also indicate that absorption after oral exposure is fairly complete for both animals and humans (Brown et al. 1974a; Fry et al. 1972; Taylor et al. 1974). 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 l-2 hours). Additional animal studies investigating the rate of dermal absorption would be useful to quantitate dermal absorption and to compare information from oral and inhalation studies.

Data are available regarding the distribution of chloroform in animals after inhalation and oral exposure to chloroform (Brown et al. 1974a; Chenoweth et al. 1962; Cohen and Hood 1969; Corley et al. 1990; Danielsson et al. 1986; Taylor et al. 1974); however, data regarding the distribution of chloroform in humans is very limited (Feingold and Holaday 1977) and warrants further investigation. It appears that distribution following oral exposure is similar to that following inhalation exposure. 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. It appears that both the mode of oral administration and the vehicle affect metabolism. Additional data investigating the mode and vehicle of administration would be useful in order to understand the role of these factors in the mechanism of chloroform's toxicity. The co-administration of other compounds (e.g., ethanol) has been shown to alter chloroform metabolism and toxicity. Further investigations of the hazards associated with exposure to complex mixtures containing chloroform would be useful.

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). The major route of chloroform elimination is pulmonary, but minor pathways are through enterohepatic circulation, urine, and feces as parent compound or metabolites. There are no human or animal data regarding excretion of dermally applied chloroform.

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Comparative Toxicokinetics. Target organs for chloroform distribution appear to be similar in humans and animals, according to inhalation studies (Corley et al. 1990; Feingold and Holaday 1977). 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). Marked sex-related differences in tissue distribution and covalent binding to tissue MMBs 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 eliminated 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 a large number of oral studies, relatively few inhalation studies, and almost no 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.

Methods for Reducing Toxic Effects. Protective clothing and protective breathing devices may be used to prevent exposure to large amounts of chloroform, although for everyday low exposures to chloroform, these methods are obviously impractical. General procedures such as flushing the skin with water following dermal exposure and emesis or gastric lavage following oral exposure may be used to reduce absorption of chloroform. However, specific medical treatments that prevent absorption of chloroform have not been identified. Such mechanisms might be beneficial because they might be more effective than general procedures and might involve less risk than procedures such as emesis. Ways to enhance elimination of chloroform from the body are not known. Although chloroform is eliminated fairly rapidly, methods to accelerate elimination without producing toxic metabolites would be helpful in reducing toxicity. The mechanism by which chloroform produces toxicity appears to involve metabolism by phenobarbital-inducible isozymes of cytochrome P-450 to phosgene (Pohl et al. 1980a, 1980b). Development of methods to selectively inhibit the P-450 isozymes responsible for this reaction might reduce chloroform toxicity. There is also evidence that GSH conjugates with reactive products of chloroform metabolism, providing protection from damaging effects (Docks and Krishna 1976; Hook and Smith 1985). Development of a method to maintain high tissue GSH levels following exposure to chloroform might have a mitigating effect on toxicity. Therefore, although no treatments are currently available to block the toxic action of chloroform or repair damage caused by this chemical, there are indications that further research in this area would enable identification of such treatments.

2.10.3 Ongoing Studies

A few ongoing studies involving chloroform have been identified. The effects of volatile anesthetics on the N-methyl-D-aspartate (NMDA) receptor-channel complex are being studied. Specific aims are to determine the effects of several volatile anesthetics (halothane, enflurane, isoflurane, diethyl ether, cyclopropane, nitrous oxide, and chloroform) on ligand binding to glutamate binding sites on the NMDA receptor complex; to study ligand binding to the glycine modulatory site, glutamate, glycine, divalent cation, and spermidine activation of NMDA receptor ion channels; and to examine NMDAreceptor mediated changes in calcium content of rat brain microvesicles (Aronstam 1994). Mechanistic work on the hepatotoxicity and toxigenic sequence will be studied *in vitro* with suspensions of hepatocytes exposed to carbon tetrachloride and other agents known to alter calcium homeostasis and stimulate phospholipase A_2 (bromotrichloromethane and chloroform) (Glende 1994). Mechanisms of toxic chemical interactions in the liver resulting in hepatotoxicity will utilize the hepatotoxic effects of chloroform to explore interactions among metals and organics in the induction of stress response proteins such as metallothionein, heme oxygenase, and nitric oxide synthase, and the induction of inflammatory cytokines (i.e., tumor necrosis factor-alpha, interleukin-1, and interleukin-6). Work by Swenburg will determine if exposure to environmental carcinogens, including 1,1,2-trichloroethylene, 1,1,2,2-tetrachloroethylene, 1,1,2-trichloroethane, chloroform, and carbon tetrachloride, induces or modulates the formation and/or repair of cyclic DNA adducts. Work by Benjamin (1995) will also continue to study the phenomenon of hepatic cell proliferation, and the effect that this response has on the initiation and promotion of cancer. Other research will investigate the toxicity and bioaccumulation of a mixture of sediment contaminants (trichlorethylene, lead, benzene, chloroform, phenol, chromium, and arsenic) in several species of invertebrates and fish. Uptake and depuration will be measured in chironomids *(Chironomus riparius),* and pharmacokinetic models will be developed to describe bioaccumulation of these sediment contaminants (Clements 1994). Studies by Yang (1994) will continue to evaluate age- and dosing-related changes in pharmacokinetics, biochemical markers, liver cell proliferation, and histopathology in male Fischer 344 rats chronically exposed (up to 2 years) to low levels of a chemical mixture of 7 organic and inorganic groundwater pollutants (including arsenic, benzene, chloroform, chromium, lead, phenol, trichloroethylene). Also, this research will further explore the pharmacokinetic modeling of chemical mixtures and incorporate time-course information on biochemical markers, cell proliferation and histopathology into pharmacokinetic and pharmacodynamic modeling. A study by the Japan Industrial Safety and Health Association (Japan Bioassay Lab) that explores the toxicity of chloroform inhaled over a 2-year period

(6 hours a day, 5 days a week) in male and female Fischer 344 rats and BDF_1 mice is reportedly close to completion (Matsushima 1994). Efforts are also being made to develop a high-efficiency activated carbon granule for drinking-water treatment that can remove water contaminants, including chloroform (Mieville 1992).