3. HEALTH EFFECTS

3.1 INTRODUCTION

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

3.2 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 (LOAELs) 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.

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.

This toxicological profile discusses health effects that result from exposure to chlorine gas. However, ATSDR feels there is a need to inform the public about the role of chlorine in the process of disinfection of water, namely, the production of hypochlorous acid and hypochlorite ion when chlorine is introduced in water. As a way to inform the public, ATSDR has decided to include some information regarding the health effects of hypochlorite solutions in the Profile for chlorine. For chlorine gas, the most important route of exposure is inhalation. For hypochlorous acid and sodium hypochlorite, the most important routes of exposure are oral and dermal.

3.2.1 Inhalation Exposure

3.2.1.1 Death

There is extensive information regarding the lethal effects of exposure to high concentrations of chlorine. Much of the information available is derived from the use of chlorine gas as a chemical weapon at the battle of Ypres, Belgium, during World War I. Approximately 150 tons of chlorine released from 6,000 cylinders killed, by some accounts, 800 soldiers and incapacitated 2,500–3,000 (Joy 1997). In a review of the effects of warfare gases, the U.S. Department of Army (DOA 1933) stated that of the total 70,752 casualties from gas poisoning in the American Expeditionary Forces, 1,843 were gassed with chlorine. A study of 838 of these subjects revealed that 4 deaths were due to later-developing effects of chlorine gassing. The causes of death were broncho-pneumonia, lobar pneumonia, purulent pleurisy, and tuberculous meningitis. In an evaluation of the after effects of chlorine gassing in 700 members of the First Canadian Division, Meakins and Priestley (1919) reported that five deaths had occurred, three

apparently due to an acute pneumonic condition. One death occurred 5 months after exposure to chlorine and, although the exact cause of death was unknown, the symptoms recorded were pain in the left chest, dyspnea, orthopnea, and pronounced cyanosis. The concentration of chlorine to which the soldiers were exposed during the gas attacks is unknown.

DOA (1933) summarized the pathology of chlorine exposure leading to death in 24 hours as follows: an acute inflammation of the trachea and bronchi is followed by congestion and edema of the entire respiratory tract. The edema and consolidation of the lungs lead to acute dilation followed by passive congestion of the abdominal viscera. Acute death is the result of the pulmonary edema and respiratory and cardiovascular failure. Subacute death is generally due to pulmonary infection with resulting bronchitis and pneumonia (DOA 1933). Postmortem findings include: acute conjunctivitis, congestion of abdominal organs (especially the liver), increased lung volume, fluid in the pleural cavity, mottled appearance on lung surface with scattered areas of emphysema, pleural hemorrhage, perivascular edema, and dilation of blood vessels, frothy fluid filling the trachea and bronchi, red mucous membranes, and heart enlargement and dilated heart chambers (especially the right side) (DOA 1933).

There are also reports of deaths due to spills of chlorine gas to the environment following railroad accidents. CDC (2005) reported that in January 2005, a train in South Carolina carrying chlorine tanker cars collided with another train and an estimated 11,500 gallons of chlorine gas were immediately released to the air causing the death of nine persons. The primary cause of death of those who died at the scene was asphyxia (Van Sickle et al. 2009). Reported findings included congested lungs, pulmonary edema, and tracheal and bronchial erythema. CDC (2005) also reported that a similar accident on June 2004 in Texas released approximately 90,000 pounds of chlorine gas resulting in two fatalities among residents near the site. Eight people died as a direct result of lung injury caused by exposure to chlorine following a freight train accident in Florida in February 1978 (Jones et al. 1986). A train accident in San Luis Potosí, Mexico, in August 1981 caused the release of an unspecified amount of chlorine gas, which caused the death of 14 people (Costero and Falcón Escobedo 1983). An 11-month-old infant died after liquid chlorine spilled as a result of a derailment in La Barre, Louisiana, in January 1961 (Joyner and Durel 1962). The child died hours after exposure and the cause of death presumably was massive pulmonary edema. Joyner and Durel (1962) indicate that 7 hours after the accident, levels of 400 ppm chlorine were measured in areas 75 yards from the wreck. Citing an unpublished report, Baxter et al. (1989) stated that the rupture of a chlorine storage vessel in Romania in 1939 caused the death of 68 people.

Dixon and Drew (1968) reported the case of a worker at a factory producing chlorine who was exposed to chlorine gas that leaked from a faulty valve for about 30 minutes and died 3–3.5 hours later; postmortem examination showed that pulmonary edema was the prime cause of death. Adelson and Kaufman (1971) reported the death of two healthy adults 25 and 76 hours after being exposed to chlorine gas that leaked into their home from a nearby water filtration plant while they were sleeping. An autopsy of the earlier death showed congested and edematous lungs whose cut surfaces released substantial amounts of water and frothy liquid on pressure, and injected tracheobronchial mucosa. Postmortem examination of the later death showed a similar picture of the lungs with the additional findings of swollen brain with flattening of convolutions and subarachnoid hemorrhage. Suzuki et al. (2001) reported the case of a worker who inhaled concentrated chlorine gas and died from pulmonary thrombosis 6 days after exposure.

Using information on lethal effects of chlorine in animals and humans from the literature, Withers and Lees (1985a) developed probit equations to derive a revised estimate of the lethal toxicity of chlorine that considers physical activity, particularly inhalation rate, effectiveness of medical treatment, and the form of the lethal toxic load function. The LC_{50} values for a 10-minute exposure with standard level of activity were estimated at 433, 173, and 364 ppm for the regular, vulnerable, and average population, respectively. For a 30-minute exposure, the corresponding LC_{50} values were estimated at 250, 100, and 210 ppm, respectively. Prater (1990) indicates that concentrations of 400 ppm can be lethal to humans in 30 minutes and that immediate death follows inhalation of a concentration of 1,000 ppm. Concentrations between 1,000 and 1,200 ppm for 30 minutes are lethal to humans according to a review of earlier data from various American authors by Withers and Lees (1985b).

Even more abundant information exists regarding lethal effects of chlorine in animals. For data regarding work beginning with World War I, the reader is referred to a review by Withers and Lees (1985b). Work conducted at the U.S. Army's Medical Research Laboratory of the Chemical Warfare Service cited by DOA (1933) indicates that acute exposures to concentrations >870 ppm were usually lethal to dogs, whereas concentrations below 656 ppm were rarely fatal. Additional studies also in dogs, also summarized by DOA (1933), indicate that dogs that died within 24 hours of gassing showed severe injury to the mucous membranes of the upper respiratory tract, congestion, and edema of the entire respiratory tract including the peribronchial tissues and the sheaths of the large blood vessels. There was also acute inflammatory reaction of the lungs that developed into pneumonia. In dogs dying 2–5 days after gassing, the most important feature was the inflammatory process, whereas in dogs dying 5–14 days after gassing death was due to pulmonary infection. Since the original sources were not available, these studies in dogs are not listed in Table 3-1.

Weedon et al. (1940) conducted lethality studies in unspecified strains of rats and mice. In a group of eight rats exposed to 1,000 ppm chlorine, the first death occurred in 20 minutes and all were dead in 1.7 hours. The exposure level of 1,000 ppm was the LC_{50} in 53 minutes, and 250 ppm was the LC_{50} in 440 minutes. Rats that died immediately after exposure showed slight brain congestion; lungs distended and hemorrhagic with cut surfaces wet and foamy; distended heart; liver congestion; distended and hemorrhagic stomach; distended intestines; and congested kidneys. In mice, the first death occurred in 21 minutes, and all eight were dead in 50 minutes. The 1,000 ppm exposure level was a 28-minute LC_{50}, whereas 250 ppm was a 440-minute LC_{50}. Mice that died immediately after exposure had slight brain congestion; lungs partly collapsed and hemorrhagic; moderately distended heart; liver congestion; distended and hemorrhagic stomach; slightly distended intestines, and congested kidneys.

In lethality studies conducted by Zwart and Woutersen (1988), 5,484 ppm was a 5-minute LC_{50} in Wistar rats, whereas 447 ppm was a 60-minute LC_{50}; in Swiss-Webster mice, 1,032 ppm was a 10-minute LC_{50} and 516 ppm was a 30-minute LC_{50}.

In a different study in mice, a 10-minute LC_{50} of 302 ppm was calculated in intact mice and 131 ppm in mice that were exposed via a cannula placed in the trachea, suggesting that the nose is an effective scrubber of chlorine (Alarie 1981). In a study in mechanically-ventilated pigs, exposure to 140 ppm chlorine for 10 minutes caused the death of four out of five pigs within 6 hours of exposure (Gunnarsson et al. 1998). Death was caused by cardiovascular failure triggered by a severe mismatching of ventilation and perfusion. Additional information on lethal concentrations of chlorine in various animal species can be found in World Health Organization (WHO 1982).

Lethality was also reported in an intermediate-duration study in rats (10/sex/exposure level) exposed to 1, 3, or 9 ppm 6 hours/day, 5 days/week for 6 weeks (Barrow et al. 1979). Three rats from the 9 ppm exposure level group died before day 30 and had lesions consistent with chlorine exposure (widespread inflammation of the respiratory tract with hyperplasia and hypertrophy of epithelial cells of the respiratory bronchioles, alveolar ducts, and alveoli). The specific times of death were not reported.

The LOAEL values for death in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	1 hr				293 (1-hour LC50)	Vernot et al. 1977	
2	Rat (NS)	1 min - 16 hr				1000 (50% killed in 53 minutes)	Weedon et al. 1940	
						250 (50% killed in 440 minutes)		
3	Rat (Wistar)	5-60 min				688 (30-minute LC50)	Zwart and Woutersen 1988	
						1926 (10-minute LC50)		
						5486 (5-minute LC50)		
						447 (60-minute LC50)		
4	Mouse (Swiss-Webster)	10 min				302 M (10-minute LC50 followed by 3-hour observation period)	Alarie 1981	
5	Mouse (NS)	16 hr				1000 (50% killed in 28 minutes)	Weedon et al. 1940	
						250 (50% killed in 440 minutes)		

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
6	Mouse (Swiss-Webster)	10-30 min				1032 (10-minute LC50) 516 (30-minute LC50)	Zwart and Woutersen 1988	
Systemic								
7	Human	4-8 hr	Resp	0.5[b]	1 (itching and burning of nose and throat; altered pulmonary function)		Anglen 1981	
			Ocular	0.5	1 (eye irritation)			
8	Human	1 hr	Resp	0.4	1 (transient alterations in pulmonary function tests)		D'Alessandro et al. 1996	
9	Human	8 hr	Resp	0.5 M[b]	1 M (changes in pulmonary function tests; runny nose; throat burning)		Rotman et al. 1983	
			Ocular	0.5 M	1 M (eye irritation)			
10	Human	3 d 6 hr/d	Resp	0.5 M[b]			Schins et al. 2000	NOAEL is for pulmonary function.
11	Human	15 min	Resp	0.5[b]			Shusterman et al. 1998	NOAEL is for nasal airway resistance and pulmonary peak flow.

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
12	Human	15 min	Resp		1 (increased nasal airway resistance)		Shusterman et al. 2003b	
13	Rat (Fischer- 344)	10 min	Resp		25 M (50% decrease in respiratory rate, RD50)		Barrow and Steinhagen 1982	
14	Rat (Sprague-Dawley)	2-10 min	Resp	100 M	200 M (slight perivascular edema in the lung)		Demnati et al. 1995	NOAEL and LOAEL are for 2-minute exposure and evaluation 72 hours later.
15	Rat (Fischer- 344)	1-10 d 5 d/wk 6 hr/d	Resp		12 M (swelling of the nose and wheezing)		Dodd et al. 1980	
			Ocular		12 M (swelling around the eyes)			
			Bd Wt			12 M (approximately 20% weight loss)		

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
16	Rat (Fischer- 344)	1-5 d 6 hr/d	Resp			9.1 M (erosion and ulceration of olfactory epithelium)	Jiang et al. 1983	
			Bd Wt			9.1 M (13% weight loss on day 5)		
17	Rat (Sprague-Dawley)	30 min	Resp		184 M (histological and biochemical evidence of lung injury)		Leustik et al. 2008	
18	Rat (Fischer- 344)	1-12 hr	Resp	2.5 M	5 M (reduced total sulfydryl content in nasal respiratory epithelium after 6-hour exposure)		McNulty et al. 1983	
19	Rat (Sprague-Dawley)	15 min	Resp			1330 M (pulmonary edema and hemorrhage)	Yildirim et al. 2004	
20	Mouse (Swiss-Webster)	10 min	Resp		9.3 M (50% reduction in respiratory rate, RD50)		Barrow et al. 1977	

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
21	Mouse (Swiss-Webster)	5 d 6 hr/d	Resp			9.3 M (exfoliation, erosion, ulceration, necrosis of nasal respiratory epithelium)	Buckley et al. 1984	
22	Mouse (Hybrid)	60 min	Resp		3.5 M (50% reduction in respiratory rate, RD50)		Gagnaire et al. 1994	
23	Mouse (Swiss-Webster)	1-5 d 6 hr/d	Resp			9.1 M (erosion and ulceration of the olfactory epithelium)	Jiang et al. 1983	
			Bd Wt			9.1 M (21% weight loss on day 5)		
24	Mouse (Hybrid)	5 min	Resp		100 M (flattening of pulmonary epithelium)		Martin et al. 2003	
25	Mouse (C57BL/6N)	15 min	Resp		2.3 F (50% decrease in breathing frequency, RD50)		Morris et al. 2005	
26	Mouse (C57BL/6N)	60 min	Resp		221 (biochemical and histological evidence of lung injury)		Tian et al. 2008	

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
27	Rabbit (NS)	30 min	Resp	50		100 (pulmonary edema followed by emphysema)	Barrow and Smith 1975	
INTERMEDIATE EXPOSURE								
Death								
28	Rat (Fischer- 344)	6 wk 5 d/wk 6 hr/d				9 (death in 3/10 before day 30 of exposure)	Barrow et al. 1979	
Systemic								
29	Rat (Fischer- 344)	6 wk 5 d/wk 6 hr/d	Resp		1 F (inflammation of nasal turbinates)	9 (erosion of nasal mucosal epithelium)	Barrow et al. 1979	
			Gastro	3		9 (focal erosion of gastric mucosa)		
			Hemato	5	9 (increased hematocrit in females and segmented neutrophils in males)			
			Hepatic	1	3 (cytoplasmic vacuolation of hepatocytes)			
			Renal	3	9 (slight to moderate kidney congestion)			
			Ocular	1	3 (ocular irritation)			
			Bd Wt	1	3 F (15% decreased final weight)	9 M (43% decreased final weight)		

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
30	Rat (Fischer- 344)	62 d 5 d/wk 6 hr/d	Resp		0.5[c] M (loss of cilia and epithelium in the trachea)	5 (severe upper respiratory irritation)	Kutzman 1983	NOAELs are for organ histopathology.
			Cardio	5				
			Hepatic	5 M				
			Renal	5 M				
			Ocular	0.5	1.5 (occasional signs of eye irritation)	5 (severe eye irritation)		
			Bd Wt		0.5 F (final weight 11% lower than controls)	5 F (weight loss; final weight 32% lower than controls)		
Immuno/ Lymphoret								
31	Rat (Fischer- 344)	62 d 5 d/wk 6 hr/d		5			Kutzman 1983	NOAEL is for histopathology of spleen and peribronchial lymph nodes.
Neurological								
32	Rat (Fischer- 344)	62 d 5 d/wk 6 hr/d		5			Kutzman 1983	NOAEL is for histopathology of the brain.
Reproductive								
33	Rat (Fischer- 344)	62 d 5 d/wk 6 hr/d		5			Kutzman 1983	NOAEL is for fertility in males and females and sperm morphology.

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
CHRONIC EXPOSURE								
Systemic								
34	Monkey (Rhesus)	1 yr 5 d/wk 6 hr/d	Resp		0.1[d] F (minimal nasal epithelial hyperplasia)		Klonne et al. 1987	NOAELs are for organ histopathology.
			Cardio	2.3				
			Gastro	2.3				
			Hemato	2.3				
			Musc/skel	2.3				
			Hepatic	2.3				
			Renal	2.3				
			Endocr	2.3				
			Dermal	2.3				
			Ocular	0.5	2.3 (conjunctival irritation)			
			Bd Wt	2.3				

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
35	Rat (Fischer- 344)	M: 2 yr, 5 d/wk, 6 hr/d F: 2 yr, 3 d/wk, 6 hr/d	Resp		0.4 (alterations of minimal severity in nasal epithelium)		Wolf et al. 1995	NOAELs are for gross and microscopic pathology of organs and tissues.
			Cardio	2.5				
			Gastro	2.5				
			Hemato	2.5				
			Musc/skel	2.5				
			Hepatic	2.5				
			Renal	2.5				
			Endocr	2.5				
			Dermal	2.5				
			Ocular	2.5				
			Bd Wt	2.5				

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
36	Mouse (B6C3F1)	2 yr 5 d/wk 6 hr/d	Resp		0.4 (minimal to moderate alterations in the nasal epithelium)		Wolf et al. 1995	NOAELs are for gross and microscopic pathology of organs and tissues.
			Cardio	2.5				
			Gastro	2.5				
			Hemato	2.5				
			Musc/skel	2.5				
			Hepatic	2.5				
			Renal	2.5				
			Endocr	2.5				
			Dermal	2.5				
			Ocular	2.5				
			Bd Wt	2.5				
Immuno/ Lymphoret								
37	Monkey (Rhesus)	1 yr 5 d/wk 6 hr/d		2.3			Klonne et al. 1987	NOAEL is for histopathology of lymph nodes and spleen.
38	Rat (Fischer- 344)	M: 2 yr, 5 d/wk, 6 hr/d F: 2 yr, 3 d/wk, 6 hr/d		2.5			Wolf et al. 1995	NOAEL is for gross and microscopic pathology of lymphoreticular tissues.

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
39	Mouse (B6C3F1)	2 yr 5 d/wk 6 hr/d		2.5			Wolf et al. 1995	NOAELs are for gross and microscopic pathology of immunoreticular organs and tissues.
Neurological								
40	Monkey (Rhesus)	1 yr 5 d/wk 6 hr/d		2.3			Klonne et al. 1987	NOAEL is for histopathology of central and peripheral components of the nervous system.
41	Rat (Fischer- 344)	M: 2 yr, 5 d/wk, 6 hr/d F: 2 yr, 3 d/wk, 6 hr/d		2.5			Wolf et al. 1995	NOAEL is for gross and microscopic pathology of central and peripheral components of the nervous system.
42	Mouse (B6C3F1)	2 yr 5 d/wk 6 hr/d		2.5			Wolf et al. 1995	NOAELs are for gross and microscopic pathology of the brain, spinal, and sciatic nerve.
Reproductive								
43	Monkey (Rhesus)	1 yr 5 d/wk 6 hr/d		2.3			Klonne et al. 1987	NOAEL is for histopathology of reproductive organs and tissues.

Table 3-1 Levels of Significant Exposure to Chlorine - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
44	Rat (Fischer- 344)	M: 2 yr, 5 d/wk, 6 hr/d F: 2 yr, 3 d/wk, 6 hr/d		2.5			Wolf et al. 1995	NOAEL is for gross and microscopic pathology of reproductive organs.

a The number corresponds to entries in Figure 3-1.

b Used to derive an acute-duration inhalation minimal risk level (MRL) of 0.06 ppm; the MRL was derived by adjusting the NOAEL of 0.5 ppm for continuous exposure (0.5 ppm x 8/24) and dividing by an uncertainty factor of 3 to account for human variability.

c Used to derive an intermediate-duration MRL of 0.002 ppm; the MRL was derived by dividing the LOAEL[HEC] of 0.14 ppm by an uncertainty factor of 90 (3 for extrapolation from animals to humans with dosimetric adjustment, 3 for use of a minimal LOAEL, and 10 for human variability).

d Used to derive a chronic-duration inhalation MRL of 0.00005 ppm; the MRL was derived by dividing the BMCL10[HEC] of 0.00136 ppm by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = Female; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LC50 = lethal concentration, 50% kill, LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; ppm = parts per million; RD50 = 50% decrease in respiration rate; Resp = respiratory; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to Chlorine - Inhalation

Acute (≤14 days)

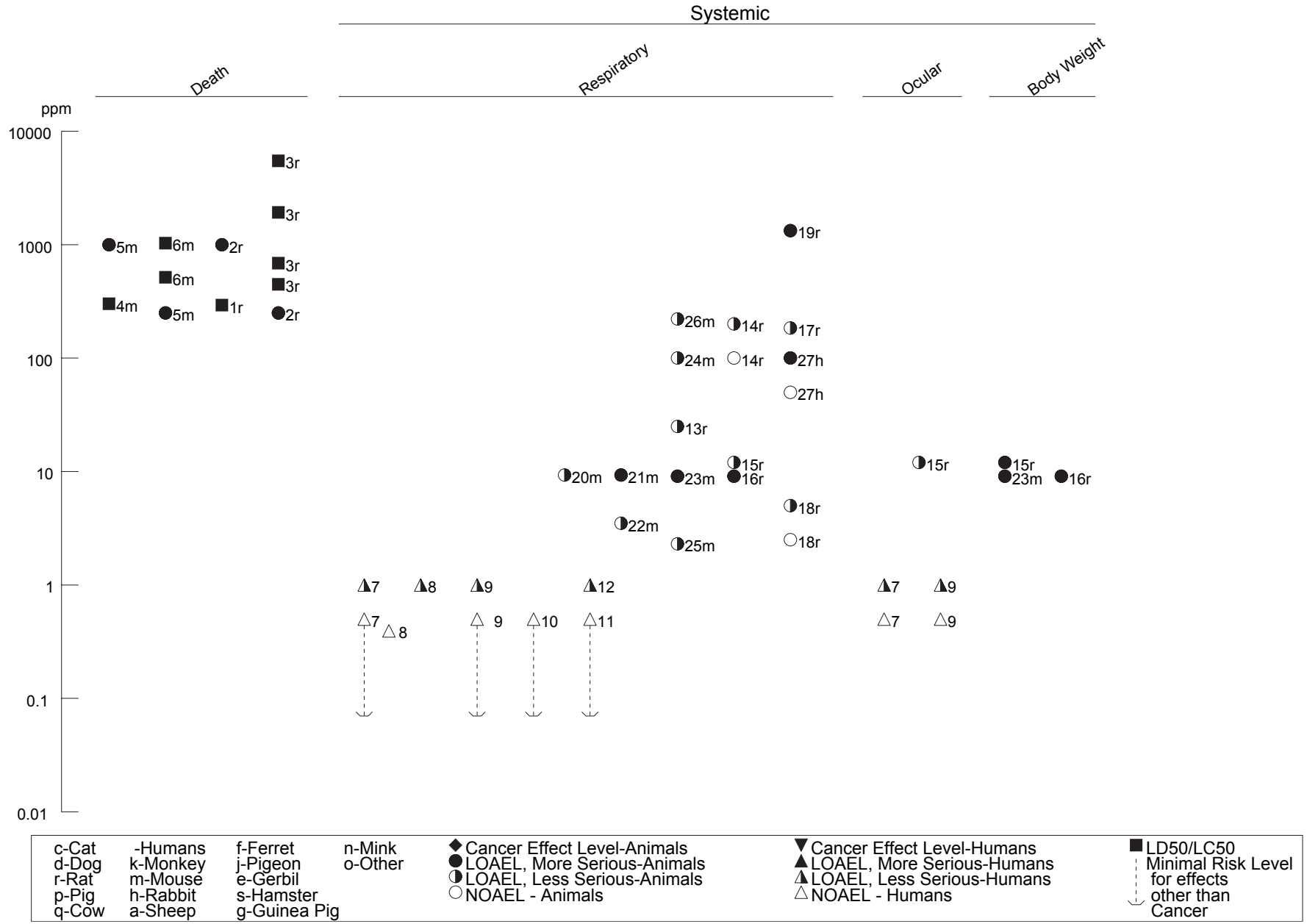


Figure 3-1 Levels of Significant Exposure to Chlorine - Inhalation *(Continued)*

Intermediate (15-364 days)

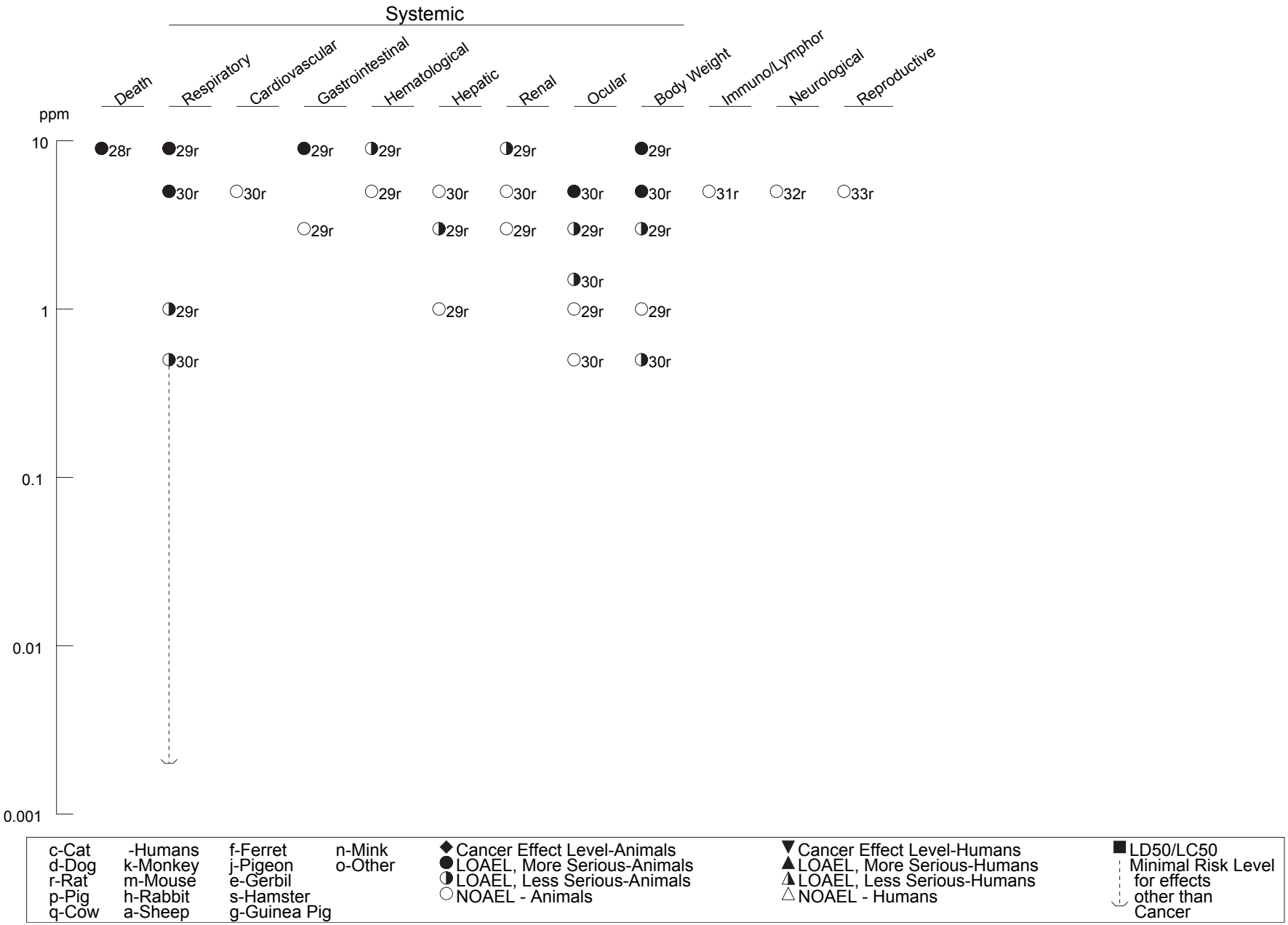


Figure 3-1 Levels of Significant Exposure to Chlorine - Inhalation *(Continued)*

Chronic (≥365 days)

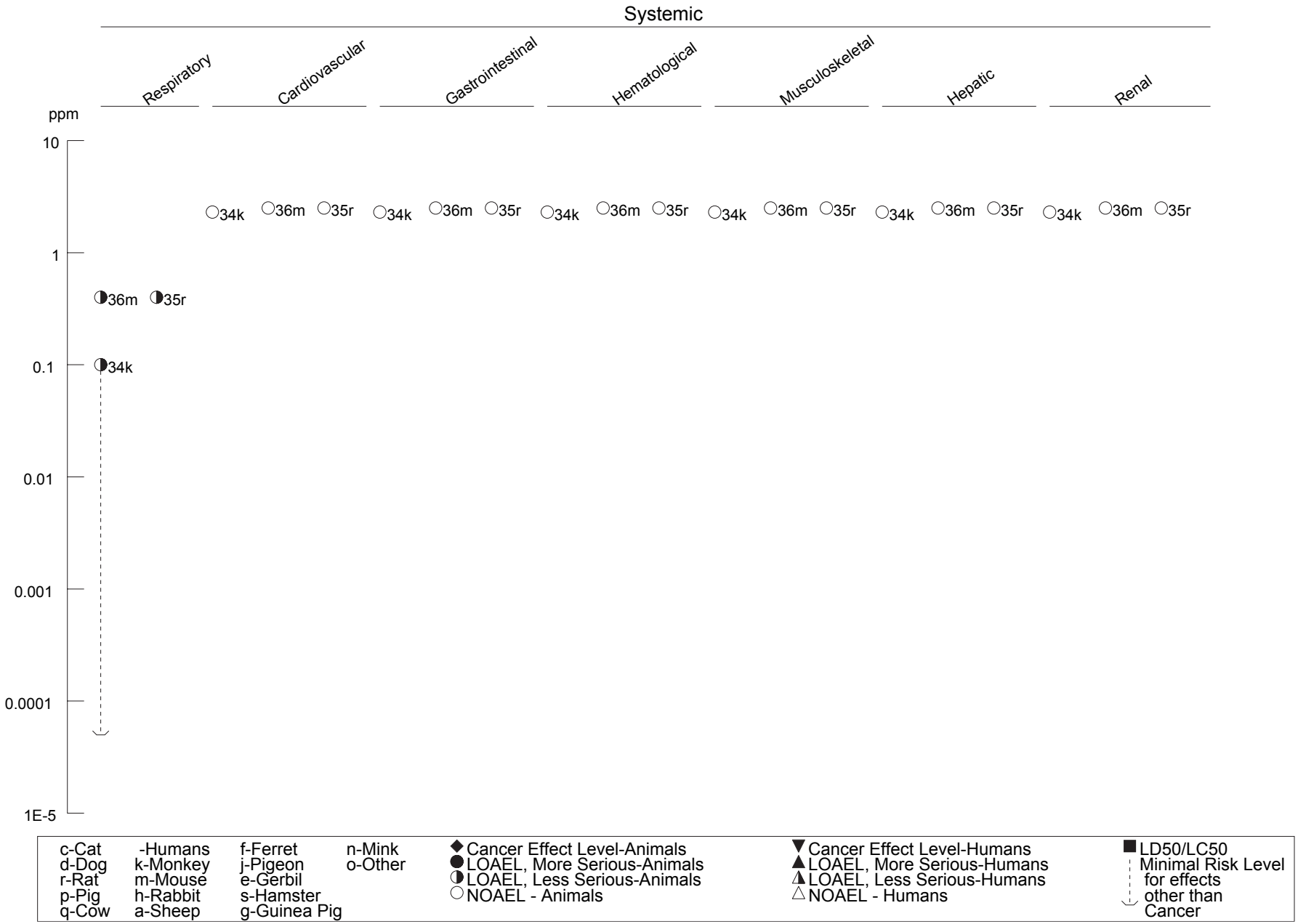
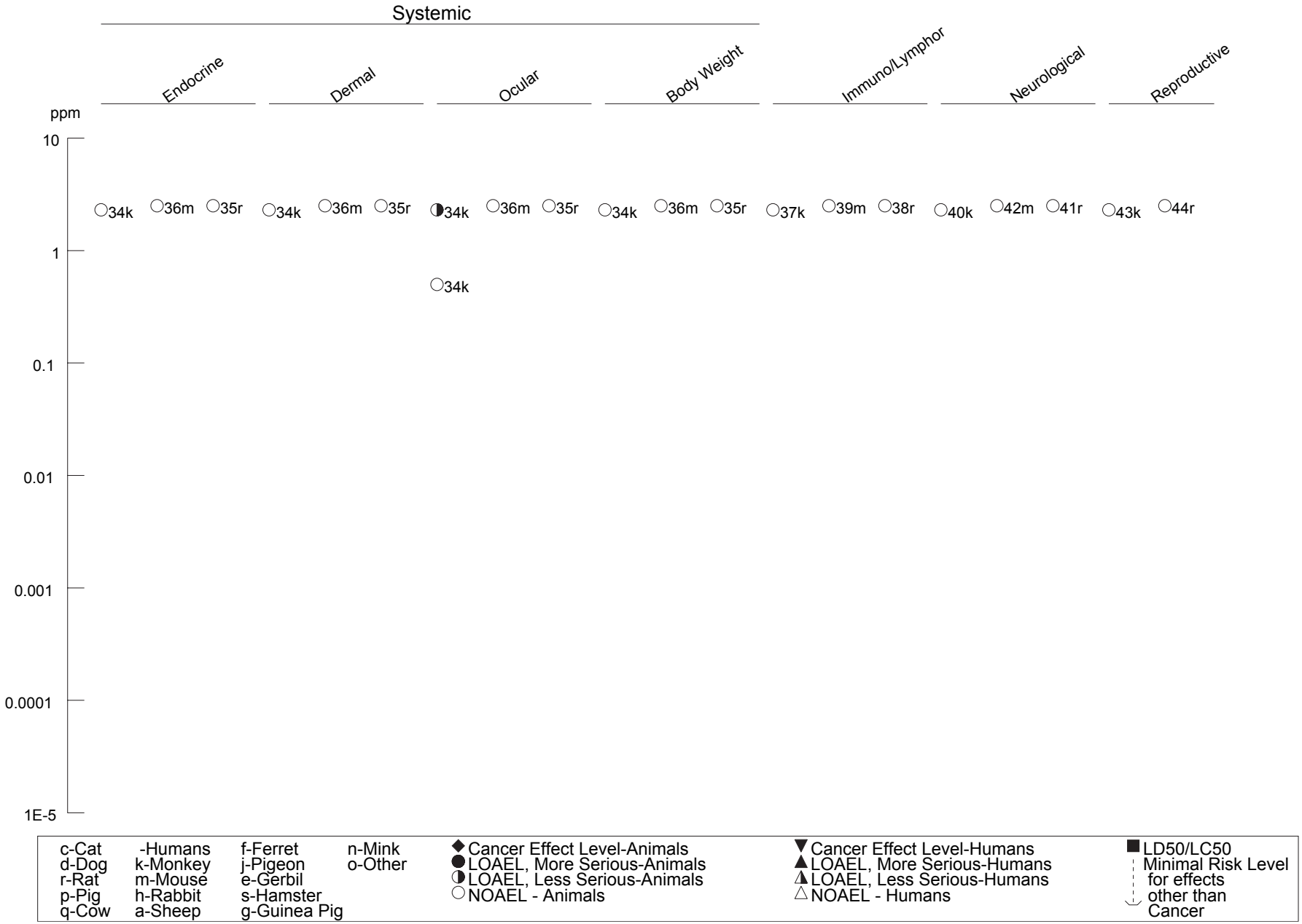


Figure 3-1 Levels of Significant Exposure to Chlorine - Inhalation *(Continued)*

Chronic (≥365 days)



3.2.1.2 Systemic Effects

The highest NOAEL and all reliable LOAEL values from each study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. The respiratory system, particularly the upper portion of the respiratory system, is the target for exposure to chlorine gas, and there is a considerable amount of information to support this observation. Chlorine is a respiratory irritant and its effects depend on its concentration, as well as the duration of exposure and the water content of the tissue involved.

Table 3-2 lists clinical effects associated with exposure concentrations compiled from epidemiological and toxicological studies by Ellenhorn and Barceloux (1988). Whether acute exposure to high levels of chlorine induces long-term respiratory effects is still a matter of debate. As mentioned below, some studies have reported a clinical picture of persistent airflow obstruction as well as increased bronchial reactivity, while many others have not found significant persistent alterations. There are many factors that can determine whether residual effects are detected, including exposure level and duration of exposure, medical treatment following exposure, length of the follow-up, underlying respiratory disease, and smoking status. The preponderance of the evidence suggests that no serious long-term respiratory alterations result from acute exposure to low to moderate (up to approximately 20 ppm) concentrations of chlorine gas.

Effects caused by several types of exposures are summarized below including exposure to high concentrations as it occurred during gassing attacks in World War I, accidental exposures of the general population, occupational exposures, and acute, low-level, controlled exposure in volunteers. Information on odor perception is also summarized. Due to the high volume of studies in some of these categories, representative examples are presented.

Odor Perception. A study in which a trained odor panel of four members was exposed to chlorine under controlled laboratory conditions reported that the first concentration at which all members cold detect the odor was 0.314 ppm (Leonardos et al. 1968). Ryazanov (1962) reported that the odor threshold of a group of volunteers was 0.3–0.4 ppm. In a study by Rupp and Henschler (1967), chlorine was slowly introduced to a test room so that the concentration increased from 0 to 1.3 ppm in a 50-minute period.

Table 3-2. Acute Effects of Chlorine Exposure on the Respiratory Tract of Humans

Concentration (ppm)	Effects
0.2–3.5	Odor detection
1–3	Mild mucous membrane irritation tolerated up to 1 hour
5–15	Moderate irritation
30	Immediate chest pain, dyspnea, cough
40–60	Toxic pneumonitis and pulmonary edema
430	Lethal over 30 minutes
1,000	Lethal within minutes

Source: Ellenhorn and Barceloux 1988

The odor of chlorine was first detected at 0.06 ppm and all the subjects (number not specified) could smell 0.2 ppm chlorine. In a summary of information on odor perception, NIOSH (1976) states that Stayzhkin reported a threshold of 0.2 ppm for chlorine in a group of 12 subjects who were asked to discern clean air from chlorine by inhaling from two tanks and that Beck found that subjects who were exposed to increasing concentrations of chlorine the length of time of perception was positively related to the exposure concentrations (i.e., the perception of higher concentrations lasted longer than that of low concentrations suggesting that tolerance to low chlorine concentrations may develop). In general, there seems to be consensus that the mean odor threshold lies between 0.2 and 0.4 ppm, which is between 1/25 and 1/50 of the concentration considered to represent immediate danger to life and health of 10 ppm (NIOSH 2005). This means that individuals with a normal sense of smell should be able to take appropriate avoidance measures before serious health effects due to chlorine exposure can occur.

War Exposures. A review of the effects of chlorine as a result of its use during World War I indicates that subjects exposed to very high concentrations (not specified, but presumably >100 ppm) experienced burning of the throat, cough, and feeling of suffocation, dyspnea, and usually death within 24 hours of acute pulmonary edema (DOA 1933). Those surviving 48 hours usually recovered, but bronchitis persisted for weeks and, in some cases, there was evidence of pulmonary emphysema. Data summarized by DOA (1933) on studies that evaluated residual effects indicate that bronchitis and asthma were common months to years after gassing. Dyspnea and chest pain after exercise were also frequent complaints. Citing a study of 838 U.S. soldiers who had been subjected to chlorine gassing, DOA (1933) states that 9 soldiers were discharged due to conditions attributable to gassing; these conditions included pulmonary tuberculosis, bronchitis, pleurisy, and dyspnea. An additional 39 cases were found to have been disabled at the time of discharge, and bronchitis and pleurisy were among the disabling conditions. The extent to which these residual effects were truly due to chlorine gassing is difficult to ascertain due to lack of information on pre-existing conditions, smoking status, and even factors such as the climatic conditions that existed at the time.

Exposures of the General Population. There is considerable information on the effects of exposures of the general population to chlorine derived from various types of situations, including storage tank leaks, railroad accidents, mishandling of bleach cleaning solutions, accidents involving swimming pool chemicals, and accidents in high school chemistry laboratories. Representative examples are summarized below.

Chasis et al. (1947) provided a comprehensive description of immediate and delayed respiratory effects in a group of subjects exposed to chlorine that leaked from a defective cylinder that was carried in a truck in Brooklyn, New York. The accident involved several hundred people. Although the exposure concentration was not known, it was high enough to cause a visible cloud. Chasis et al. (1947) report on 33 persons admitted to a local hospital. Immediate symptoms included rhinorrhea, cough, choking sensation, and substernal burning, pain, and constriction. Substernal pain, burning, and constriction, and choking sensation were present within 2 hours of exposure and, in most patients, subsided completely within 72 hours. Respiratory distress subsided within 3 days in 27 patients and within 6 days in 5 patients. During the first week, almost all 33 patients complained of substernal soreness, which was interpreted as indicating the presence of tracheobronchitis. Physical examination on admission revealed acutely ill patients in moderate to marked respiratory distress, increased respiratory rate of costal abdominal type, and both dry and moist rales. Laboratory data showed sputum with large numbers of epithelial cells showing pronounced degenerative changes. Most cultures showed microorganisms representative of the normal pharyngeal flora. Chest x-rays showed mottling of the lungs and patches of irregular density and differences in the degree of aeration between the two pulmonary fields. Spirometry was conducted on 8 patients 48 hours after exposure and showed markedly reduced vital capacity (VC) and maximal breathing capacity (1 minute); these changes showed improvement in subsequent days. The diagnosis of pulmonary changes was: pulmonary edema, tracheobronchitis, and pneumonia. In 29 patients who were followed for up to 16 months after exposure, there was no evidence of permanent pulmonary disease.

Hasan et al. (1983) reported that exposure of 28 subjects to chlorine that leaked from a storage tank caused cough, dyspnea, and nasopharyngeal irritation. Pulmonary tests conducted 18 hours after exposure showed diminished forced expiratory volume in 1 second (FEV_1), and low forced expiratory flow rate at 50 and 25% vital capacity (FEF_{50} and FEF_{25}) and $FEF_{25–75\%}$. These abnormalities were still present 14 days after exposure in subjects whose chief initial complaint was dyspnea. Evaluation of nine subjects 5 months after chlorine exposure showed pulmonary parameters within normal limits.

In contrast to the findings of the above two studies, some studies have reported long-term effects of acute high chlorine exposure. For example, Chester et al. (1977) reported the case of a woman who was exposed following a leak in a liquid storage tank and suffered severe cough and chest pain within minutes after exposure. Chest x-rays at the time showed bilateral infiltrates in the midpulmonary zones, but 1 year after the accident x-rays were normal. However, dyspnea and chronic cough with occasional production of white to yellow sputum persisted over the next 4 years. Schönhofer et al. (1996) studied three cases

that experienced nose and throat irritation, cough, shortness of breath, wheezing, chest tightness, and a feeling of suffocation minutes after exposure to chlorine gas that leaked from a tank. Chest x-rays showed no evidence of pulmonary edema. Four months after the accident, bronchoalveolar lavage showed inflammatory changes, but no such changes were seen 16 months later. However, moderate to severe bronchial hyperresponsiveness was observed up to 30 months after the accident. Schönhofer et al. (1996) noted that the condition showed the typical feature of the reactive airways dysfunction syndrome (RADS), defined as an asthma-like occupational illness after an acute exposure to concentrated respiratory irritants characterized by increased responsiveness to methacholine (Brooks et al. 1985).

Weill et al. (1969) studied 12 subjects who were exposed to chlorine after the derailment of a tank car containing 30 tons of liquid chlorine near the town of Morganza, Louisiana. Chlorine concentrations of 400 ppm were measured 75 yards from the wreck 7 hours after the accident. Immediate effects included shortness of breath, cough, and x-rays revealed pulmonary edema. Three and/or 7 years after the accident, all patients were free of respiratory symptoms and pulmonary tests, including total lung capacity (TLC), VC, residual volume (RV), FEV_1, and single-breath diffusing capacity for carbon monoxide (DL_{CO}) were within 2 standard deviations of the predicted value. Weill et al. (1969) concluded that acute exposure to chlorine does not result in significant permanent lung damage. A previous account of this accident was given by Joyner and Durel (1962).

The Agency for Toxic Substances and Disease Registry (1998) conducted a health assessment of a population in Alberton, Montana after tanker cars derailed and released chlorine gas, solid sodium chlorate, and potassium cresylate. Readings at the site of the accident occasionally reached 1,000 ppm, but it was estimated that members of the community had been exposed to up 20 ppm chlorine. Analysis of soil and wipe samples determined that chemicals other than chlorine had not migrated offsite. A total of 682 persons were interviewed within 2 weeks of the accident. The most frequent conditions were cough, nose and throat irritation, and difficulty breathing. The report also indicates that children aged 0–5 years had the highest prevalence of respiratory infections, that persons who had previous respiratory problems (i.e., asthma, chronic bronchitis, hay fever) reported a higher prevalence of respiratory health problems, and that the reported frequencies of respiratory symptoms were higher among current and former smokers than in nonsmokers.

Accidental domestic exposure to chlorine gas can occur when bleach (sodium hypochlorite) is mixed with other cleaning substances that contain an acid, for example, phosphoric acid cleaners (used to remove hard water deposits); the chemical reaction generates chlorine gas. Many such incidents have been

reported. For example, CDC (1991) reported five episodes in which subjects experienced nose irritation, sore throat, chest tightness, cough, and difficulty breathing; in most cases, respiratory symptoms subsided within a day. Gapany-Gapanavičius et al. (1982) reported two similar cases in which subjects inhaled chlorine after mixing bleach and hydrochloric acid and immediately experienced a burning sensation in the throat, cough, and shortness of breath. Chest x-rays taken hours after the accident showed air in the mediastinal space (space in the middle of the chest separating the two pleurae), probably the result of severe coughing, which resolved 7–10 days after the accident. None of these cases were followed to evaluate possible long-term effects. Additional information from similar exposure scenarios can be found in a review by Mrvos et al. (1993).

Chlorine gas can be released around swimming pools when chlorinating agents are handled improperly or due to malfunction of the chlorination equipment, which underscores a strong need for training pool owners and operators in pool maintenance. Pertinent information can be found in CDC (2009).

Sexton and Pronchik (1998) described the effects of such an exposure on 13 children who presented to the emergency department. On admission to the emergency department, most patients complained of throat irritation, chest pain, shortness of breath, wheezing, and chest tightness. Five patients who were admitted to the hospital had normal chest x-rays. At follow-up interviews 2 weeks later, the patients did not complain of residual respiratory symptoms. Ploysongsang et al. (1982) studied four patients who inhaled, for 2–5 minutes, an undetermined amount of chlorine gas that leaked from a container at a public swimming pool and experienced cough, a feeling of irritation of the upper respiratory tract, and tightness in the chest. Pulmonary function studies conducted 12–14 hours after the accident showed values within normal ranges. However, tests done 1 month later showed a significant increase in measurements of volumes, suggesting that there had been an acute reduction of lung volumes after the exposure. Ploysongsang et al. (1982) concluded that exposure to chlorine had produced an insignificant and inconsistent obstruction in large airways. Agabiti et al. (2001) reported the effects of accidental inhalation of chlorine gas among a total of 282 subjects attending a pool. Cough and shortness of breath were common acute symptoms after the accident and 27% reported some respiratory symptoms 15–30 days after exposure. Lung function measurements at that time revealed a tendency to lower levels among those with the highest perceived exposure, but only a decrease in FEV_1 was significant. The study also found that among children (approximately half the sample), the incidences of all symptoms tended to be higher among those who had a history of chronic respiratory disease, among those who were engaged in physical exercise when the accident occurred, among those who were slow to evacuate the pool, and among those who reported higher exposure (as judged by eye irritation). Also, incidences were higher

among smokers and former smokers than among never smokers. A recent study of 18 children exposed to chlorine in a swimming pool accident found that a biomarker of pulmonary inflammation, leukotriene B_4, was still significantly increased in exhaled breath condensate 2 months after exposure, long after pulmonary function parameters had returned to normal values (Bonetto et al. 2006). Immediately after exposure, the children had experienced dyspnea and burning of the throat and spirometry tests done within the first 24 hours showed reduced forced vital capacity (FVC) and FEV_1. The authors also found that hours after exposure a Clara cell-specific protein, CC16, was significantly elevated in serum compared to non-exposed children, and suggested that this may be interpreted as a sign of injury to the lung epithelial permeability barrier. Transient increases in serum CC16 have been reported following exposure to certain pulmonary irritants (see Lakind et al. 2007 for review). Additional information regarding swimming pool accidents can be found in Almagro et al. (2008), Babu et al. (2008), Grasemann et al. (2007), and Thomas and Murray (2008).

Controlled Low-level Exposure of Volunteers. Anglen (1981) exposed up to 29 male and female volunteers to 0, 0.5, 1, or 2 ppm chlorine for either 4 or 8 hours. Sensations were recorded before and during exposure and pulmonary function was monitored by measuring FVC and FEV_1 before and at various times during exposure. Itching and burning of the throat were the highest responses and were most prevalent by the end of an 8-hour exposure to 1 ppm chlorine. Responses for sensations of itching or burning of the nose and eyes were also prevalent at 1 ppm chlorine. In general, males provided stronger irritation responses than females. Exposure to 1 or 2 ppm chlorine for 8 hours produced significant changes in pulmonary function but similar exposures to 0.5 ppm did not. Exposure to 2 ppm for up to 30 minutes produced no increase in subjective irritation and exposure to 2 ppm for 2 hours did not alter pulmonary function. In another study from the same group of investigators (it is unclear whether a follow-up to Anglen [1981] or a separate study), eight healthy male volunteers exposed to target concentrations of 0, 0.5, or 1 ppm chlorine (Rotman et al. 1983). Pulmonary tests were conducted before exposure, after a 4- and 8-hour exposure period and again 2 and 24 hours after exposure ceased. During exposure, the subjects exercised on a treadmill for 15 minutes of each hour to simulate light-to-moderate work that raised the heart rate to 100 beats per minute. Specific respiratory parameters measured included FVC, FEV_1, forced expired volume in 1 second as percent FVC (FEV_1%), peak expiratory flow rate (PEFR), FEF_{50} and FEF_{25}, TLC, expiratory reserve volume (ERV), functional residual capacity (FRC), residual volume, airway resistance (Raw), single-breath DL_{CO}, closing volume, and difference in nitrogen concentrations between 750 and 1,250 mL of inhaled vital capacity (ΔN_2). Exposure to 1 ppm chlorine caused runny nose and mild burning in the throat, but no such effects were reported at 0.5 ppm. Significant changes in pulmonary function tests were mostly restricted to the 1 ppm exposure level and

were evident after 4 hours of exposure. Changes were observed in FEV_1, PEFR, FEF_{50}, FEF_{25}, TLC, Raw, and ΔN_2. Greater changes in some of these parameters were seen after 8 hours of exposure. Few changes were still evident 24 hours after exposure, but most parameters had returned to pre-exposure values by that time. It should be noted that one volunteer who was atopic experienced severe distress during exposure to 1 ppm and was forced to exit the chamber before the full 8-hour period due to shortness of breath and wheezing.

D'Alessandro et al. (1996) evaluated pulmonary function in subjects with (n=10) and without (n=5) airway hyperresponsiveness (HR, defined by baseline methacholine hyperresponsiveness). The HR subjects were exposed to 0.4 or 1.0 ppm chlorine, whereas the healthy subjects were exposed to 1.0 ppm chlorine. All exposures lasted 60 minutes. Airflow and airway resistance were measured immediately before and immediately after exposure. Also, lung volumes, airflow, diffusing capacity, airway resistance, and responsiveness to methacholine were measured 24 hours before and 24 hours after exposure. Exposure of the HR group to 0.4 ppm chlorine resulted in no significant change in airflow or resistance either immediately or 24 hours after exposure. Exposure to 1.0 ppm chlorine resulted in an immediate decrease in FEV_1 and $FEF_{25–75\%}$ and increase in airway resistance among normal and HR subjects, but the magnitude of the effects among HR subjects was significantly greater than in healthy subjects. Twenty-four hours after exposure, there were no significant changes for healthy or HR subjects in airflow, lung volumes, diffusing capacity, resistance, or methacholine responsiveness. Comparing relative changes from baseline immediately after exposure between normal and HR subjects showed that HR subjects had much greater changes in pulmonary function tests.

A similar study was conducted in eight volunteers exposed to chlorine 6 hours/day on 3 consecutive days to each of the four exposure conditions, 0, 0.1, 0.3, and 0.5 ppm chlorine (Schins et al. 2000). Pulmonary function including effort-dependent parameters and effort-independent parameters were evaluated before and after exposures. In addition, nasal lavage measurements were performed before and after each exposure and 1 and 4 days after each exposure. The nasal lavage fluid was examined for total cells, epithelial cells, neutrophils, lymphocytes, eosinophils, monocytes, albumin (an indicator of epithelial permeability), and interleukin-8 (indicator of inflammatory response). Subjective complaints by the subjects were judged to be not treatment-related, but objective physiological measures of nasal irritant response were not included. Examination of the nasal lavages gave no indication of an inflammatory response or irritant effects on the nasal epithelium. The results of the pulmonary function tests showed that the only significant effect related to chlorine exposure was a difference in maximal mid expiratory

flow (MMEF) between 0 and 0.5 ppm exposure; however, this was attributed to an unexplained shift in baseline values during control exposure (0 ppm).

Shusterman et al. (2003b) measured nasal airway resistance in 52 healthy adults (24 males and 28 females) before and after exposure to 0 or 1 ppm chlorine for 15 minutes. Subjects were stratified on age (18–34, 35–51, 52–69 years), gender, and allergic rhinitis status (27 were positive). Nasal airway resistance was measured by active posterior rhinomanometry. Exposures to air and chlorine were a week apart. Subjects with allergic rhinitis showed a significantly greater increase in nasal airway resistance (49% increase from baseline) than healthy subjects (10% increase from baseline) 15 minutes after exposure. The increase in nasal airway resistance was most pronounced in older subjects and least pronounced in the youngest group. No significant differences were seen between males and females. In an earlier study, the same group of investigators had reported that subjects with SAR exposed to 0.5 ppm chlorine for 15 minutes experienced a much greater increase in nasal airway resistance than subjects without SAR, as measured by active posterior rhinomanometry (Shusterman et al. 1998). However, when subjective responses to odor, nasal irritation, and nasal congestion were analyzed separately by rhinitis status, no significant exposure-related changes were observed for rhinorrhea, postnasal drip, or headache either on a pool or stratified basis. In addition, within either the SAR or non-SAR group, there was no relationship between subjective and objective congestion after chlorine exposure. Pulmonary peak flow tests showed that none of the subjects exhibited clinically significant changes in peak flow, nor did they complain of cough, wheezing, or chest tightness on chlorine exposure days.

As a whole, these studies indicate that acute-duration exposures to 1 ppm chlorine can induce upper respiratory tract irritation and transient alterations in parameters of respiratory function and exposure concentrations of 0.5 ppm are generally devoid of such effects and, therefore, 0.5 ppm can be considered an acute (1–8 hours) NOAEL for sensory irritation and pulmonary function. The 0.5 ppm level caused no significant effects in an atopic subject (Rotman et al. 1983) or in subjects with airway hyper-responsiveness (D'Alessandro et al. 1996), and, while increased nasal airway resistance measured instrumentally in subjects with SAR, the latter did not clearly perceive the effect as an adverse effect (Shusterman et al. 1998). These studies also show that individuals with compromised respiratory function constitute a susceptible group for exposure to chlorine. The NOAEL of 0.5 ppm and LOAEL of 1 ppm from these studies as a group, serve as the basis for derivation of an acute-duration inhalation MRL for chlorine.

Long-term, Low-level Occupational Exposures. Relatively few studies have examined the effects of long-term exposure to low levels of chlorine in humans, and the ones that have done so have not provided conclusive answers largely because of study limitations.

Patil et al. (1970) studied the health effects of chlorine in 600 workers from 25 plants producing chlorine in North America. A group of 382 workers not considered to be routinely exposed to chlorine served as controls. The average duration of exposure was 11.9 years. Each worker received one physical examination that included evaluation of medical and occupational histories, blood and urine tests, pulmonary function tests and electrocardiogram (EKG). Tobacco and alcohol use were also monitored. The concentration of chlorine was monitored in each plant every 2 months over a period of 1 year in several representative areas, but otherwise unspecified. Exposure data were available for 332 workers and showed a time-weighted average (TWA) 8-hour mean of 0.15±0.29 ppm (range, 0.006–1.42 ppm). It also showed that almost all workers were exposed to <1 ppm chlorine, 94% were exposed to ≤0.5 ppm, and 70% were exposed to ≤0.2 ppm. Evaluation of the 332 workers who had exposure data showed that none of the end points examined (those subjected to recall or measured) showed a dose-response relationship. The mean concentration of 0.15 ppm may be considered a NOAEL for the study, but there are limitations such as unclear analytical methodology, no clear definition of the case/control populations, and insufficient detail regarding the method of analysis that render the NOAEL questionable; thus, it is not included in Table 3-1.

Ferris et al. (1967) examined the prevalence of chronic respiratory disease among 147 workers in a pulp mill and 124 controls who worked in a paper mill and found no significant differences in respiratory symptoms or in tests for FVC and FEV_1 (tests were conducted without a nose clip) between the two groups. Duration of exposure was not provided. Chlorine levels were measured on three different occasions in 3 years; in one occasion, the mean was 7.4 ppm and only traces were reported in the other two occasions. The limit of detection of the method was 1 ppm. Examination of the same cohort 10 years later did not reveal any increased mortality or increased specific cause of death (Ferris et al. 1979). Evaluation of 200 men seen at both times did not reveal any differences in respiratory symptoms or chronic nonspecific respiratory disease.

Enarson et al. (1984) evaluated respiratory effects and pulmonary function in a group of 392 male pulp mill workers exposed to chlorine, sulfur dioxide, hydrogen sulfide, and methylmercaptan, in addition to various particulates (i.e., wood dust, ash, lime dust), for a mean duration of 101.5±86.6 months. A control, unexposed group, consisted of 310 male rail yard workers who lived in the same community and

who performed similar manual labor. End points examined included prevalence of respiratory symptoms (usual cough, usual phlegm, wheezing without a cold, dyspnea when hurrying, chest tightness, and chest illness). Pulmonary function tests conducted included FEC, FEV_1, $FEF_{25–75\%}$, and FEV_1/FVC ratio. Chlorine was the main contaminant in two areas of the pulp mill, the bleach plant and the machine room (mean 8-hour TWA 0.18 and 0.02 ppm, respectively). Overall, pulp mill workers complained more frequently of usual phlegm, wheeze without cold, and chest illness than rail workers. However, the most significant finding was that among bleach workers (n=15) and machine room workers (n=22), nonsmokers (n=4) had a significantly lower $FEF_{25–75\%}$ and FEV_1/FVC ratio than rail yard workers. Given the small number of workers involved, the possibility of exposure to multiple chemicals, and the lack of information on chlorine peak exposure levels, the validity of the 0.18 ppm as an effect level is questionable.

A study at a chlorine plant in Sweden compared the changes in vital capacity (VC) and FEV_1 that occurred between measurements separated by 10 years among 44 workers exposed to chlorine and 33 white-collar workers matched for age and smoking status (Hyback 1999). The author stated that the concentration of chlorine was measured continuously over the years and was always below 0.5 ppm, as a set standard. The results of the tests showed that in fact, over the years, VC and FEV_1 declined more in white-collar workers (significantly for FEV_1) than in the workers exposed to chlorine. Hyback (1999) speculated that perhaps the low concentrations of chlorine gas may protect workers from contracting respiratory infections that over time contribute to a decline in respiratory function.

The limited information available does not suggest that long-term exposure to low levels of chlorine gas affects respiratory function, but additional, better-conducted studies are necessary to confirm this view.

High-level Occupational Exposure. Schwartz et al. (1990) studied a group of 20 workers who were briefly (minutes) exposed in a pulp mill to chlorine gas when liquid chlorine leaked from a tank and evaporated. Acute symptoms included burning of the nose and throat, and dry cough with chest tightness. Pulmonary tests were conducted within 24 hours of exposure and several times over the next 12 years. The most significant findings were a high prevalence of airflow obstruction (FEV_1/VC ratio <65%) that persisted over the observation period and a prevalence of low residual volume (RV) that increased during the follow-up period. Schwartz et al. (1990) also found that 5 of 13 subjects tested at year 12 had increased airway reactivity to inhaled methacholine. While the findings were suggestive of long-term pulmonary complication, the investigators acknowledged that without pre-exposure pulmonary function

tests and individual measures of exposure, it is difficult to determine whether the changes were due to chlorine exposure.

Moulick et al. (1992) evaluated 82 patients exposed to approximately 66 ppm chlorine that leaked from a storage tank at a chemical factory in Bombay, India. Acute symptoms of exposure included dyspnea, cough, and irritation of the throat. Pulmonary tests performed in 62 cases within 48 hours of exposure indicated obstruction in 17 cases, restriction in 2 cases, and a mixed pattern in 33 cases. Also, bronchoscopy showed tracheobronchial mucosal congestion and hemorrhagic spots. Four out of 16 patients who were followed for 1 year showed persistent cough 4–6 weeks after exposure, but after 1 year, there were no residual symptoms and x-rays and pulmonary function tests were normal. Evaluation of five nonsmoking patients 3 years after exposure did not reveal any residual symptoms and pulmonary function tests were normal (tests were not specified).

Lemière et al. (1997) reported the case of a nonsmoking worker at a water-filtration plant man who was exposed to chlorine levels high enough to induce immediate burning of the nose and throat and retrosternal burning and wheezing. Five years earlier, he experienced similar symptoms after chlorine inhalation, but the symptoms had been transient. Two days after exposure, FEV_1 was significantly reduced (66% of predicted) and the response to methacholine provocation was slightly abnormal. A bronchial biopsy showed almost complete replacement of the epithelium by a fibrinohemorrhagic exudate. Subsequent biopsies taken over a 5-month period showed considerable epithelial desquamation 15 days after exposure followed by signs of regeneration 5 weeks after exposure and considerable improvement 5 months after exposure, although an inflammatory infiltrate was still present. The airway responsiveness to methacholine paralleled the inflammatory changes, but could be significantly improved by inhaled steroids.

Kowitz et al. (1967) described the effects of chlorine exposure that occurred when a cylinder containing chlorine that was being unloaded from a freighter leaked. Neither exposure concentration nor exposure duration was available. At least 150 men were involved and almost all experienced acute symptoms. Eleven of 17 subjects who were admitted to a hospital were evaluated over a 3-year period. All showed respiratory distress on admission; other common signs included rales, wheeze, or rhonchi, or both, and pulmonary edema. Pulmonary function testing conducted over the 3-year evaluation period showed a persistent decrease in lung volume and diffusing capacity and increased airway resistance. According to Kowitz et al. (1967), these alterations were compatible with an alveolar-capillary injury.

There are also a number of studies that describe the effects of occasional occupational exposures to elevated concentrations of chlorine over a background of low level exposure ("gassing" incidents). Some examples are summarized below.

In a study of 321 workers at a British Columbia pulp mill, 189 reported one or more gassing incidents, although no data were available to determine the severity of the exposure incidents (Kennedy et al. 1991). The average chlorine levels in the pulp mill were <1 ppm, but estimates of concentrations during the gassing incidents are not available. Pulp mill workers who reported having being gassed were significantly more likely to report wheezing on occasion than individuals who had not been gassed. While there were no significant differences in lung function tests between the overall pulp mill group and a control group, nonsmoking and formerly smoking pulp mill workers who reported being gassed had significantly lower MMEF and FEV_1/FVC ratio than those who had not been gassed. Kennedy et al. (1991) hypothesized that the first accidental high exposure incident may cause an inflammatory reaction in the small airways that does not completely resolve because of continuous or repeated presence of the stimulus. A longitudinal analysis over a 7-year period (1981–1988) of 67 pulp mill workers that underwent pulmonary function tests both in 1981 and 1988 reported that there was a significantly greater decline in FEV_1/FVC ratio and MMEF in the gassed group than in the unexposed group matched for age and smoking status (Salisbury et al. 1991).

In a survey of 281 construction workers involved in renovation work at a pulp mill, 257 workers reported an average of 24 gassing episodes over a 3–6-month period (Courteau et al. 1994). Exposure data during the gassings were not available, but 36% of 483 air samples taken in the bleach plant after the episodes showed chlorine concentrations of <0.5 ppm, 58% were between 0.5 and 8 ppm, and 6% were >8 ppm. The most commonly reported symptoms were irritation of the throat (78%), cough (67%), and shortness of breath (54%); the latter was not associated with age, smoking state, or history of asthma or chronic bronchitis. Over 60% of the workers described a flu-like syndrome that lasted for an average of 11 days. Visits to the first aid department were associated with greater reporting of most symptoms including dyspnea and cough. Seventy-one workers identified as having moderate to high risk based on exposure data and onset of respiratory symptoms were evaluated 18–24 months later (Bhérer et al. 1994). At this time, a questionnaire completed by 64 workers (90%) suggested that 58 (91%) still had respiratory symptoms and 51 underwent spirometry and methacholine challenge tests. Bronchial obstruction (FEV_1 <80% predicted) was observed in 16 workers, whereas 29 showed significantly increased airway responsiveness. Based on the fact that workers who had been to an emergency room were more likely to be left with airway hyper-responsiveness, Bhérer et al. (1994) speculated that severity of one or more

gassing episodes may be a more significant determinant of the likelihood of developing permanent alterations than the number of episodes.

In a study of 300 pulp and paper workers in New Hampshire, 105 reported ever having experienced an episode of high exposure to chlorine gases (Henneberger et al. 1996). Spirometry (FEV_1, FVC, FEV_1/FVC, MMEF) showed that the prevalence of obstructive pattern was >3 times greater for the gassed subjects compared with the others. For workers who had 26-pack years of cigarette smoking, an obstructive pattern (abnormally low FEV_1 and FEV_1/FVC) was observed only among those with a history of gassing. In addition, the combination of high cigarette smoking and gassing had a greater-than-additive effect on obstruction. Monitoring data were not available in this report.

Gautrin et al. (1999) evaluated 239 workers in a metal production plant who, 3 years earlier, had taken part in a cross-sectional study (Gautrin et al. 1995). In the first evaluation, the authors reported that FVC was higher in workers who had no symptoms after a gassing episode compared with those who had mild symptoms. Also, FEV_1 and FVC were significantly lower in workers who experience >10 gassing incidents with mild symptoms than in workers who experienced no symptoms. In both cases, the differences were significant only in workers who had ever smoked. Increased airway responsiveness was also found in subjects who experienced >10 gassing incidents with mild symptoms. In 98% of the accidental exposures, chlorine was reported as the gas involved. Among 211 workers seen at follow-up (Gautrin et al. 1999), heavy smokers showed a decrease in FEV_1/FVC% that was predicted by the number of gassing episodes causing mild symptoms between the two evaluations. Nineteen workers showed increased airway responsiveness which was associated with accidents reported to the first aid unit during the previous 2 years. The same group of investigators also reported that chronic rhinitis reported in the second assessment was significantly associated with acute exposure to chlorine and that chronic lower airway symptoms were more frequent in the second assessment among workers reporting chronic rhinitis on both assessments than in others (Leroyer et al. 1999).

Effects in Animals. The studies in animals support the findings in humans, particularly the nature of the acute effects of exposure to high concentrations of chlorine gas. A great deal of information regarding the toxicity of chlorine in animals was generated during World War I and the years that followed triggered by the use of chlorine as a chemical weapon during the war. A summary of the earlier studies conducted in various European countries as well as in the United States and Russia can be found in Withers and Lees (1985b). Additional information on the early literature, particularly from extensive studies in dogs, can be found in DOA (1933).

More recently, studies in rodents have confirmed the earlier observations regarding high exposures and have provided valuable information regarding the irritant properties of chlorine.

Acute exposure to low-to-moderate concentrations of chlorine induces a reduction in the respiratory rate, a protective reflex response mediated by stimulation of trigeminal nerve endings in the nasal mucosa. The concentration of the chemical that induces a 50% decrease in respiratory rate is termed RD_{50}. For example, RD_{50} values of 9.3 and 3.5 ppm were determined in mice exposed for 10 and 60 minutes, respectively (Barrow et al. 1977; Gagnaire et al. 1994). An RD_{50} of 25 ppm was determined in male F344 rats exposed to chlorine for 10 minutes (Barrow and Steinhagen 1982). This study also demonstrated the development of tolerance to chlorine since in rats pre-exposed to chlorine at 1, 5, or 10 ppm intermittently for 32 weeks, the RD_{50} values were 90, 71, and 454 ppm, respectively, measured 16–24 hours after the last day of pre-exposure. Barrow and Steinhagen (1982) speculated that the mechanism of tolerance may involve reactions of chlorine with sulfhydryl groups in the receptors or that chlorine exposure may damage the free nerve endings in the respiratory nasal mucosa. Rats pre-exposed to chlorine also developed cross-tolerance to formaldehyde (Chang and Barrow 1984). Interestingly, rats pre-exposed to 15 ppm formaldehyde did not develop tolerance to formaldehyde, but did develop cross-tolerance to chlorine, which suggested the existence of different reactive sites for the two gases (Chang and Barrow 1984).

A study by the same group of investigators examined the effects of chlorine on lung -SH content and on the enzymes that maintain non-protein -SH levels, glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase (GSSG-RED) in rats exposed to 0 or 12 ppm chlorine for up to 2 weeks and sacrificed at various times after cessation of exposure (Dodd et al. 1980). The results showed no significant alterations in lung protein -SH, non-protein -SH, G6PD, or GSSG-RED in rats sacrificed immediately after 1, 5, or 10 days of exposure. Rats sacrificed 3 or 6 days after exposure showed an increase in lung -SH, G6PD, and GSSG-RED. These parameters returned to control values after 10 days of recovery. The investigators concluded that the increase in lung -SH and enzymatic activities observed during the recovery periods may reflect reparative processes subsequent to damage induced by chlorine. A different study by the same group showed that exposures to up to 10 ppm chlorine for 12 hours did not alter the total sulfhydryl content (TSH) of the olfactory mucosa but lower concentrations did reduce TSH in the respiratory mucosa, suggesting that inhaled chlorine can oxidize tissue sulfhydryl groups at the point of entry, but not at deeper regions of the respiratory tract (McNulty et al. 1983). McNulty et al. (1983) also found that exposure to 5 ppm for 6 hours or 10 ppm for 3 hours (concentration times

exposure=30) produced similar reductions in TSH, but exposure to 2.5 ppm for up to 12 hours did not significantly affect TSH content. The investigators speculated that a threshold concentration may be needed to overwhelm the protective mechanism in the respiratory mucosa (perhaps the mucociliary flow) allowing chlorine to penetrate deeper into the underlying tissue.

Acute studies also have examined respiratory function in animals.

Barrow and Smith (1975) evaluated inspiratory-expiratory flow rate ratios (Vi/Ve) and volume-pressure relationships (lung compliance) in rabbits exposed to 0, 50, 100, and 200 ppm chlorine for 10 minutes. The tests were conducted 0.5 hours after exposure and after 3, 14, and 60 days without exposure. After the last test, the rabbits were killed and the lungs were removed for gross and microscopic examination. Rabbits exposed to 50 ppm showed mild pneumonitis, which was also observed in control animals; this exposure level did not induce significant changes in air flow ratios, but transiently decreased lung compliance. Exposure to 100 or 200 ppm induced transient concentration related increases in Vi/Ve and a decrease, followed by an increase, in pulmonary compliance; these changes are related to gross signs of pulmonary edema and microscopic changes characterized by chronic pneumonitis and anatomic emphysema. Rabbits allowed to recover for 14 or 60 days showed no “specific airway pathology.”

In another study, mice exposed for 15 minutes to 0.8, 2, 3.1, or 3.8 ppm chlorine showed concentration-related decreases in respiratory frequency and increases in specific airway resistance (Morris et al. 2005). Pretreatment with atropine did not alter the increase in airway resistance, suggesting that this response does not involve parasympathetic cholinergic endings. However, pretreatment with capsaicin, a sensory nerve toxin, dramatically reduced respiratory irritation and the obstructive response, suggesting the involvement of sensory nerves. Mice exposed to much higher concentrations of chlorine (100–800 ppm) for 15 minutes showed increased airways resistance and increased responsiveness to methacholine and microscopic examination of the lungs showed flattening of the epithelium and epithelial cell loss and changes associated with oxidative stress (Martin et al. 2003). Since the increased responsiveness to methacholine could be prevented by inhibition of nitric oxide synthase, it appeared that nitric oxide (NO) production may have contributed to the airway response to inhaled chlorine.

Jiang et al. (1983) studied the time course of the histopathological alterations in the respiratory tract of rats and mice exposed to the RD_{50} of 9.1 ppm chlorine 6 hours/day for 1–5 days. The animals were killed immediately after the last exposure and the nose, larynx, trachea, and lungs were processed for microscopic examination. In both species, lesions were seen in the nasal passages with less severe

changes in the nasopharynx, larynx, trachea, and lungs. The lesions in the nasal passages involved both the olfactory and respiratory epithelia; the nasal squamous epithelium showed minimal change. Lesions in the respiratory epithelium included acute epithelial degeneration with epithelial cell exfoliation, erosion, and ulceration after 1 and 3 days of exposure, infiltration by neutrophils after 3 and 5 days, and squamous metaplasia after 5 days. After 3 and 5 days of exposure, the epithelial lesions and inflammatory response found in the respiratory and olfactory mucosa became progressively more severe and extended more posteriorly. The most severe changes occurred in the olfactory mucosa of the anterior portion of the dorsal meatus, which showed extensive epithelial erosion and ulceration. Less severely affected areas showed necrosis and variable loss of sensory cells. Scanning electron microscopy showed loss of olfactory cilia and cellular exfoliation. The larynx and trachea showed acute degeneration of respiratory epithelial cells, whereas the lungs showed moderate to severe peribronchiolitis. Exposure of mice to the RD_{50} of 9.3 ppm for 5 days induced severe exfoliation, erosion, ulceration, and necrosis of the nasal respiratory epithelium (Buckley et al. 1984). It also induced minimal inflammation and squamous metaplasia. In the olfactory epithelium, chlorine induced severe ulcerations and necrosis and degeneration of sensory nerve endings. In the trachea, the lesions ranged from mild to moderate epithelial exfoliation, hyperplasia, and squamous metaplasia. In the lungs, chlorine induced a moderately severe terminal bronchiolitis with occlusion of affected bronchioles by serocellular exudate. Mice killed 72 hours after the last exposure had reduced inflammation and exudate, but there was little difference in the extent of ulceration and degenerative lesions, suggesting that the 3-day period was insufficient for complete repair of the lesions.

Exposure of rats to much higher concentrations of chlorine (50–1,500 ppm for 1–10 minutes) induced increasing lung damage that partially resolved within 24 hours of exposure (Demnati et al. 1995). In rats exposed to 1,330 ppm chlorine for 15 minutes, pulmonary changes observed 45 days after exposure included interstitial fibrosis and thickening of the alveolar septa due to thickening of the basement membrane (Yildirim et al. 2004). Exposure of rats to ≥184 ppm chlorine for 30 minutes induced hypoxemia, respiratory acidosis, and hypercapnia, and increased the surfactant surface tension and protein content of the bronchoalveolar lavage fluid, suggesting alveolar epithelial injury (Leustik et al. 2008). Less evidence of lung injury was seen in rats that were injected before exposure to chlorine with a mixture of ascorbate, deferoxamine, and N-acetyl-L-cysteine, suggesting that damage could be ameliorated by low-molecular antioxidants (Leustik et al. 2008). Exposure of mice to 221–289 ppm chlorine for 60 minutes caused severe lung inflammation as evidenced by widespread neutrophil influx into the lung parenchyma 6 hours after exposure followed by a clustering of neutrophils around damaged

airways 24 hours after exposure (Tian et al. 2008). Histologically, chlorine caused massive sloughing of the airway epithelium that was evident 6 hours after exposure.

Two intermediate-duration studies in animals were located.

Barrow et al. (1979) evaluated the respiratory response in F344 rats exposed to 0, 1, 3, or 9 ppm chlorine 6 hours/day, 5 days/week for 6 weeks. Nasal discharge was seen occasionally in rats exposed to 1 ppm, but was common in rats exposed to 3 and 9 ppm. Respiratory difficulty was also apparent in some rats exposed to 9 ppm. At termination, gross necropsy revealed accumulation of inflammatory reactions in the upper nasal passages in rats exposed to 3 and 9 ppm chlorine. Microscopic evaluations showed indications of inflammatory reactions in the upper and lower respiratory tract of high-dose males and females. The nasal turbinates showed mucopurulent inflammation with secretory material and erosions of the mucosal epithelium. Changes in the trachea and bronchi consisted mostly of hyperplasia of the epithelial lining and inflammatory reactions. The alveolar sacs contained macrophages and secretory material and epithelial cells showed necrosis, hypertrophy and hyperplasia. Alterations in rats exposed to 1 and 3 ppm were less extensive and were limited to focal mucopurulent inflammation of the nasal turbinates in females. Males exposed to 1 or 3 ppm showed deeper pulmonary changes consisting of slight to moderate inflammatory reaction around the respiratory bronchioles and alveolar ducts, increased alveolar macrophages, and isolated areas of atelectasis (incomplete expansion). A LOAEL of 1 ppm for respiratory effects can be defined in this study based on the presence of inflammatory changes in the nasal turbinates of females and in the lungs of males; no NOAEL was established.

A similar study examined clinical signs, lung function, and histopathology of the nasal turbinates and lungs from F344 rats exposed to 0, 0.5, 1.5, or 5 ppm chlorine 6 hours/day, 5 days/week for 62 days (Kutzman 1983). Pulmonary function tests (plethysmograph-based assessment of multiple end points, including lung and tidal volumes, breathing frequency, transpulmonary pressure, lung compliance, N_2 washout, diffusing capacity for CO, maximum expiratory flow volume, peak expiratory flow, and airway resistance) were conducted in 21–24 anesthetized males 6 hours after the last exposure. Respiratory tissues from these rats were prepared for histopathology. The lungs from some of these rats were also examined for collagen, elastin, total protein, and DNA. Exposure to 5 ppm cause severe upper respiratory irritation; exposure to 1.5 ppm showed occasionally less severe signs of irritation, whereas exposure to 0.5 ppm caused no obvious signs of irritation or discomfort. The tests of pulmonary function did not reveal marked abnormalities. The most significant effect was a reduction in airflow at 75% of exhaled vital capacity in all exposed groups, indicating some degree of small airway involvement. There were no

histopathological alterations in the lungs and nasal turbinates, but there was a tendency in the trachea for loss of cilia and epithelium at 0.5 and 5 ppm chlorine. The lung biochemistry only showed an increased collagen concentration at 1.5 and 5 ppm. Based on upper respiratory irritation and loss of cilia and epithelium in the trachea, the exposure level of 0.5 ppm can be defined as a LOAEL for respiratory effects; no NOAEL was defined in this study. This study was used as the basis for derivation of an intermediate-duration inhalation MRL for chlorine.

Two studies have examined the effects of chronic exposure to chlorine on respiratory parameters in animals.

Wolf et al. (1995) exposed groups of F344 rats and $B6C3F_1$ mice (approximately 70/sex/exposure level) to 0, 0.4, 1, or 2.5 ppm chlorine gas for 2 years. Males from both species and female mice were exposed 6 hours/day, 5 days/week, whereas female rats were exposed 6 hours/day, 3 days/week. The reduced exposure of female rats was based on unpublished data from the investigators that showed female rats to have a greater sensitivity to repeated long-term exposure to chlorine. End points evaluated included gross and microscopic examination of the respiratory tract; the nasal passages were examined microscopically at five different levels. Both in rats and mice, there were no gross lesions attributable to exposure to chlorine, and microscopic evaluation of the respiratory tract showed that chlorine-related effects were restricted to the nasal passages. In the study, the incidences were presented as percentages of all animals for which the nasal passages were adequate for microscopic examination, but the numbers of animals examined were not provided. No lesions were seen in the larynx, trachea, bronchi, or bronchioles. In general, rats and mice exhibited similar types of lesions. For the most part, the nasal lesions were site-specific, but the severity and/or incidence were not always concentration-dependent. The majority of the nasal responses exhibited a rostral-to-caudal severity gradient. The lesions rarely extended to the nasopharyngeal meatus. Lesions observed included respiratory and olfactory epithelial degeneration, septal fenestration, mucosal inflammation, respiratory epithelial hyperplasia, squamous metaplasia, and goblet cell (rats only) hypertrophy and hyperplasia, and secretory metaplasia of the transitional epithelium of the lateral meatus. Also observed was intracellular accumulation of eosinophilic proteinaceous material involving the respiratory, transitional, and olfactory epithelia. Lesions were also observed in controls, but the incidences were significantly lower than in the treated groups. One of the lesions with the lowest incidence in controls was goblet cell hyperplasia in female rats (4%); the respective incidences in the 0.4, 1, and 2.5 ppm group were 71, 90, and 91%. In mice, olfactory epithelium atrophy exhibited one of the lowest incidences in controls (3%); the respective incidences in the 0.4, 1, and 2.5 ppm group were 20, 21, and 39%. In both cases, severity also was concentration-related. Based on the increased

incidence of various types of lesions in the nasal passages, the exposure level of 0.4 ppm constitutes a LOAEL for respiratory effects in rats and mice; a NOAEL was not defined.

Klonne et al. (1987) exposed Rhesus monkeys to 0, 0.1, 0.5, or 2.3 ppm chlorine 6 hours/day, 5 days/week for 1 year. Pulmonary diffusing capacity for CO and distribution of ventilation were evaluated monthly during the study. At termination, the nasal tissues (at the first palatine ridge and just posterior to the third, fifth, and seventh palatine ridges), trachea, and lungs were examined. There was no evidence of treatment-related effects on pulmonary function at any interval during the study. The only treatment-related histopathological effects consisted of focal epithelial hyperplasia characterized by increased cell numbers and loss of cilia and goblet cells in the respiratory epithelium of the nose and trachea. The affected areas of the nasal passages showed hypercellularity with loss of goblet cells and cilia. In some of these areas, the nuclei showed altered polarity. Lesions were more frequent on the angular margins of the turbinates and less frequent on the lateral wall or septum adjacent to these margins. In some cases, the respiratory epithelial hyperplasia was associated with mild suppurative inflammatory response. Lesions in the trachea resembled those in the nose, but were less severe and involved only a small circumferential section of the ventral and ventrolateral trachea. The combined incidences of lesions in the nasal mucosa, characterized as trace and mild in males and females, were 1/8, 3/8, 6/8, and 8/8 in the control, 0, 0.1, 0.5, and 2.3 ppm exposure groups, respectively. The lowest exposure concentration of 0.1 ppm chlorine is a LOAEL for nasal lesions in monkeys. This study was used as the basis for derivation of a chronic-duration inhalation MRL for chlorine.

Ibanes et al. (1996) re-examined the respiratory tissues from the chronic studies in monkeys, rats, and mice summarized above to better characterize the lesions and to improve human risk assessments based on these data sets. In general, the re-evaluation found a good correlation between the subjective scores of tissue responses in the original studies and quantitative analyses in the re-evaluation. The investigators also noted that the lesions in monkeys and rodents exhibited both differences and similarities. One notable difference was that monkeys showed less severe lesions, but extended more distally in the respiratory tract. The investigators concluded that respiratory tract airflow characteristics play a major role in lesion distributions in monkeys and rodents. Yet, with appropriate exposure and response adjustments, both rodents and Rhesus monkeys appear to be valid models for human risk assessment.

Cardiovascular Effects. It is not uncommon to find reports of tachycardia and elevated blood pressure in individuals admitted to emergency rooms following accidental exposure to high concentrations of chlorine, but how much of this can be attributed to chlorine exposure or to a stressful

situation is not clear. Some cases in which EKGs were performed shortly after exposure found no significant alterations (Chasis et al. 1947; Güloğlu et al. 2002; Ramachandran et al. 1990). Tachypnea and hypertension were frequently observed in persons who were hospitalized following a train derailment that released chlorine gas in South Carolina in 2005 (Van Sickle et al. 2009). In most persons in whom the conditions were observed, tachypnea and tachycardia persisted across examination. No specific mention of cardiovascular effects was found in studies that evaluated possible long-lasting effects of chlorine exposure.

Rats and mice that were exposed to lethal concentrations of chlorine had distended hearts (Weedon et al. 1940), but little additional information is available regarding cardiovascular effects in animals following exposure to chlorine. It is reasonable to assume that some cardiovascular parameters would be affected after acute exposure to high concentrations of chlorine as a result of the severe ventilation-perfusion mismatching and hypoxia caused by pulmonary edema; however, no evaluations were made in the studies available.

In an intermediate-duration study, rats exposed to 5 ppm chlorine for 62 days had no significant gross or microscopic alterations in the heart (Kutzman 1983). Intermittent exposure of monkeys to 2.3 ppm chlorine for 1 year (Klonne et al. 1987) or of rats and mice to 2.5 ppm chlorine for 2 years (Wolf et al. 1995) did not cause any significant gross or microscopic alterations in the heart and aorta.

Gastrointestinal Effects. Nausea and vomiting are common acute symptoms following exposure to high concentrations of chlorine and are believed to be reflex reactions and not a specific effect of chlorine.

Barrow et al. (1979) reported that rats exposed to 9 ppm chlorine 6 hours/day, 5 days/week for 6 weeks had focal erosion of the gastric mucosa, which was usually accompanied by focal inflammation of the adjacent submucosal areas. No such effects were seen in rats exposed to 3 ppm chlorine. The investigators suggested that general stress or perhaps ingestion of hydrochloric acid and/or hypochlorous acid as a result of grooming or deposition in the oral cavity may have caused the condition. The results of Barrow et al. (1979) could not be confirmed or refuted in a study of very similar design by Kutzman (1983) because the latter did not examine the gastrointestinal tract.

Intermittent exposure of monkeys to up to 2.3 ppm chlorine for 1 year or of rats and mice to up to 2.5 ppm for 2 years did not produce gross or microscopic alterations in the gastrointestinal tract (Klonne et al. 1987; Wolf et al. 1995).

Hematological Effects. Leukocytosis is a common finding in individuals acutely exposed to high concentrations of chlorine, and is most likely a clinical manifestation of acute inflammation and tissue destruction rather than a specific effect of chlorine. Hypoxemia is also characteristic of exposures high enough to cause pulmonary edema and consequently, interference in gas exchange.

Experiments in dogs conducted by Underhill (1920) following World War I showed that following gassing with high concentrations of chlorine, there was a relative increase in red blood cells and hemoglobin that reached a peak approximately 5 hours after exposure and was due to a decrease in intravascular fluid content. He also observed that in dogs exposed to relatively low concentrations of chlorine, there was slight leukocytosis, which was caused solely by an increase in polymorphonuclear cells. An intermediate-duration study in rats reported that exposure to 9 ppm chlorine for 6 weeks induced a significant increase in hematocrit (females) and segmented neutrophils (males and females) (Barrow et al. 1979). No significant changes in hematological parameters were observed in monkeys, rats, or mice exposed to 2.3–2.5 ppm chlorine in chronic duration studies (Klonne et al. 1987; Wolf et al. 1995).

Musculoskeletal Effects. The only information regarding musculoskeletal effects and exposure to chlorine is a report in which a 25-year-old man was diagnosed with myasthenia gravis manifested as laryngeal stridor after accidental exposure to chlorine gas (Foulks 1981). Myasthenia gravis is an autoimmune disorder that causes a reduction in the number and/or sensitivity of acetylcholine receptors at the neuromuscular junction resulting in muscle weakness. Foulks (1981) speculated that the massive exposure of the laryngeal muscles to chlorine may have altered the neuromuscular junction in a way that caused an autoimmune stimulus that resulted in myasthenia gravis.

Exposure of monkeys to up to 2.3 ppm chlorine for 1 year or of rats and mice to up to 2.5 ppm for 2 years did not produce gross or microscopic alterations in skeletal muscle (Klonne et al. 1987; Wolf et al. 1995).

Hepatic Effects. Very limited information was located regarding evaluation of liver end points in patients after acute high exposure to chlorine. Leube and Kreiter (1971) reported that 1 day after exposure to chlorine, 26 out of 65 patients (40%) had elevated serum ALT and 2 out of 13 (15%) had elevated

serum aspartate aminotransferase (AST) values. Without any information on other factors that impact the levels of these enzymes in serum, such as alcohol consumption, it is difficult to ascertain the toxicological significance of this finding. Moulick et al. (1992) mention that measurements of serum AST activity in patients admitted to the hospital following exposure to chlorine did not show any significant abnormality. The exposure concentration was not known, but 2 hours after the massive leak, the level of chlorine was found to be 66 ppm. Hasan et al. (1983) reported that subjects exposed to chlorine gas following a leak from a liquid storage tank had normal liver function tests; the tests were not specified.

Liver congestion has been observed in rodents exposed to acute lethal concentrations of chlorine (i.e., Weedon et al. 1940), but generally, other low-concentration, acute-duration studies did not examine the liver, or if they did, the findings were not reported. In an intermediate-duration study, rats exposed to ≥3 ppm chlorine, but not 1 ppm, 6 hours/day, 5 days/week for 6 weeks had cytoplasmic vacuolation in the hepatocytes (Barrow et al. 1979). This was accompanied by a significant increase in serum alkaline phosphatase at 3 and 9 ppm in males and females and an increase in serum alanine aminotransferase (ALT) in females exposed to 9 ppm. In contrast, in a study of very similar design, rats exposed to up to 5 ppm chlorine 6 hours/day, 5 days/week for 62 days showed no significant histological alterations in the liver (Kutzman 1983). The reason for this apparent discrepancy is not apparent.

Intermittent exposure of monkeys to up to 2.3 ppm chlorine for 1 year or of rats and mice to up to 2.5 ppm for 2 years did not produce gross or microscopic alterations in the liver (Klonne et al. 1987; Wolf et al. 1995).

Renal Effects. No reports were located of renal effects in humans following inhalation exposure to chlorine. However, it would have not been unexpected to find some abnormal indices of renal function in individuals acutely exposed to high levels of chlorine as a consequence of severe cardiopulmonary alterations.

Slight to moderate congestion of the kidneys was reported in male and female rats exposed intermittently to 9 ppm chlorine for 6 weeks, but no significant alterations were seen in rats exposed to 3 ppm (Barrow et al. 1979). Kutzman (1983) examined the kidneys of rats exposed to up to 5 ppm chlorine for 62 days and reported no significant gross or microscopic alterations. Intermittent exposure of monkeys to up to 2.3 ppm chlorine for 1 year or of rats and mice to up to 2.5 ppm for 2 years did not produce gross or microscopic alterations in the kidney (Klonne et al. 1987; Wolf et al. 1995).

Endocrine Effects. No information was located regarding endocrine effects in humans following inhalation exposure to chlorine. No gross or microscopic alterations were reported in the adrenals, pancreas, parathyroid, pituitary, or thyroid gland from monkeys exposed to up to 2.3 ppm chlorine for 1 year (Klonne et al. 1987). Similarly, no gross or histological alterations were found in the pancreas, parathyroid, pituitary, or thyroid from rats and mice exposed to up to 2.5 ppm chlorine for 2 years (Wolf et al. 1995). No further information on endocrine effects in animals exposed to chlorine was located in the literature.

Dermal Effects. Considering that chlorine is an irritating gas, very few reports have described skin effects in subjects exposed to high concentrations of the gas. A health assessment of a population that may have been exposed to up to 200 ppm chlorine after tanker cars derailed reported that skin rashes and skin burns affected 16–25% of a total of 682 persons interviewed 2 weeks after the accident (Agency for Toxic Substances and Disease Registry 1998). In another train derailment, some exposed subjects had minor first-degree skin burns resulting from the vapor exposure (Joyner and Durel 1962). A health evaluation report of firefighters that responded to a chlorine gas leak in Henderson, Nevada, mentions that many of the firefighters complained of skin irritation (NIOSH 1995). During the incident, chlorine concentrations in the air ranged from <0.2 to 17 ppm.

Studies in animals have not described skin alterations following exposure to chlorine gas, even in high exposure acute-duration studies in which animals experienced whole-body exposures. In monkeys exposed to up 2.3 ppm chlorine for 1 year, histological examination of samples of skin did not reveal any significant exposure-related alterations (Klonne et al. 1987). The same negative observations were made in rats and mice exposed to up to 2.5 ppm chlorine for 2 years (Wolf et al. 1995).

Ocular Effects. Almost all reports of acute exposure to high concentrations of chlorine mention eye irritation (lacrimation, conjunctivitis, burning sensation) as one of the most prevalent complains among the patients. These effects usually resolve within a few days after exposure. In a controlled exposure study with volunteers, complaints of itching or burning of the eyes were significantly greater during exposures to 1 ppm chlorine than during control exposures (Anglen 1981).

Swelling around the eyes was reported in rats exposed to 12 ppm chlorine 6 hours/day, 5 days/week for 10 days, but not for 5 days (Dodd et al. 1980). Ocular irritation was also reported in rats exposed intermittently to 3 ppm chlorine for 6 weeks, but no irritation was apparent at 1 ppm (Barrow et al. 1979). This is more or less consistent with the results of another intermediate-duration study that reported

occasional signs of eye irritation in rats exposed to 1.5 ppm for 62 days and severe irritation in rats exposed to 5 ppm chlorine (Kutzman 1983). Monkeys exposed intermittently to 2.3 ppm for 1 year showed conjunctival irritation with some exudation at the end of the study, but there was no gross or microscopic evidence of chronic changes in the conjunctiva or in the cornea (Klonne et al. 1987). No ocular effects were observed in rats or mice exposed to up to 2.5 ppm chlorine for 2 years (Wolf et al. 1995).

Body Weight Effects. No relevant information was located regarding body weight effects in humans following inhalation exposure to chlorine. Reduced body weight gain or weight loss has been commonly reported in acute high-exposure studies in animals of enough duration to allow evaluation of weight changes. Although rarely reported, the reduced growth or weight loss is largely due to reduced food consumption. For example, rats exposed to 12 ppm chlorine 5 days/week for 2 weeks lost approximately 20% of their body weight during the second week relative to their starting weight (Dodd et al. 1980). Pair-fed rats also lost weight, confirming that the effect in the chlorine-exposed rats was due to reduced food intake. In another acute-duration study, rats exposed to 9.1 ppm chlorine for 3 or 5 days lost 11 and 13%, respectively, of the initial body weight (Jiang et al. 1983). Under the same exposure conditions, mice lost 17 and 21% of the initial body weight (Jiang et al. 1983).

Similar results have been obtained in intermediate-duration studies. Final body weight of rats exposed to 9 ppm chlorine for 6 weeks (Barrow et al. 1979) or to 5 ppm for 62 days (Kutzman 1983) were >30% lower than controls. Even a relatively low concentration of chlorine of 0.5 ppm for 62 days lowered body weight in female rats by approximately 11% relative to controls (Kutzman 1983). Neither study provided information on food or water consumption. Exposure of monkeys to 2.3 ppm chlorine for 1 year (Klonne et al. 1987) or of rats and mice to 2.5 ppm for 2 years (Wolf et al. 1995) reduced body weight gain during the studies, but the final weights, judging by figures in the papers, appeared to be <10% lower than the respective controls.

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located that evaluated immunocompetence or effects on lymphoreticular organs in humans following exposure to chlorine gas.

Minimal information is available from studies in animals. Barrow et al. (1979) reported that the spleen and thymus from rats exposed to 9 ppm chlorine for 6 weeks had a decreased content of lymphoid

elements, but no such changes were seen in rats exposed to 1 or 3 ppm. According to the investigators, this may have been a function of the poor physical condition and decreased nutritional state of the rats in that dosing group. In a very similar study, no histological alterations were reported in the spleen and peribronchial lymph nodes from rats exposed to up to 5 ppm chlorine for 62 days (Kutzman 1983). Studies in monkeys, rats, and mice also have found no gross or microscopic lesions in lymphoreticular organs and tissues following intermittent chronic exposure to 2.3–2.5 ppm chlorine (Klonne et al. 1987; Wolf et al. 1995).

The highest NOAEL values and all LOAEL values from each reliable study for lymphoreticular effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.4 Neurological Effects

Symptoms effects such as headache, dizziness, anxiety, and syncope are commonly reported following acute high exposures to chlorine and are thought to be due, at least in part, to asphyxia induced by chlorine.

In a case of high exposure to chlorine that resulted in the death of the patient, postmortem examination showed a swollen brain with flattening of convolutions and subarachnoid hemorrhage (Adelson and Kaufman 1971). The investigators speculated that the lesions could have been caused by hypoxia that resulted from the severe pulmonary effects. In another case report, a 60-year-old man who accidentally inhaled chlorine gas in a swimming pool accident had a magnetic resonance scan of the head conducted 2 years after the accident that showed multiple areas of decreased signal in the periventricular white matter (Levy et al. 1986). Other neurological tests showed no evidence of cranial nerve abnormalities or sensory deficits. This brief communication does not mention what might have prompted the subject to undergo the scan.

Kilburn (1995, 2000, 2003b) published a series of reports describing long-lasting neurological effects in subjects accidentally exposed to high concentrations of chlorine gas under various scenarios. The earliest study (Kilburn 1995) reported that six subjects exposed to an undetermined concentration of chlorine for 3 minutes to 5 hours had difficulty concentrating and sleeping, dizziness, loss of balance, excessive fatigue, loss of strength, depression, and irritability during a period of 1–3 years after the accident. Neurobehavioral tests were conducted 15–50 months after exposure and the results were compared to a control group matched for sex, age, and education. It should be noted that the testers were aware of the

exposure status of the subjects. The results showed impaired balance with the eyes closed and hearing loss in all of the exposed subjects. Five had decreased vibration sensitivity, color discrimination, and verbal recall; four had prolonged blink reflex latency; three had prolonged simple and choice reaction times, and three had nerve defects or constricted visual fields. In a subsequent study, 22 patients exposed briefly (the reports mentions seconds to a few minutes in one section and minutes to a few hours in another section) to chlorine gas were evaluated with a battery of tests 7–48 months after exposure. A total of 296 unexposed subjects served as controls. The results showed significant impairment among the exposed group in a number of areas including balance, reaction time, color identification, visual field performance, blink latency, cognition, verbal recall, and making trails. A similar study was conducted with subjects exposed to chlorine as a result of a train derailment (see Agency for Toxic Substances and Disease Registry [1998] under Respiratory Effects) (Kilburn 2003b). Ninety-seven subjects were tested 7 weeks after exposure and 57 were tested 3 years later; 26 were tested on both occasions. Seven weeks after exposure, the exposed subjects showed impairment in five neurobehavioral functions compared with unexposed subjects recruited for the 3-year testing. At 3 years, the patients showed impairment in seven additional tests compared with controls. Because of lack of exposure data, these studies are not listed in Table 3-1.

There are no studies in animals that could confirm or refute Kilburn's findings mentioned above. The only information regarding neurological effects of chlorine in animals is limited to reports of no gross or microscopic alterations in the brain of rats exposed intermittently to up to 5 ppm chlorine for 62 days (Kutzman 1983), or in the brain, spinal cord, and sciatic nerve of rats and mice exposed to up to 2.5 ppm chlorine for 2 years (Wolf et al. 1995). Also, in monkeys exposed intermittently for 1 year to up to 2.3 ppm chlorine, there were no gross or histological alterations in central or peripheral nervous system tissues (Klonne et al. 1987). The investigators also mentioned that clinical observational neurological examinations conducted in the monkeys prior to sacrifice were unremarkable, but the scope of these tests was not specified.

The highest NOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

The only information available regarding effect in humans is that evaluation of the outcome of 15 pregnancies among female workers at a chlorine plant in 1932–1933 did not provide any evidence of reproductive toxicity (Skljanskaya et al. 1935).

In an intermediate-duration inhalation study, male and female rats were exposed intermittently to up to 5 ppm chlorine for 62 days (Kutzman 1983). At the end of the exposure period, 8 exposed males were mated with unexposed females and 10 exposed females were mated with unexposed males and all females were sacrificed on gestation day 19 for evaluation of reproductive end points. The results showed no significant effects of chlorine exposure on fertility, number of corpora lutea, viable embryos, early or late deaths, or pre-implantation loses. In addition, in males exposed for 62 days, there were no histological alterations in the testes, and sperm morphology was unremarkable.

In chronic-duration studies, exposure of male and females monkeys for 1 year or male and female rats and male mice for 2 years to up to 2.5 ppm chlorine did not result in gross or microscopic alterations of the reproductive organs (Klonne et al. 1987; Wolf et al. 1995). Female mice developed a concentration-related increase in ovarian abscesses and of suppurative inflammation of the uterus. Without a further discussion, the investigators stated that it was unlikely that chlorine induced the inflammatory response, but no alternative explanation was provided.

The highest NOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

In a summary of the limited information available, WHO (1982) reports that early studies from the Russian literature found no evidence of teratogenic in offspring from effects female workers at a chlorine plant in 1932–1933 and that rabbits exposed to low chlorine concentrations (0.6–1.6 ppm) during pregnancy gave birth to healthy offspring. No additional information was located regarding developmental effects in animals following inhalation exposure to chlorine.

3.2.1.7 Cancer

There are several studies of cancer in humans that involve exposure to chlorine, among other chemicals, in occupational settings.

A study of 28 employees at a Texas chemical plant who had died of primary intracranial neoplasms found no evidence that exposure to chlorine may have played a role (Bond et al. 1983). Chlorine was one of the chemicals to which cases and controls had the most frequent potential for exposure. Bond et al. (1985) conducted a case-control study of 26 renal cancer deaths among employees of a multiple process chemical production facility. Although an increased odds ratio (OR) for renal cancer was found in a chlorine production area, it was not attributed to chlorine exposure, but to asbestos and caustic materials. In addition, in the magnesium processing area, where large amounts of chlorine were used, there was a decreased risk of renal cancer. A nested case-control study of 306 lung cancer deaths among 19,608 employees of a chemical plant provided no evidence that chlorine had a role in the deaths (Bond et al. 1986). The OR for chlorine exposure was 1.08 (95% confidence interval [CI], 0.81–1.44).

A study of 51 lung cancer cases at a dye and resin manufacturing plant reported an increased OR for lung cancer for employees who were seen at the plant infirmary for acute exposure to chlorine after adjusting for smoking (OR=27; 95% CI, 3.5–205) (Barbone et al. 1992). However, the number of cases was small (6 cases versus 3 out of 102 controls), and four of the six cases worked in the anthraquinone dye and epichlorohydrin production area. Barbone et al. (1992) stated that based on small number of cases, the small association between lung cancer and acute exposure to chlorine may be due to chance or to confounding by other unidentified exposures.

In a larger study of 2,391 male workers producing magnesium metal, Heldaas et al. (1989) found 4 cases of lung cancer versus 1.3 expected in a subset of workers who experienced chlorine intoxication and had at least 20 years since first employment (95% CI, 0.8–7.8). However, the rate ratios for lung cancer were higher in those workers who were not registered in the chlorine exposure list. The authors speculated that the use of respiratory protective gear (mouthpieces) may have been a reason for the difference.

In a study of 1,190 workers at chlor alkali plants, there was a marginally significant excess of lung cancers (10 observed versus 4.9 expected; 95% CI, 1.0–3.8) which, according to the authors, was possibly due to previous use of asbestos (Barregård et al. 1990). Jäppinen et al. (1987) conducted a retrospective cohort study of 3,545 workers in the Finnish pulp and paper industry and found 78 cases of lung cancer

versus 62.6 expected (95% CI, 98–155). The excess was most prominent in board mill workers (40 observed versus 18.1 expected; 95% CI, 158–302), particularly after 20 years of latency (25 observed versus 7.8 expected; 95% CI, 209–476). However, there is no mention in the study of the chemicals to which the various subcohorts (based on work histories) may have been more intensely exposed.

Only the study by Wolf et al. (1995) provided information on carcinogenicity of inhaled chlorine in animals. In that study, male and female rats and mice were exposed intermittently to up to 2.5 ppm chlorine for 2 years. Gross and histological examination of all major tissues and organs, including the nasal cavity at five levels did not show any biologically or statistically significant increase in neoplasms.

The EPA, the International Agency for Research on Cancer (IARC), and the Department of Health and Human Services (DHHS) have not classified chlorine gas as to its carcinogenicity.

3.2.2 Oral Exposure

Studies that used tap water as the medium for delivery of chlorine to experimental animals are not included in this section to eliminate potential confounding effects of chlorination byproducts.

Although people are not likely to be exposed to chlorine itself through oral routes, there are reports of accidental or intentional exposure to bleach (typically a 3–6% solution of sodium hypochlorite). Information from some of those reports is also included in this section.

3.2.2.1 Death

Human deaths have been reported following ingestion of sodium hypochlorite. In a review of the literature, Racioppi et al. (1994) state that the lethal dose of sodium hypochlorite in adults has been reported to be approximately 200 mL of a solution containing 3–6% chlorine. Racioppi et al. (1994) also indicate that the aspiration of bleach in the lungs following ingestion as been reported as the cause of fatalities. Ross and Spiller (1999) described a fatal case of a 66-year-old woman who ingested an unknown quantity of bleach and died of cardiac arrest 4.5 hours after the bleach ingestion. Autopsy revealed esophageal and gastric mucosal erosions, perforations at the gastroesophageal junction, and extensive necrosis of adjacent soft tissue. Jakobsson et al. (1991) reported the case of a fatal poisoning in a 1-year-old girl after ingestion of a household cleanser containing 4.5% sodium hypochlorite in an alkaline solution. Examination of the upper gastrointestinal tract showed severe gross and microscopic damage. According to unpublished information reviewed by Racioppi et al. (1994), the oral LD_{50} for a

5.25% solution of sodium hypochlorite household bleach in rats is 13,000 mg/kg; for a 13% solution, LD_{50} values of 5,000 and 8,200 mg/kg are listed. A review of the literature by BG-Chemie (1991) indicates that LD_{50} values of 6,800 and 5,800 mg/kg were determined for male and female ddY rats, respectively.

3.2.2.2 Systemic Effects

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-2.

Respiratory Effects. Respiratory rate was not altered in a group of 10 volunteers who drank 0.5 L/day of drinking water containing 5 mg/L chlorine for 12 weeks (Lubbers et al. 1982). This corresponds approximately to doses of 0.036 mg Cl/kg/day, assuming a body weight of 70 kg. Evaluations of the respiratory rate were done weekly during the dosing period and continued for an additional 8 weeks post-dosing. Since the study did not control for non-experimental ingestion of chlorine by the volunteers, the total daily dose of chlorine consumed is likely to have been higher than 0.036 mg Cl/kg/day. In a review of the literature, Racioppi et al. (1994) mention the case of a woman who developed aspiration pneumonitis after ingesting an unknown amount of hypochlorite bleach. Bracco et al. (2005) described a similar case. In this case, chest x-rays performed 2 hours after intoxication showed bilateral bibasal infiltrate suggestive of aspiration pneumonia. Over the next 24 hours, the patient's respiratory condition declined and she required ventilatory support. The patient eventually recovered after 26 days of mechanical ventilation. In the fatal case of bleach ingestion described by Jakobsson et al. (1991), microscopic examination of the lungs showed aspiration of epithelium derived from the upper respiratory tract, and signs of acute bronchitis.

Limited data on respiratory effects are available in animals. Ninety-day drinking water studies in Sprague-Dawley rats (Daniel et al. 1990) and $B6C3F_1$ mice (Daniel et al. 1991) dosed with up to 24.9 and 39.2 mg Cl/kg/day, respectively, reported no gross or microscopic alterations in the lungs, trachea, and nasal turbinates. Furukawa et al. (1980) also reported no significant histological alterations in the lungs and bronchial tube of F344 rats dosed with up to 85 mg Cl/kg/day for 92 days. Similar results were reported in chronic-duration studies in F344 rats (Hasegawa et al. 1986; NTP 1992) and $B6C3F_1$ mice (NTP 1992). Rats received doses of up to 133 mg Cl/kg/day and mice received doses up to 24.2 mg Cl/kg/day for 2 years.

Table 3-3 Levels of Significant Exposure to Hypochlorite Solution - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL: Less Serious (mg/kg/day)	LOAEL: Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Systemic								
1	Rat (Sprague-Dawley)	once (G)	Hepatic		20 M (increased triacylglycerols in whole liver homogenate)		Chang et al. 1981	No other end points were evaluated.
2	Rat (Wistar)	14 d (GW)	Cardio	200 F			Cunningham 1980	Only organ weight was assessed.
			Hepatic	200 F				
			Renal	200 F				
			Bd Wt	200 F				
Neurological								
3	Rat (Wistar)	14 d (GW)		200 F			Cunningham 1980	Only brain weight was assessed.
INTERMEDIATE EXPOSURE								
Systemic								
4	Human	4 wk (W)	Hepatic	0.4			Wones et al. 1993	NOAELs are for serum thyroid hormones and serum lipid profile.
			Endocr	0.4				
5	Rat (Sprague-Dawley)	12 mo (W)	Hemato	12 M			Abdel-Rahman et al. 1984	

Table 3-3 Levels of Significant Exposure to Hypochlorite Solution - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
6	Rat (Long- Evans)	66-76 d (GW)	Hemato	3.4			Carlton et al. 1986	NOAELs are for blood counts and serum thyroid hormone levels.
			Endocr	3.4				
			Bd Wt	3.4				
7	Rat (Wistar)	6 wk (W)	Bd Wt	15.7 M			Cunningham 1980	
8	Rat (Sprague-Dawley)	90 d (W)	Resp	24.9 F			Daniel et al. 1990	NOAELs are for gross and microscopic evaluation of tissues and organs, and hematology and clinical chemistry.
			Cardio	24.9 F				
			Gastro	24.9 F				
			Hemato	24.9 F				
			Musc/skel	24.9 F				
			Hepatic	24.9 F				
			Renal	24.9 F				
			Endocr	24.9 F				
			Dermal	24.9 F				
			Bd Wt	24.9 F				

Table 3-3 Levels of Significant Exposure to Hypochlorite Solution - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
9	Rat (Sprague-Dawley)	8 wk (W)	Bd Wt	4.1 M			Exon et al. 1987	
10	Rat (Fischer- 344)	92 d (W)	Resp	85 M			Furukawa et al. 1980 hypochlorous acid and sodium hypochlorite	NOAELs are for histopathology of tissues and organs, clinical chemistry and hematology.
			Cardio	52 F	84 F (endocardial hyperplasia; myocardial fibrosis)			
			Gastro	85 M				
			Hemato	85 M				
			Hepatic	85 M				
			Renal	85 M				
			Endocr	85 M				
			Dermal	85 M				
			Bd Wt	26 M	50 M (19% reduction in final body weight)	85 M (46% reduction in final body weight)		
11	Rat (Fischer- 344)	13 wk (W)	Bd Wt	76	152 M (final body weight reduced 19%)	305 M (final body weight reduced 47%)	Hasegawa et al. 1986	Only body weight is listed due to incomplete reporting of other end points.

Table 3-3 Levels of Significant Exposure to Hypochlorite Solution - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
12	Mouse (B6C3F1)	90 d (W)	Resp	39.2 F			Daniel et al. 1991	NOAELs are for gross and histological evaluation of tissues and organs, adn hematology and clinical chemistry.
			Cardio	39.2 F				
			Gastro	39.2 F				
			Hemato	39.2 F				
			Musc/skel	39.2 F				
			Hepatic	39.2 F				
			Renal	39.2 F				
			Endocr	39.2 F				
			Dermal	39.2 F				
			Bd Wt	39.2 F				
13	Gn Pig (albino)	5 wk (W)	Bd Wt	26 M			Cunningham 1980	
Immuno/ Lymphoret								
14	Rat (Sprague-Dawley)	90 d (W)		24.9 F			Daniel et al. 1990	NOAEL is for gross and microscopic evaluation of lymphoreticular organs.

Table 3-3 Levels of Significant Exposure to Hypochlorite Solution - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
15	Rat (Sprague-Dawley)	8 wk (W)		4.1 M			Exon et al. 1987	Some reported changes in immune parameters of unknown toxicological significance were not considered adverse.
16	Rat (Fischer- 344)	92 d (W)		85 F			Furukawa et al. 1980 hypochlorous acid and sodium hypochlorite	NOAEL is for gross and microscopic evaluation of lymphoreticular organs.
17	Mouse (B6C3F1)	90 d (W)		39.2 F			Daniel et al. 1991	NOAEL is for gross and histological evaluation of lymphoreticular organs.
Neurological								
18	Rat (Sprague-Dawley)	90 d (W)		24.9 F			Daniel et al. 1990	NOAEL is for gross and microscopic evaluation of the brain and sciatic nerve.
19	Rat (Fischer- 344)	92 d (W)		85 F			Furukawa et al. 1980 hypochlorous acid and sodium hypochlorite	NOAEL is for gross and microscopic examination of the brain.

Table 3-3 Levels of Significant Exposure to Hypochlorite Solution - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
20	Mouse (B6C3F1)	90 d (W)		39.2 F			Daniel et al. 1991	NOAEL is for gross and histological evaluation of the brain and sciatic nerve.
Reproductive								
21	Rat (Long- Evans)	66-76 d (GW)		3.4			Carlton et al. 1986	NOAEL is for fertility and histopathology of the reproductive tract.
22	Rat (Sprague-Dawley)	90 d (W)		24.9 F			Daniel et al. 1990	NOAEL is for gross and microscopic evaluation of reproductive organs.
23	Rat (Fischer- 344)	92 d (W)		85 F			Furukawa et al. 1980 hypochlorous acid and sodium hypochlorite	NOAEL is for gross and microscopic evaluation of reproductive organs.
24	Mouse (B6C3F1)	90 d (W)		39.2 F			Daniel et al. 1991	NOAEL is for gross and histological evaluation of reproductive organs.
Developmental								
25	Rat (Long- Evans)	42 d (GW)		3.4			Carlton et al. 1986	NOAEL is for litter viability and size and pup weight.

Table 3-3 Levels of Significant Exposure to Hypochlorite Solution - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
CHRONIC EXPOSURE								
Systemic								
26	Rat (Fischer- 344)	2 yr (W)	Resp	133 F			Hasegawa et al. 1986	NOAELs are for gross and microscopic alterations in organs and tissues.
			Cardio	133 F				
			Gastro	133 F				
			Hemato	133 F				
			Hepatic	133 F				
			Renal	133 F				
			Endocr	133 F				
			Dermal	133 F				
			Ocular	133 F				
			Bd Wt		67 F (11% reduction in final body weight)			

Table 3-3 Levels of Significant Exposure to Hypochlorite Solution - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
27	Rat (Fischer- 344)	2 yr (W)	Resp	14.4 F			NTP 1992	NOAELs are for histopathology of tissues and organs and hematology parameters.
			Cardio	14.4 F				
			Gastro	14.4 F				
			Hemato	14.4 F				
			Hepatic	14.4 F				
			Renal	14.4 F				
			Endocr	14.4 F				
			Dermal	14.4 F				
			Bd Wt	14.4 F				
			Other	14.4 F				

Table 3-3 Levels of Significant Exposure to Hypochlorite Solution - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
28	Mouse (B6C3F1)	2 yr (W)	Resp	24.2 F			NTP 1992	NOAELs are for histopathology of tissues and organs and hematology parameters.
			Cardio	24.2 F				
			Gastro	24.2 F				
			Hemato	24.2 F				
			Hepatic	24.2 F				
			Renal	24.2 F				
			Endocr	24.2 F				
			Dermal	24.2 F				
			Bd Wt	24.2 F				
			Other	24.2 F				
Immuno/ Lymphoret								
29	Rat (Fischer- 344)	2 yr (W)		133 F			Hasegawa et al. 1986	NOAEL is for gross and microscopic appearence of the spleen.
30	Rat (Fischer- 344)	2 yr (W)		14.4 F			NTP 1992	NOAEL is for histopathology of lymphoreticular organs.

Table 3-3 Levels of Significant Exposure to Hypochlorite Solution - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
31	Mouse (B6C3F1)	2 yr (W)		24.2 F			NTP 1992	NOAEL is for histopathology of lymphoreticular organs.
Neurological								
32	Rat (Fischer- 344)	2 yr (W)		133 F			Hasegawa et al. 1986	NOAEL is for gross and microscopic appearence of the brain.
33	Rat (Fischer- 344)	2 yr (W)		14.4 F			NTP 1992	NOAEL is for histopathology of various brain areas.
34	Mouse (B6C3F1)	2 yr (W)		24.2 F			NTP 1992	NOAELs are for histopathology of various brain areas.
Reproductive								
35	Rat (Fischer- 344)	2 yr (W)		133 F			Hasegawa et al. 1986	NOAEL is for gross and microscopic appearence of the reproductive organs.
36	Rat (Fischer- 344)	2 yr (W)		14.4 F			NTP 1992	NOAEL is for histopathology of reproductive organs.

Table 3-3 Levels of Significant Exposure to Hypochlorite Solution - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
37	Mouse (B6C3F1)	2 yr (W)		24.2 F			NTP 1992	NOAEL is for histopathology of reproductive organs.

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

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = Female; Gastro = gastrointestinal; Gn pig = guinea pig; (GW) = gavage in water; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; (W) = drinking water; wk = week(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to Hypochlorite Solution - Oral

Acute (≤14 days)

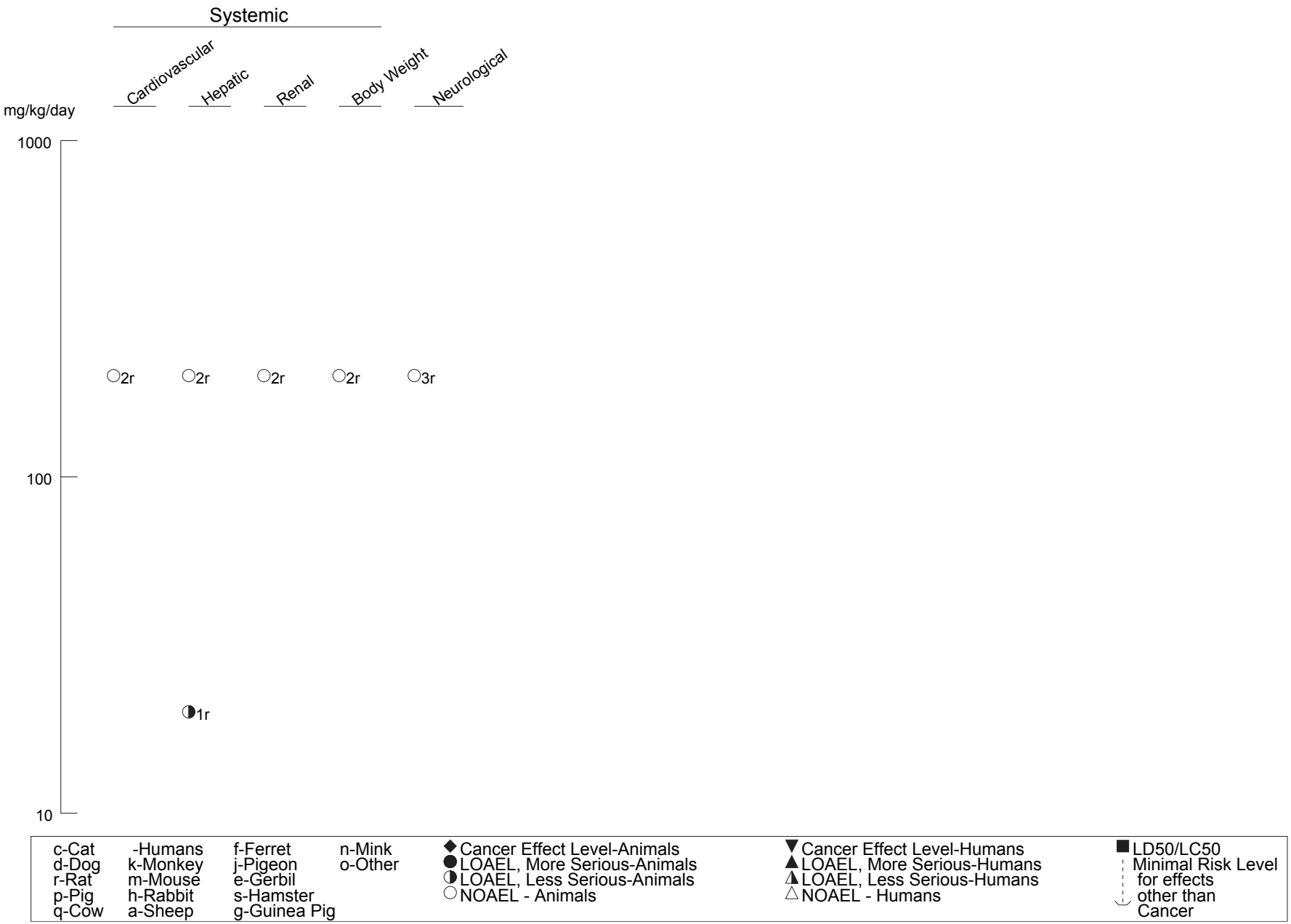


Figure 3-2 Levels of Significant Exposure to Hypochlorite Solution - Oral *(Continued)*

Intermediate (15-364 days)

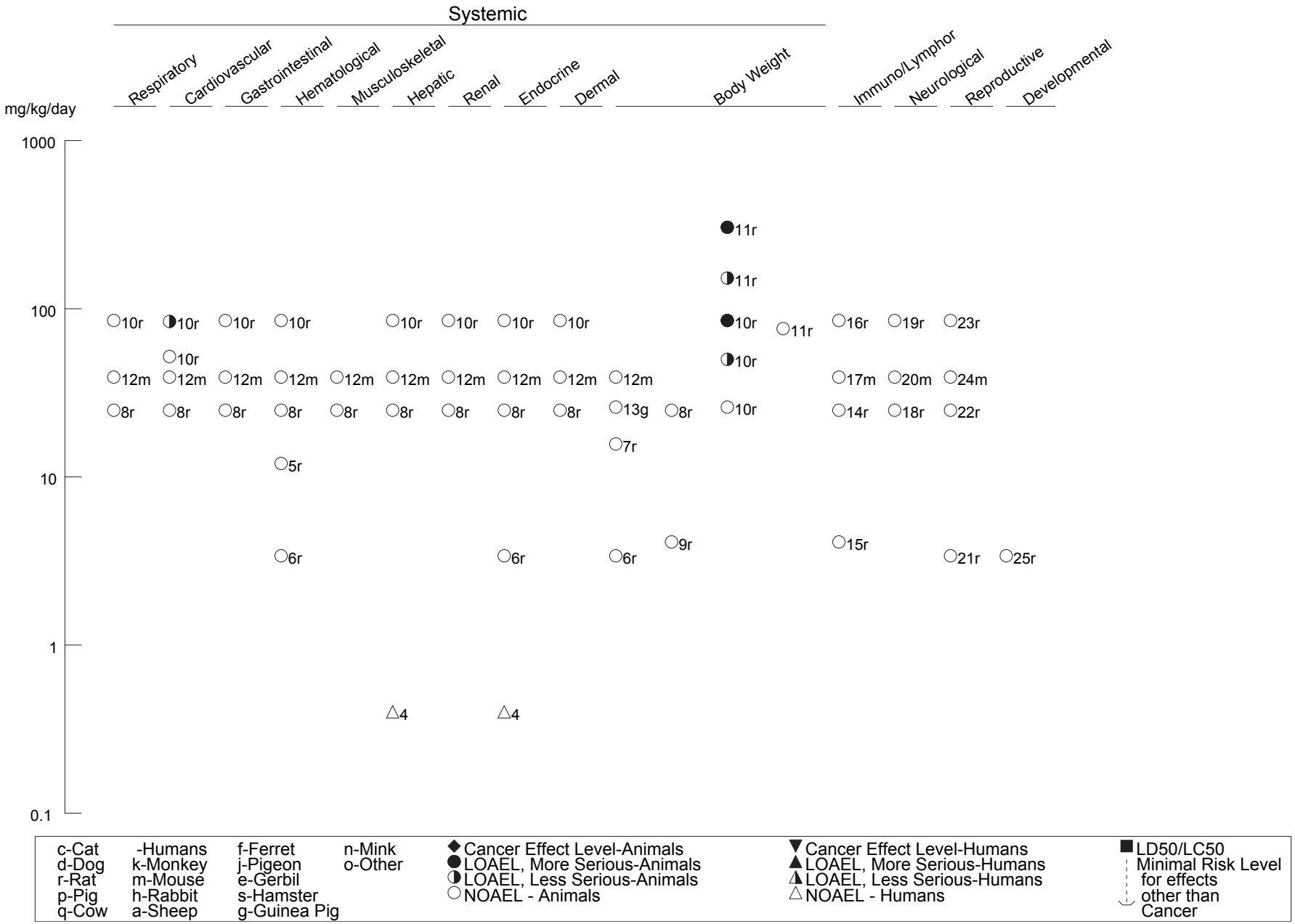
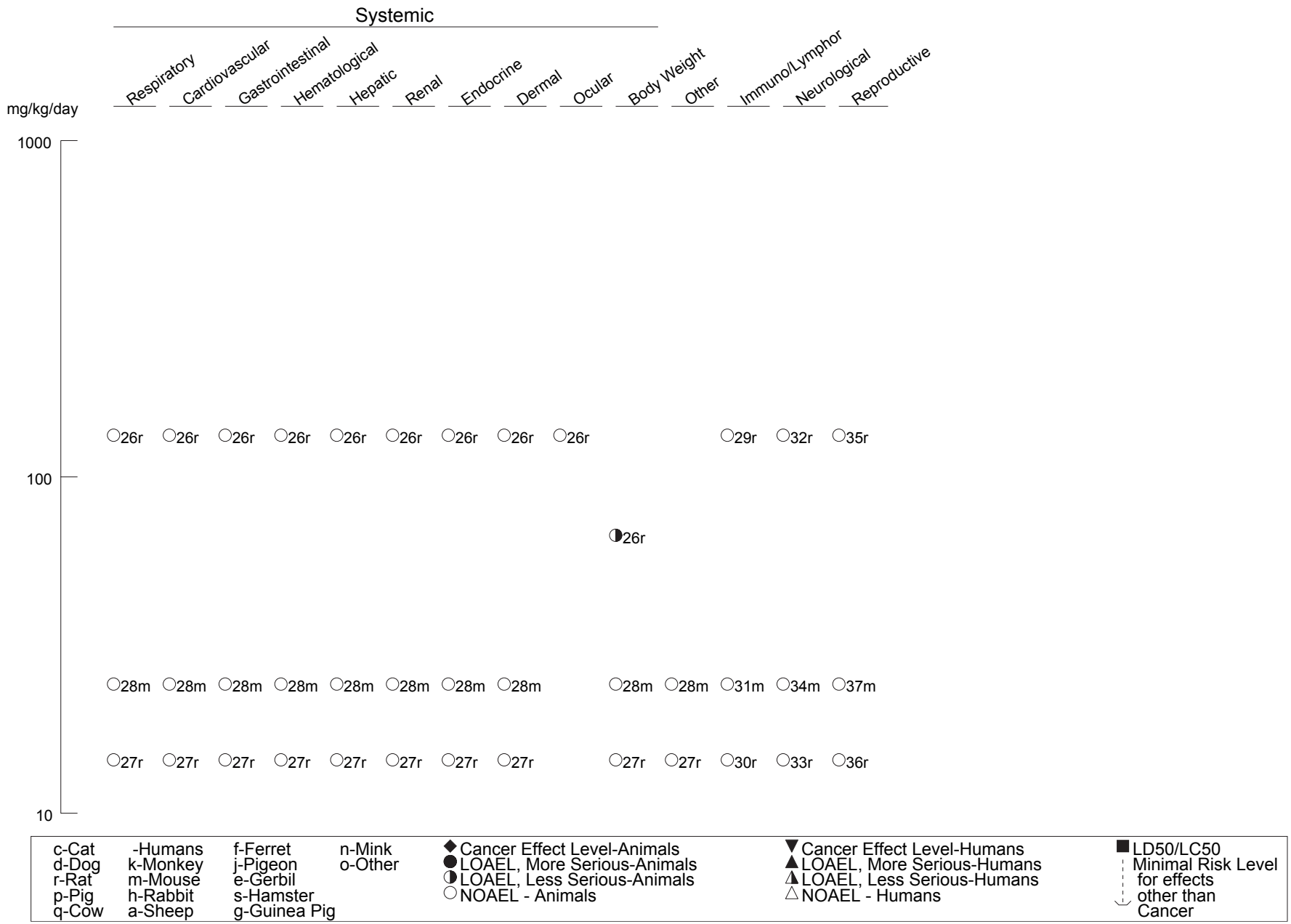


Figure 3-2 Levels of Significant Exposure to Hypochlorite Solution - Oral *(Continued)*

Chronic (≥365 days)



Cardiovascular Effects. In the study by Lubbers et al. (1982) in volunteers mentioned above, drinking water that provided 0.036 mg Cl/kg/day for 12 weeks did not alter systolic or diastolic blood pressure or pulse rate, and electrocardiograms were unremarkable. No additional relevant information was located regarding cardiovascular effects in humans following oral exposure to hypochlorite solutions.

A 14-day study in which Wistar rats were dosed by gavage with sodium hypochlorite in milk (up to 200 mg Cl/kg/day) reported no significant alterations in heart weight, but no additional cardiovascular parameters were examined (Cunningham 1980). Two intermediate-duration studies reported no significant gross or microscopic alterations in the heart and aorta from rats and mice exposed to up to 24.9 and 39.2 mg Cl/kg/day, respectively, for 90 days (Daniel et al. 1990, 1991). Furukawa et al. (1980) reported that endocardial hyperplasia and fibrosis of the myocardium were observed in male and female F344 rats dosed with approximately 84 mg Cl/kg/day in the drinking water for 92 days. No such effects were seen in rats dosed with approximately 50 mg Cl/kg/day. Two-year studies also did not find histological alterations in the heart from rats and mice that received doses of up to 133 and 24.2 mg Cl/kg/day, respectively (Hasegawa et al. 1986; NTP 1992).

Gastrointestinal Effects. In general, ingestion of small amounts (less than a cup) of sodium hypochlorite bleach (approximately 5.3% sodium hypochlorite) does not cause serious or permanent damage to the upper gastrointestinal tract. Pike et al. (1963) reviewed 129 cases of children who ingested Clorox® and reported that no complications of consequences were found. Sixty-five cases were examined by esophagoscopy within 96 hours of the ingestion and only 2 showed evidence of esophageal injury. The children were between 12 month and 7 years old and the amounts of bleach ingested ranged from "½ ounce to 1 cup." Landau and Saunders (1964) state that among 393 children who ingested bleach and were seen at a hospital, there were no esophageal strictures or perforations, and about 50% of the patients received no treatment. Hook and Lowry (1974) reported that among 23 definite cases of children who ingested Clorox®, severe irritation of the esophageal mucosa was observed in only 1 case. Minor transient irritation was observed in some of the patients. A report from the German literature of 23 children who accidentally ingested 3–5% sodium hypochlorite indicates that there was only 1 case with signs of superficial burns in the esophagus, which had disappeared 2 weeks later when controlled by esophagoscopy (Mühlendahl et al. 1978). Liquid bleach is a strong emetic, which helps reduce the time of residence in the stomach, but on the other hand, it increases the potential for aspiration.

Examination of fatal cases following ingestion of unknown quantities has revealed esophageal and gastric mucosal erosions, perforations at the gastroesophageal junction, and extensive necrosis of adjacent soft tissue (Ross and Spiller 1999). In a fatal case of a child who drank 4.5% sodium hypochlorite in an alkaline solution (pH 12), severe gross lesions were seen in the mouth, tongue, glottis, epiglottis, esophagus, and stomach (Jakobsson et al. 1991). Glottic and subglottic edema was described by Babl et al. (1998) in a child who drank household bleach from a cup.

In some earlier studies in animals, commercial bleach was administered through a tube directly into the esophagus and, in some cases, the distal end of the esophagus was artificially occluded to prolong and control the contact time between the solution and the mucosa (Hook and Lowry 1974; Landau and Saunders 1964; Strange et al. 1951; Yarington 1970). For example, commercial bleach placed in the esophagus of 151 dogs for several minutes caused the immediate death of 8 dogs from perforations into their pleural cavities (Landau and Saunders 1964). Necropsy performed 3 months later on the seven dogs that survived revealed no abnormalities. Yarington (1970) reported that, in dogs, the minimum amount of bleach that caused a burn in the esophagus was 10 cm^3 applied over a 5-minute period. A volume of 30 cm^3 applied for 2 minutes caused minimal edema of the esophagus.

Few more recent studies are available. Gross and microscopic examination of multiple levels of the gastrointestinal tract of Sprague-Dawley rats that drank water that provided up to 24.9 mg Cl/kg/day for 90 days did not reveal any significant gross or microscopic alterations (Daniel et al. 1990). The same negative results were reported in F344 rats and $B6C3F_1$ mice that drank water with up to 85 mg Cl/kg/day or 39.2 mg Cl/kg/day, respectively, for 90 days (Daniel et al. 1991; Furukawa et al. 1980). Two-year studies also did not find histological alterations in the gastrointestinal tract from F344 rats and $B6C3F_1$ mice that received doses of up to 133 and 24.2 mg Cl/kg/day, respectively (Hasegawa et al. 1986; NTP 1992).

Hematological Effects. No significant treatment-related alterations in a comprehensive number of hematological parameters were reported in volunteers who drank 0.5 L/day of drinking water containing 5 mg/L chlorine (approximately 0.036 mg Cl/kg/day) for 12 weeks (Lubbers et al. 1982).

A 12-month study in Sprague-Dawley rats exposed to approximately 12 mg Cl/kg/day reported alterations in red blood cell fragility at various times during the study; however, the alterations were sporadic and not dose- or duration-related, and varied in direction suggesting that they may have not been caused by exposure to chlorine (Abdel-Rahman et al. 1984). Red blood cell count and hematocrit were reduced

significantly after 3 months of treatment with 12 mg Cl/kg/day, but not at lower doses of chlorine (0.12 and 1.2 mg Cl/kg/day). Evaluations conducted after 6 months of treatment showed no significant difference in hematology parameters between treated and control groups (Abdel-Rahman et al. 1984). In another intermediate-duration study, exposure of male and female Long-Evans rats to up to 3.4 mg Cl/kg/day did not induce significant alterations in blood cell counts (Carlton et al. 1986). In yet additional studies, standard hematology parameters were not significantly altered in rats or mice dosed with up to 85 and 39.2 mg Cl/kg/day, respectively, for 90 days (Daniel et al. 1990, 1991; Furukawa et al. 1980). Hematology parameters were also evaluated in F344 rats and B6C3F$_1$ mice after 14–15 and 66 months of treatment with chlorine in the 2-year drinking water bioassay (NTP 1992) and at termination in the Hasegawa et al. (1986) study in F344 rats. The results showed no significant treatment-related alterations in rats dosed with ≤133 mg Cl/kg/day or in mice dosed with ≤24.2 mg Cl/kg/day.

Musculoskeletal Effects. No information was located regarding musculoskeletal effect in humans following oral exposure to hypochlorite and limited information is available in animals. No significant gross or microscopic alterations were seen in skeletal muscle and sternebrae from Sprague-Dawley rats following exposure to up to 24.9 mg Cl/kg/day in the drinking water for 90 days (Daniel et al. 1990). Similar results were reported in B6C3F$_1$ mice exposed to up to 39.2 mg Cl/kg/day for 90 days (Daniel et al. 1991). The 2-year NTP (1992) study reported no significant alterations in the femur from F344 rats or B6C3F$_1$ mice that received up to 14.4 or 24.2 mg Cl/kg/day, respectively, in the drinking water.

Hepatic Effects. Serum chemistry tests used to evaluate liver function did not reveal any significant alteration in volunteers during 12 weeks of exposure to approximately 0.036 mg Cl/kg/day in the drinking water and during a period of 8 weeks after exposure ceased (Lubbers et al. 1982). In another study of controlled exposure in volunteers, exposure to approximately 0.4 mg Cl/kg/day in the drinking water for 4 weeks had no significant effect on the lipid profile in serum (Wones et al. 1993).

Very limited information is available regarding liver effects in acute-duration oral studies in animals. Liver weight was not significantly altered in Wistar rats after 14 days of daily gavage doses of sodium hypochlorite in milk (up to 200 mg Cl/kg/day) (Cunningham 1980); no other liver parameter was monitored in this study. In another study of limited scope, triacylglycerols were significantly increased in whole liver homogenates from rats 2 days after a single gavage dose of approximately 20 mg Cl/kg (Chang et al. 1981). Ten days after dosing, the levels of triacylglycerols had returned to pre-dosing levels.

No significant gross or microscopic alterations were seen in the liver of Sprague-Dawley rats, F344 rats, or B6C3F$_1$ mice exposed to up to 24.9, 85, and 39.2 mg Cl/kg/day, respectively, in the drinking water for 90 days (Daniel et al. 1990, 1991; Furukawa et al. 1980). Levels of serum liver enzymes also were not significantly affected by treatment. Similarly, no significant hepatic effects were reported in F344 rats or B6C3F$_1$ mice that received up to 133 or 24.2 mg Cl/kg/day, respectively, in the drinking water for 2 years (Hasegawa et al. 1986; NTP 1992). Clinical chemistry tests conducted at termination in rats in the Hasegawa et al. (1986) study did not provide any indication of liver toxicity.

Renal Effects. The only relevant information regarding renal effects in humans following oral exposure to hypochlorite is that urinalysis performed weekly on volunteers during a 12-week controlled intake of approximately 0.036 mg Cl/kg/day and during a subsequent period of 8 weeks after treatment was unremarkable (Lubbers et al. 1982).

The only information available in acute-duration oral studies in animals is that kidney weight was not altered in rats in a 14-day study in which received daily doses of up to 200 Cl/kg/day by gavage in milk (Cunningham 1980). No other renal parameter was evaluated. No significant gross or microscopic alterations were seen in the kidney of Sprague-Dawley rats, F344 rats, or B6C3F$_1$ mice exposed to up to 24.9, 85, and 39.2 mg Cl/kg/day, respectively, in the drinking water for 90 days (Daniel et al. 1990, 1991; Furukawa et al. 1980). However, Furukawa reported increased incidence of gross abnormalities (no further details provided) in the bladder of rats in all treated groups (8–85 mg Cl/kg/day) in the 90-day study. No significant kidney effects were reported in F344 rats or B6C3F$_1$ mice that received up to 133 or 24.2 mg Cl/kg/day, respectively, in the drinking water for 2 years (Hasegawa et al. 1986; NTP 1992).

Endocrine Effects. A study in male and female volunteers who consumed daily for 4 weeks 1.5 L of distilled water that provided a dose of approximately 0.4 mg Cl/kg/day reported that there was a slight reduction in serum levels of thyroxine (T4) and triiodothyronine (T3) in men, which was not accompanied by any meaningful change in thyroid-stimulating hormone (TSH) levels (Wones et al. 1993). In another study in volunteers, ingestion of approximately 0.036 mg Cl/kg/day in the drinking water for 12 weeks did not significantly alter serum levels T3 or T4 (Lubbers et al. 1982).

No information was located regarding endocrine effects in acute-duration studies in animals. In an intermediate-duration reproductive study in male and female Long-Evans rats, dosing with up to 3.4 mg Cl/kg/day by gavage (hypochlorous acid) had no significant effect on serum thyroid hormone (unspecified) levels in males or females (Carlton et al. 1986). In the 90-day drinking water studies in

Sprague-Dawley and F344 rats and B6C3F$_1$ mice administered up to 24.9, 85, and 39.2 mg Cl/kg/day, respectively, there were no gross or microscopic alterations in the pancreas, adrenals, pituitary, thyroid, and parathyroid glands (Daniel et al. 1990, 1991; Furukawa et al. 1980). Similar results were obtained in the 2-year drinking water bioassay in F344 rats and B6C3F$_1$ mice (NTP 1992). Rats were exposed to up to 14.4 mg Cl/kg/day and mice were exposed to up to 24.2 mg Cl/kg/day. Hasegawa et al. (1986) also reported no alterations in the adrenal glands from F344 rats exposed to up 133 mg Cl/kg/day in the drinking water for 2 years.

Dermal Effects. No information was located regarding dermal effects in humans following oral exposure to hypochlorite solutions. The skin of rats and mice was examined in the 90-day (Daniel et al. 1990, 1991; Furukawa et al. 1980) and 2-year drinking water studies (Hasegawa et al. 1986; NTP 1992) and no significant gross or microscopic alterations related to treatment with chlorine were reported. In the 90-day studies, high doses in rats and mice were approximately 85 and 39.2 mg Cl/kg/day, respectively. In the 2-year studies, high doses in rats and mice were 133 and 24.2 mg Cl/kg/day.

Ocular Effects. No studies were located regarding ocular effects in humans following oral exposure to hypochlorite solutions. The only information from studies in animals is that exposure of F344 rats to up to 133 mg Cl/kg/day for 2 years did not increase the incidence of cataracts (Hasegawa et al. 1986). The eyes were not examined in rats or mice in the 90-day studies (Daniel et al. 1990, 1991) or in the NTP (1992) 2-year study.

Body Weight Effects. No studies were located regarding body weight effects in humans following oral exposure to hypochlorite solutions. Reduced growth was not observed in Wistar rats that received 200 mg Cl/kg/day (from sodium hypochlorite) by gavage in milk for 14 days (Cunningham 1980). Body weight was also not affected in Sprague-Dawley rats and B6C3F$_1$ mice exposed to up to 24.9 and 39.2 mg Cl/kg/day, respectively, in the drinking water for 90 days (Daniel et al. 1990, 1991) or in F344 rats and B6C3F$_1$ mice exposed to up to 14.4 and 24.2 mg Cl/kg/day, respectively, in the drinking water for 2 years (NTP 1992). Hasegawa et al. (1986) reported that male Fisher-24 rats exposed to approximately 152 or 305 mg Cl/kg/day (from sodium hypochlorite) in the drinking water for 90 days had reductions of 19 and 47% in final body weight compared with control rats; however, neither food nor water consumption data were provided in the study. In another 90-day study, male F344 dosed with approximately 50 mg Cl/kg/day (as sodium hypochlorite) in water had a final body weight 19% lower than controls; this group of rats also consumed 42% less water daily than controls (Furukawa et al. 1980). Rats dosed with 84 mg Cl/kg/day consumed 66% less water than controls and their final body weight was 46% lower than

controls (Furukawa et al. 1980). In the 2-year study conducted by the same investigators, final body weights were reduced 11 and 20% in female rats dosed with 67 and 133 mg Cl/kg/day, respectively. The investigators stated that in high-dose females water intake was somewhat lower (no quantitative data provided) during the first year, but not in the second year.

Metabolic Effects. Hypernatremia and hyperchloremic acidosis were reported in a woman who intentionally drank about 500 mL of a strong bleach solution (10% sodium hypochlorite) (Ward and Routledge 1988). Treatment with 5% dextrose gradually improved her electrolytes and 5 days later, she had recovered. Hyperchloremic metabolic acidosis also was reported in a woman who intentionally ingested an unknown amount of bleach (5.25% sodium hypochlorite, pH 11.4) and eventually died of cardiac arrest 4.5 hours after ingestion of the bleach solution (Ross and Spiller 1999). No other relevant information was located regarding metabolic effects in humans or animals following oral exposure to hypochlorite solutions.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans following oral exposure to hypochlorite solutions. Limited information is available in animals. In an 8-week study in Sprague-Dawley rats, exposure to 4.1 mg Cl/kg/day (the highest dose tested) in the drinking water (as sodium hypochlorite) resulted in alterations in several immunological parameters including reduced delayed-type hypersensitivity (DTH) reaction, increased prostaglandin E2 synthesis by macrophages, and reduced oxidative metabolism by macrophages following stimulation with phorbol myristate acetate (Exon et al. 1987). In the absence of systemic toxicity in rats dosed with much higher dose of chlorine in longer-term studies, the toxicological significance of these changes is unknown. In 90-day (Daniel et al. 1990, 1991; Furukawa et al. 1980) and 2-year (Hasegawa et al. 1986; NTP 1992) studies in rats and mice dosed with chlorine in the drinking water, there were no significant gross or microscopic alterations in the spleen, thymus, and lymph nodes. In the 90-day studies Sprague-Dawley and F344 rats and $B6C3F_1$ mice were exposed to up to 24.9, 85, and 39.2 mg Cl/kg/day, respectively. In the 2-year studies, high-doses in F344 and rats and $B6C3F_1$ mice were 133 and 24.2 mg Cl/kg/day, respectively. In none of these long-term studies was immunocompetence evaluated.

The highest NOAEL values from each reliable study for immunological and lymphoreticular effects in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans following exposure to hypochlorite solutions. One acute-duration study reported that exposure of Wistar rats to up to 200 mg Cl/kg/day (as sodium hypochlorite) by gavage in milk had no significant effect on brain weight, but no other neurological end point was evaluated in this study (Cunningham 1980). No gross or microscopic alterations were reported in the brain and sciatic nerve of Sprague-Dawley or F344 rats and $B6C3F_1$ mice in 90-days drinking water studies (Daniel et al. 1990, 1991; Furukawa et al. 1980). In these studies, rats and mice received doses of up to 85 and 39.2 mg Cl/kg/day, respectively. In the NTP (1992) 2-year drinking water study in F344 rats and $B6C3F_1$ mice, gross and microscopic examination of several brain areas did not show any significant alteration that could be attributed to treatment with chlorine. Hasegawa et al. (1986) also reported no histological alterations in the brain of F344 rats dosed with up to 133 mg Cl/kg/day (as sodium hypochlorite) in the drinking water for 2 years. None of these studies reported any adverse neurological sign in the animals throughout the studies, but no neurological tests were performed.

The highest NOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No information was located regarding reproductive effects in humans following oral exposure to hypochlorite solutions. In an acute-duration drinking water study, exposure of male $B6C3F_1$ mice to ≥1.6 mg Cl/kg/day (as sodium hypochlorite) for 5 days resulted in significant increases in sperm abnormalities in mice sacrificed 3 weeks after dosing ceased, but not in mice sacrificed 1 or 5 weeks after dosing (Meier et al. 1985). In addition, no sperm abnormalities were seen in mice that were treated in the same manner with hypochlorous acid. According to the investigators, the results were somewhat surprising since sodium hypochlorite should be converted in to hypochlorous acid in acid pH of the stomach. In the absence of corroborating information from other studies and lack of internal consistency of the results, the toxicological significance of these results is difficult to ascertain; therefore, this study is not listed in Table 3-3.

In an intermediate-duration study, exposure of male and female Long-Evans rats to up to 3.4 mg Cl/kg/day (as hypochlorous acid) by gavage before and during breeding and of the females throughout gestation and lactation did not affect fertility, the gross and microscopic appearance of the reproductive

organs of males or females, and did not induce sperm abnormalities (Carlton et al. 1986). Neither the 90-day (Daniel et al. 1990, 1991; Furukawa et al. 1980) nor the 2-year (Hasegawa et al. 1986; NTP 1992) drinking water studies reported any gross or histological alterations in the reproductive organs of male and female rats and mice. These studies, however, did not assess fertility.

The highest NOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No information was located regarding developmental effects in humans following oral exposure to hypochlorite solutions. Abdel-Rahman et al. (1982) exposed female Sprague-Dawley rats to 0, 0.1, 1, or 10 mg Cl/kg/day (as hypochlorous acid) in the drinking water for 2.5 months before mating with untreated males and continuing throughout pregnancy until sacrifice on gestation day 20. Exposure to chlorine had no significant effects on fetal viability or on mean fetal body weight. Skeletal anomalies were increased at 1 and 10 mg/kg/day and total soft tissue defects at 10 mg/kg/day relative to controls. However, since neither maternal body weight nor water consumption data were provided in the study, it also appeared that the incidence of fetal anomalies in the control group were higher than in the low-dose group, interpretation of these results is problematic; therefore, this study is not listed in Table 3-3. In the study by Carlton et al. (1986) mentioned above under *Reproductive Effects*, exposure of rats during gestation to maternal doses of 3.4 mg Cl/kg/day had no significant effect on neonate viability, weight gain, or on the incidence of gross external abnormalities. Pups sacrificed at 21 days of age had normal blood counts and serum levels of thyroid hormones. Developmental landmarks such as mean day of eye opening and average day of observed vaginal patency were unaltered in pups evaluated at age 28 and 40 days. The developmental NOAEL of 3.4 mg Cl/kg/day is listed in Table 3-3 and plotted in Figure 3-2.

3.2.2.7 Cancer

Studies of the carcinogenicity of trihalomethanes or other organic chemicals that form in water as a result of the chlorination of drinking water are not discussed in this section since these studies were not intended to assess whether chlorine itself is responsible for cancer. For reviews on this issue, the reader is referred to IARC (1991), Koivusalo and Vartiainen (1997), and EPA (1994b).

Cancer bioassays of chlorine in drinking water have been conducted in rats and mice. In the NTP (1992) bioassay, F344 rats (70/sex/dose group) were exposed to 0, 70, 140, or 275 ppm sodium hypochlorite in

the drinking water for 103–104 weeks. This provided doses of 0, 4.2, 7.3, or 13.6 mg Cl/kg/day to males and 0, 4.2, 7.8, or 14.4 mg Cl/kg/day to females. The water used in the study was deionized charcoal-filtered water. Interim sacrifices (10 rats/sex/dose) were conducted at 14 and 66 weeks. The only significant finding was an increased incidence of leukemia in female rats. The incidences were: 8/50, 7/50/, 19/51, and 16/50 in the control, low-, mid-, and high-dose females, respectively. Pair-wise comparison showed a statistically significant difference between controls and the mid-dose (p=0.014) and a trend test was also significant (p=0.037). In males, the respective incidences were 25/51, 25/51, 27/50, and 29/51. These results led NTP (1992) to conclude that there was equivocal evidence of carcinogenicity in female rats based on the fact that there was no clear dose-related response or reduced latency, there was no decrease in tumor latency, and the incidence in concurrent controls (16%) was significantly lower than in historical controls (25%). In a similar study, Hasegawa et al. (1986) administered sodium hypochlorite in distilled water to groups of F344 rats (50/sex/dose) in concentrations of 0, 500, or 1,000 ppm to males and 0, 1,000, or 2,000 ppm to females for 104 weeks; this was followed by a period of 8 weeks of drinking untreated water. This corresponds to doses of approximately 0, 33, or 67 mg Cl/kg/day for males and 0, 67, or 133 mg Cl/kg/day for females. The results showed no significant treatment-related increased incidence of neoplasms or alterations in latency of neoplasms.

In the NTP (1992) study, $B6C3F_1$ mice (70/sex/dose group) drank water with 0, 70, 140, or 275 ppm sodium hypochlorite for 103–104 weeks. This corresponds to doses of approximately 0, 7.4, 14, or 24 mg Cl/kg/day for males and 0, 7.6, 14.2, or 24.2 mg Cl/kg/day for females. The water used in the study was deionized charcoal-filtered water. Interim sacrifices (10 mice/sex/dose) were conducted at 15 and 66 weeks. The results showed that treatment with chlorine did not induce significant treatment-related increases in neoplasms. In a study by Kurokawa et al. (1986), groups of $B6C3F_1$ mice (50/sex/dose) were exposed to sodium hypochlorite in drinking water in concentrations of 0, 0.5, or 1% for 103 weeks. These concentrations provided doses of approximately 0, 33, or 55 mg Cl/kg/day to males and 0, 27, or 52 mg Cl/kg/day to females. Controls consisted of 73 males and 72 females. At termination, examination of tissues and organs did not show any statistically significant differences in tumor incidences between controls and treated mice.

Neither the EPA nor the DHHS has classified chlorine (elemental) or hypochlorite salts as to their carcinogenicity. Based on inadequate evidence for carcinogenicity of hypochlorite salts in animals and no data from studies in humans, IARC determined that hypochlorite salts are not classifiable as to their carcinogenicity in humans (Group 3) (IARC 1991).

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding death in humans or in animals following dermal exposure to hypochlorite solutions.

3.2.3.2 Systemic Effects

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

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-4.

Dermal Effects. Nixon et al. (1975) reported that patch application of bleach containing 5.25% sodium hypochlorite, pH 10.7, to intact human skin for 4 hours was severely irritating. Habets et al. (1986) reported that a solution of 2% sodium hypochlorite in water caused weak to moderate skin irritation in 15 out of 69 individuals tested; the volume applied was not disclosed. Twenty individuals who were tested with 0.1 and 0.5% solutions showed no irritation. Hostynek et al. (1989) tested 10 subjects with 100 μL of a 6% strength sodium hypochlorite solution (pH 11.2) and 4 of them developed a non-immunologic form of contact urticaria within 20 minutes of the application to skin of the forehead. The same group of investigators studied the skin irritation of 20 or 100 μL of hypochlorite bleach containing 1% sodium hypochlorite and various amounts of sodium hydroxide following 24 hours exposure under occluded patch conditions in 50 volunteers (Hostynek et al. 1990). The results showed that 20 μL 1% sodium hypochlorite and 1% sodium hydroxide produced no irritation, whereas 100 μL produced significant irritation. Goffin et al. (1997) reported that patch test exposure of 15 women to 150 μL of a commercial bleaching agent containing 4% sodium hypochlorite and 0.2% sodium hydroxide for up to 90 minutes produced no clinical signs of irritation. However, instrumental tests (reflectance colorimetry, transepidermal water loss, and skin conductance) revealed subclinical damage to the stratum corneum. The investigators concluded that a 4% solution of sodium hypochlorite can alter the superficial part of the stratum corneum without modifying the barrier function of the skin.

Table 3-4 Levels of Significant Exposure to Hypochlorous Acid and/or Sodium Hypochlorite - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
ACUTE EXPOSURE							
Systemic							
Human	90 min (C)	Dermal	4 F Percent (%)			Goffin et al. 1997	Instrumental tests revealed subclinical damage to the stratum corneum.
Human	48 hr (C)	Dermal	0.5 Percent (%)	2 Percent (%) (weak to moderate skin irritation)		Habets et al. 1986	
Human	20 min (C)	Dermal		6 Percent (%) (non-immunologic contact urticaria)		Hostynek et al. 1989	Non-immunologic contact urticaria developed within 20 minutes of application.
Human	24 hr (C)	Dermal	1 Percent (%)			Hostynek et al. 1990	Application of 100 microliters induced irritation; 20 microliters did not.
Human	4 hr (C)	Dermal		5.25 Percent (%) (severe skin irritation in 4/7 subjects)		Nixon et al. 1975	
Mouse (CD-1)	2 d 8 x/d (C)	Dermal		0.53 F Percent (%) (histologic changes indicating moderate irritation response)		Hess et al. 1991	The solution was applied as a spray.

Table 3-4 Levels of Significant Exposure to Hypochlorous Acid and/or Sodium Hypochlorite - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
Gn Pig (Hartley)	4 hr (C)	Dermal	5.25 Percent (%)			Nixon et al. 1975	
Rabbit (New Zealand)	once (C)	Ocular		5 B Percent (%) (moderate eye irritation)		Griffith et al. 1980	Median day to clear varied from 7 to over 21 depending on volume applied.
Rabbit (NS)	4 hr (C)	Dermal	5.25 Percent (%)			Nixon et al. 1975	

B = both male and female; (C) = capsule; d = day; F = female; Gn pig = guinea pig; hr = hour; LOAEL = lowest-observed-adverse-effect level; min = minute; NOAEL = no-observed-adverse-effect level

Nixon et al. (1975) conducted 4-hour patch tests with a 5.25% hypochlorite bleach solutions in rabbits and guinea pigs and reported that in both species, hypochlorite caused only slight irritation to both intact and abraded skin and concluded that neither species provide an accurate model for human skin. Strange et al. (1951) studied the effect of Clorox® mixed with various biological media on the skin of rabbits and rats. Clorox® was mixed with water, saliva, gastric juice, or plasma in ratios of 2:1, 1:1, and 1:2 and applied to the shaved abdominal skin of anesthetized rabbits in inverted tubes for 15 and 30 minutes. The mixtures with saliva and water were the most damaging, whereas the mixture with plasma was the least damaging, regardless of the dilution. Strange et al. (1951) speculated that the proteins in solutions containing plasma had a buffer effect on the harmful action of Clorox® on tissue. Strange et al. (1951) also reported that submerging the feet of anesthetized rats for 15 minutes into Clorox® mixed with proteins (plasma, egg white, milk) protected the tissue from the action of Clorox®. Mixtures of Clorox® with saliva or Clorox® with water induced edema, bleeding, and actual ulceration and destruction of tissue.

Hess et al. (1991) sprayed 0.8 mL of a 1:10 dilution of a commercial bleach solution onto a shaved abdominal area of female CD-1 mice 8 times/day for 2 consecutive days. Eighteen to 24 hours after the last application, the mice were sacrificed and the skin was processed for microscopic examination of the epidermis, dermis, and hypodermis. Exposure to bleach caused a “moderate” response. Grossly, the skin appeared dry with scattered brown crusty patches. Acanthosis, intraepithelial edema, hyperkeratosis, and atypical epithelial cells were seen in the epidermis. The dermis showed some evidence of edema, whereas the hypodermis showed a mild infiltration of neutrophils, macrophages, and lymphocytes.

Ocular Effects. Very limited information was located regarding ocular effects of direct contact of the eye with hypochlorite solutions. In their text *Toxicology of the Eye*, Grant and Schuman (1993) state that “because most accidental splashes in the eye have been with the relatively weak 5% household solutions of sodium hypochlorite, very few human eye injuries have been reported, and recovery has been rapid and complete.”

Experiments conducted in male and female New Zealand albino rabbits showed that instillation of 0.1 mL of household bleach directly to the central corneal surface and followed over a 21-day period produced moderate irritation (Griffith et al. 1980). The median day to clear was 7 days. In a review of the literature, Racioppi et al. (1994) mention unpublished data indicating that in rabbits, 0.1 mL of an 8% solution of sodium hypochlorite (without rinsing) caused moderate irritation and that the recovery time

was 7 days; under similar conditions, 0.01 mL of the same solution had low irritation potential and the recovery time was 3 days.

3.2.3.3 Immunological and Lymphoreticular Effects

Although sodium hypochlorite generally is not considered a contact sensitizer, several cases of allergic contact dermatitis have been reported. Osmundsen (1978) reported that case of a woman had a strong reaction to patch testing with 0.5% sodium hypochlorite in water years after having had dermal contact with chloramine. Further tests showed positive reactions to sodium hypochlorite in 3 out of 225 patients. Habets et al. (1986) reported two cases of hand dermatitis related to sodium hypochlorite allergy, as diagnosed by patch tests. Both patients showed a positive reaction to sodium hypochlorite up to a concentration of 0.1%. Van Joost et al. (1987) reported one additional case among 40 housewives who apparently had used bleaching agents for long periods. Eun et al. (1984) also reported a case of allergic contact dermatitis in a veterinarian who occasionally washed his hands with a commercial solution containing 4–6% sodium hypochlorite.

No information was located regarding immunological and lymphoreticular effects in animals following dermal exposure to hypochlorite solutions.

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

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

No information was located regarding cancer in humans due to dermal exposure to hypochlorite solutions. No bioassays have been conducted in animals by the dermal route of exposure, but the co-carcinogenic properties of hypochlorite have been examined. In a two-stage study in female ddN mice using 4-nitroquinoline 1-oxide in benzene as initiator, application of a commercial sodium hypochlorite solution (45 times) to a shaved area in the back of the mice after 20 applications of the nitroquinoline solution resulted in an incidence of 9/32 skin tumors compared with 0/29 in the nitroquinoline alone group and 0/27 in the hypochlorite group alone (Hayatsu et al. 1971). Although there is suggestive evidence of

promotion activity for sodium hypochlorite, the study is limited by insufficient information on dose, survival, and age at termination. In another initiation-promotion study, skin application of single initiating doses of 7,12-dimethylbenz[a]anthracene in acetone to female Sencar mice followed by twice weekly applications of sodium hypochlorite resulted in squamous cell carcinomas in one mouse initiated with 7,12-dimethylbenz[a]anthracene followed by sodium hypochlorite applications (Kurokawa et al. 1984). No skin carcinomas were seen in uninitiated mice promoted with sodium hypochlorite or in control groups. In yet another study, application of sodium hypochlorite to the shaved back of female NMRI mice twice per week for 10 weeks before the mice were applied benzo[a]pyrene twice per week for 10 weeks resulted in a decrease (approximately 40%) in the number of skin carcinomas induced by benzo[a]pyrene (Pfeiffer 1978). Mice that were treated with sodium hypochlorite during or after benzo[a]pyrene had tumor incidences comparable to benzo[a]pyrene alone.

3.3 GENOTOXICITY

No studies were located regarding genotoxic effects of chlorine gas in humans. The only information available in animals exposed to chlorine gas is that from an intermediate-duration study by Kutzman (1983). In that study, rats were exposed to 0, 0.5, 1.5, or 5 ppm chlorine 6 hours/day, 5 days/week for 62 days. At termination, samples of blood and bone marrow were collected and evaluated for cytogenetic effects. The results showed no evidence on increased incidence of sister chromatid exchanges or of cellular proliferation in the bone marrow from exposed rats. There was also no evidence of increased sister chromatid exchanges or of chromosomal aberrations in peripheral lymphocytes.

There is limited information regarding the *in vivo* genotoxicity of hypochlorite ions. A study in which male $B6C3F_1$ mice were administered chlorine in the drinking water as sodium hypochlorite or hypochlorous acid for 5 days and that provided doses of up to 8 mg Cl/kg/day found no evidence of increased incidences of chromosomal aberrations or micronuclei in bone marrow (Meier et al. 1985). In another study, administration of a single intraperitoneal dose of up to 2,500 mg/kg sodium hypochlorite (1,175 mg Cl/kg/day) to male ddY mice did not increase the incidence of micronuclei in bone marrow evaluated 24 hours after dosing (Hayashi et al. 1988). Exposure of newt larvae to sodium hypochlorite in the surrounding water (0.12 or 0.25 μg/mL) for 12 days increased the frequency of micronuclei in blood erythrocytes (Le Curieux et al. 1993). However, the study did not specify in what type of water the larvae were kept. If the larvae were kept in tap water, it is possible that chlorination byproducts rather than chlorine or the hypochlorite anion were the clastogenic agents. Table 3-5 summarizes the genotoxicity of sodium hypochlorite *in vivo*. Studies of the genotoxicity of sodium hypochlorite *in vitro* are summarized

Table 3-5. Genotoxicity of Sodium Hypochlorite *In Vivo*

Species (test system)	End point	Results	Reference
Mice bone marrow cells	Micronuclei	−	Meier et al. 1985
Mice bone marrow cells	Chromosomal aberrations	−	Meier et al. 1985
Mice bone marrow erythrocytes	Micronuclei	−	Hayashi et al. 1988
Newt (*Pleurodeles waltl*) larvae erythrocytes	Micronuclei	+	Le Curieux et al. 1993

– = negative result; + = positive result

in Table 3-6. As the table shows, the results have been mixed and no general statements can be made. The variability of the results may be due to differences in the experimental protocols used.

3.4 TOXICOKINETICS

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Nodelman and Ultman (1999a) measured the fraction of an inspired chlorine bolus cleared during a single breath as a function of the bolus penetration into the respiratory system of five nonsmoker males and females during both nasal and oral breathing at a respiratory flow of 250 mL/second using a noninvasive procedure. Measurements of the chlorine concentrations were made by means of a fast-responding thermionic chlorine analyzer. Peak concentrations of 0.5 and 3 ppm chlorine were used in nasal breathing experiments and 3 ppm in oral breathing experiments. The results indicated that almost all of the chlorine inhaled was absorbed in the upper airways (above the cords) whether the subjects inhaled through the nose or through the mouth. By comparing mass transfer parameters, the investigators also determined that total absorption rates for the mouth and nose were similar. When the peak concentration in the nasal breathing experiments was increased from 0.5 to 3 ppm, the mass transfer parameters remained unchanged, indicating that the dissolution, diffusion, and chemical reactions governing the absorption of the gas by the nasal mucosa are all linear processes. In other words, over the 0.5–3 ppm concentration range, absorption appeared to be non-saturable. In a separate experimental series, the investigators determined the longitudinal distribution of a bolus of 3 ppm chlorine as a function of the flow rate (Nodelman and Ultman 1999b). Using flow rates of 150, 250, or 1,000 mL/second, the authors determined that irrespective of the mode of breathing, nasal or oral, and respiratory flow rate, >95% of the inspired chlorine was absorbed in the upper airways and <5% was delivered to the lower airways.

Some studies in animals also have provided indirect evidence that the upper respiratory tract is an efficient scrubber of chlorine. For example, Alarie (1981) reported that the 10-minute LC_{50} in intact mice was 302 ppm, but in mice that breathed through a cannula in the trachea, the LC_{50} was only 131 ppm. In another study, the surgically isolated upper respiratory tract of anesthetized mice was shown to scrub chlorine with a mean efficiency of approximately 98% (Morris et al. 2005). Preliminary studies in F344 rats similar to those conducted by Nodelman and Ultman (1999a, 1999b) in humans also showed that >90% of a chlorine dose (0.5, 1.0, 2.5 ppm) is cleared in the upper respiratory tract (Roberts et al. 2007).

Table 3-6. Genotoxicity of Sodium Hypochlorite *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Salmonella typhimurium (TA100, TA102, TA98)	Gene mutation	Not tested	−	Le Curieux et al. 1993
S. typhimurium (TA100)	Gene mutation	Not tested	+	Ishidate et al. 1984
S. typhimurium (TA1530)	Gene mutation	Not tested	+	Wlodkowski and Rosenkranz 1975
S. typhimurium (TA1538)	Gene mutation	Not tested	−	Wlodkowski and Rosenkranz 1975
Escherichia coli (PQ37)	DNA repair	Not tested	−	Le Curieux et al. 1993
E. coli (DNA polymerase deficient)	DNA damage	Not tested	+	Rosenkranz 1973
Human fibroblasts cell line HE2144	Sister chromatid exchange	Not tested	+	Sasaki et al. 1980
Humans fibroblasts cell line HE1244	Chromosome breakage	Not tested	−	Sasaki et al. 1980
Syrian hamster embryo cells	Sister chromatid exchange	Not tested	+	Miyachi and Tsutsui 2005
Chinese hamster fibroblast cell line	Chromosomal aberrations	Equivocal	+	Ishidate et al. 1984
Chinese hamster lung cell line	Chromosomal aberrations	Toxic	+	Matsuoka et al. 1979
Syrian hamster embryo cells	Chromosomal aberratons	+	−	Hagiwara et al. 2006

— = negative result; + = positive result; DNA = deoxyribonucleic acid

3.4.1.2 Oral Exposure

No relevant data were located regarding oral absorption of hypochlorite ions in humans and limited information exists in studies in animals. Administration of a single dose of approximately 3.3 mg/kg of radiolabeled hypochlorous acid (^{36}Cl) to fasted Sprague-Dawley resulted in a peak ^{36}Cl in plasma of 7.9 μg/mL 2 hours after dosing (Abdel-Rahman et al. 1983). In non-fasted rats, administration of the same dose resulted in a peak concentration of 10.7 μg/mL ^{36}Cl in plasma 4 hours after dosing, suggesting that food residues slow down absorption perhaps due to the reaction of chlorine with food components (Fukayama et al. 1986). For both fasted and non-fasted rats, the absorption half-life was 2.2 hours. The elimination half-life from plasma was 44.1 and 88.5 hours in fasted and non-fasted rats, respectively.

3.4.1.3 Dermal Exposure

No information was located regarding absorption of hypochlorite ions in humans or in animals after dermal exposure to hypochlorite solutions. Studies of dermal exposure of humans or animals to aqueous chlorine have focused almost exclusively of effects on the skin; therefore, no systemic effects have been described that could provide indirect evidence of dermal absorption of hypochlorite ions.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No information was located regarding distribution of chlorine following inhalation exposure to chlorine gas.

3.4.2.2 Oral Exposure

In rats, 96 hours after the administration of a single gavage dose of approximately 3 mg/kg of hypochlorous acid labeled with ^{36}Cl, the plasma had the highest amount of radioactivity (1.92 μg/g) followed by whole blood, bone marrow, testes, skin, kidneys, lungs, packed cells, duodenum, stomach, spleen, thyroid, thymus, liver, carcass, and fat (0.09 μg/g) (Abdel-Rahman et al. 1983). Examination of the subcellular distribution of radioactivity in the liver 24 hours after dosing with hypochlorous acid showed that 75% of the total radioactivity of the whole homogenate was associated with the cytosol, 2.5% with the microsomal fraction, 1.5% with the nuclear fraction, and <0.1% with the mitochondrial fraction.

3.4.2.3 Dermal Exposure

No information was located regarding distribution of chlorine following dermal exposure to chlorine gas or hypochlorite ions.

3.4.3 Metabolism

Limited information is available regarding the metabolism of chlorine. In a study in which rats received a single oral gavage dose of radiolabeled (^{36}Cl) hypochlorous acid showed that, 96 hours after dosing, 81% of radioactivity detected in plasma was chloride ion (Abdel-Rahman et al. 1983).

Hypochlorous acid is a very reactive chemical and has been shown to react with biomolecules found in food (Fuyakama et al. 1986). Hypochlorous acid reacts with proteins, amino acids, and unsaturated lipids to form chlorinated compounds, whereas the reaction with carbohydrates yields oxidation products. Scully et al. (1986) reported that chlorination of the stomach from rats resulted in the production of N-chloramines, tentatively identified as N-chloroalanine, N-chloroglycine, and N-chlorophenylalanine. Chemicals such as chloroform, dichloroacetonitrile and di- and trichloroacetic acids were shown to form *in vivo* in the stomach of rats following oral administration of sodium hypochlorite (Mink et al. 1983).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

No information was located regarding elimination and excretion of chlorine following inhalation exposure to the gas.

3.4.4.2 Oral Exposure

The excretion of ^{36}Cl was studied in rats following administration of a single gavage dose of approximately 2.6 mg/kg of radiolabeled hypochlorous acid (Abdel-Rahman et al. 1983). Urine, feces, and expired air were collected over a 4-day period after dosing. During the first 24 hours, 7 and 7.5% of the administered radioactivity was excreted in the urine and feces, respectively. At the end of the 4-day period, 36 and 15% of the administered radioactivity had been recovered in the urine and feces, respectively, for a combined total of about 51% of the administered dose. No radioactivity was recovered in expired air during the study period. Since all or some of the amount recovered in the feces could have

been un-absorbed radioactivity, the 36% recovered in the urine represents the minimum that was absorbed.

3.4.4.3 Dermal Exposure

No information was located regarding elimination and excretion of chlorine gas or hypochlorite ions in humans or in animals following dermal exposure to these chemicals.

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

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

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

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical 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. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-3 shows a conceptualized representation of a PBPK model.

If PBPK models for chlorine 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.

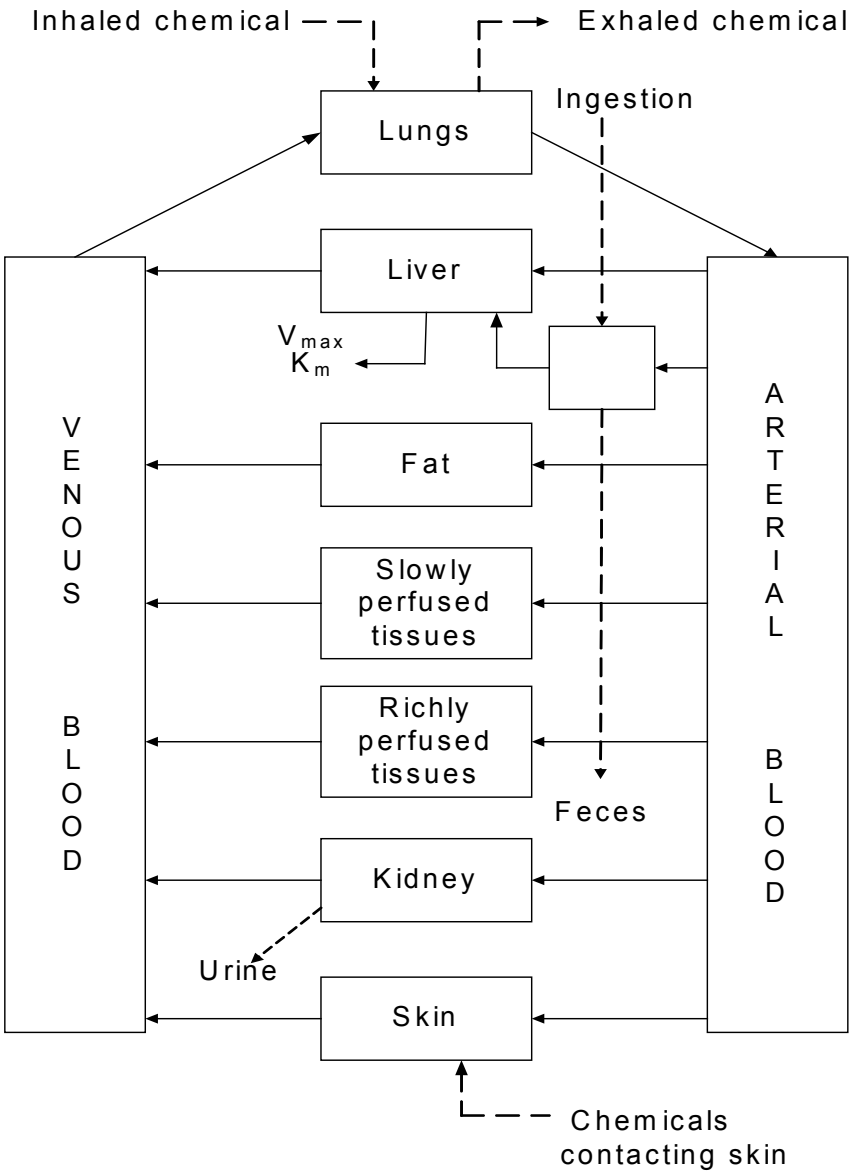
A computational fluid dynamics-physiologically based pharmacokinetic model is being developed for chlorine (Jarabek et al. 2007). The model is intended to address experimental dosimetry data on chlorine in rats, including uptake of chlorine delivered unidirectionally at various flow rates and concentrations measured in the isolated upper respiratory tract of F344 rats, and measurement of chlorotyrosines (as biomarkers) in samples taken from four regions from the respiratory and olfactory tissues.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Chlorine is a strong oxidizer that hydrolyzes in water forming hydrochloric and hypochlorous acids. Because of the high water content of the epithelial lining fluid and the local concentration of chloride and pH, the hydrolysis reaction of chlorine has a large equilibrium constant, such that the concentration of chlorine in the form of hypochlorite is 120,000 times that of chlorine gas (Nodelman and Ultman 1999a,

Figure 3-3. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



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.

Source: Adapted from Krishnan and Andersen 1994

1999b). This means that the effective solubility of chlorine between the inhaled gas and the mucus phase is 5 orders of magnitude larger than the physical solubility, which explains why >95% of the inhaled chlorine was absorbed in the upper airways in the studies with volunteers mentioned above (Nodelman and Ultman 1999a).

In a review of the toxicological significance of the chemical reactions of hypochlorite ions, Scully et al. (1989) point out that because hypochlorite is a potent oxidant, pharmacokinetic studies of radiolabeled hypochlorous acid (^{36}Cl) in animals do not reveal what happens to the parent compound, but rather to the product of the reactions of these compounds *in vivo*. However, as discussed by Scully et al. (1986), since hypochlorous acid undergoes rapid isotope exchange with unlabeled chloride, it is unclear whether the radioactive chloride detected in plasma of rats in the study by Abdel-Rahman et al. (1983) is due to complete reduction of the hypochlorous acid or to isotope exchange followed by elimination of chloride.

3.5.2 Mechanisms of Toxicity

Chlorine Gas. The toxicity of chlorine is strongly related to its oxidizing capacity. Chlorine reacts with water in the epithelial lining of the upper respiratory airways according to the following equation:

$$Cl_2 + H_2O \leftrightarrow HCl + HOCl$$

Chlorine gas has been shown to be 33 times more potent as a sensory irritant in mice than hydrochloric acid (HCl) (Barrow et al. 1977), which led the investigators to suggest that, in terms of sensory irritation, the response observed must be due to hypochlorous acid (HOCl) rather than to hydrochloric acid. More recently, Morris et al. (2005) reported that in mice, (a) an aerosol of sodium hypochlorite and (b) chlorine gas, at equivalent air concentrations, induced similar decreases in respiratory rate and increases in specific airway resistance, which suggested to the investigators that the oxidant properties of chlorine alone are sufficient to account for the observed responses. The precise mechanism by which this might occur is not known, but the assumption is that products of the reaction of chlorine with water are able to interact with functional groups in components from cells in the respiratory epithelium. At low concentrations, only sensory receptors may be affected, triggering only changes in respiratory dynamics, but higher concentrations produce frank tissue damage due to disruption of cellular components.

Hypochlorous Acid/Hypochlorite. The mechanism of toxicity of hypochlorous acid/hypochlorite is basically the same as that for chlorine gas. However, hypochlorous acid is a stronger oxidant than

chlorine gas as reflected by its higher redox potential. Damage to the upper gastrointestinal tract, as may occur following ingestion of sodium hypochlorite bleach, is likely the result of oxidation reactions of hypochlorous acid with a range of biological molecules. Exposure to dilute solutions of bleach usually result in only minor esophageal irritation, but ingestion of concentrated solutions of bleach can produce serious tissue damage. These properties of hypochlorous acid form the basis for the use of this chemical as a disinfectant (i.e., Lapenna and Cuccurullo 1996; Schraufstätter et al. 1990; Wang et al. 2007). Due to its high reactivity, ingested hypochlorous acid also reacts with organic compounds present in the stomach fluid such as proteins, polysaccharides, lipids, and vitamins, which may result in the formation of potentially harmful compounds (for review, see Fukayama et al. 1986; Mink et al. 1983; Scully et al. 1989).

3.5.3 Animal-to-Human Extrapolations

The respiratory system is the target for exposure to chlorine gas in humans and animals, and for the most part, humans and animals exhibit similar effects, particularly following acute-duration high exposures. Less is known regarding long-term effects of acute high exposures and of prolonged low level exposures, especially with regard to pulmonary function parameters. An evaluation of respiratory lesions in monkeys, rats, and mice following chronic exposure to comparable concentrations of chlorine noted that respiratory tract airflow characteristics play a major role in the distribution of the lesions and that the lesions in monkeys and rodents exhibited both differences and similarities (Ibanes et al. 1996). The conclusion was that with appropriate exposure and response adjustments, both rodents and Rhesus monkeys appear to be valid models for human risk assessment.

The gastrointestinal tract is the target for oral exposure to hypochlorite in humans and animals. Ingestion of concentrated solutions of hypochlorite bleach induces similar effects in humans and animals, and no single animal species has emerged as a preferred animal model for human gastric toxicity. A comparative study of dermal exposure of humans, rabbits, and guinea pigs to a 5.25% hypochlorite bleach solution reported that rabbits and guinea pigs were much more sensitive to the irritating properties of sodium hypochlorite, and therefore, neither species provide an accurate model for human skin (Nixon et al. 1975).

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate

terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

There are no studies that have tested whether chlorine gas has properties of endocrine disruptor, but given its mechanism of toxicity, such effects are very unlikely.

Oral exposure to hypochlorite solutions has provided no evidence of endocrine disruption in humans or in animals, but the available studies have not been designed to carefully evaluate that possibility. A study in male and female volunteers who consumed for 4 weeks 1.5 L of distilled water that provided a dose of approximately 0.4 mg Cl/kg/day reported that there was a slight reduction in serum levels of T4 and T3 in men which was not accompanied by any meaningful change in thyroid-stimulating hormone levels (Wones et al. 1993). In another study in volunteers, ingestion of approximately 0.036 mg Cl/kg/day in the drinking water for 12 weeks did not significantly alter serum levels T3 or T4 (Lubbers et al. 1982).

Relevant information in animals is very limited. Exposure of rats during gestation to maternal doses of up to 3.4 mg Cl/kg/day (as hypochlorous acid) had no significant effect on pups' serum levels of thyroid hormones at 21 days of age (Carlton et al. 1986). In addition, developmental landmarks such as mean day of eye opening and average day of observed vaginal patency were unaltered in pups evaluated at age 28 and 40 days. Studies in rats and mice found no significant gross or microscopic alterations in endocrine glands following long-term exposure to chlorine the drinking water (Daniel et al. 1990, 1991; Furukawa et al. 1980; Hasegawa et al. 1986; NTP 1992).

3.7 CHILDREN'S SUSCEPTIBILITY

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

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek

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

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

The respiratory tract is also the target for chlorine gas toxicity in children, and children exposed to chlorine exhibit the same signs and symptoms observed in adults detailed in Section 3.2.1.2. Whether children are more susceptible than adults to the effects of chlorine exposure is not known with certainty, but there are some reports that suggest that they might be. There are many reports of intoxication of children following a variety of exposure scenarios: tank explosions, train derailments, accidents at swimming pools, and accidents during school science class. Some representative examples are summarized below.

Following a train derailment in which members of a community, including over 100 children, may have been exposed to up 20 ppm chlorine, the most frequent conditions were coughing, headache, throat irritation, and a burning sensation in the eyes (Agency for Toxic Substances and Disease Registry 1998). Children aged 0–5 years had the highest prevalence of respiratory infections, rash, and vomiting. Fleta et al. (1986) reported the case of a leak of chlorine from a tank that exposed 76 children. The most prevalent symptoms were irritative cough (91%), nasal-pharyngeal pruritus (66%), chest pain (25%),

tachypnea (20%), and dyspnea (14%). Other conditions included headache, vomiting, and nausea. The report indicates that the symptoms of most children subsided naturally upon leaving the center of contamination. Seventy of the 76 children were released within 2 hours of the accident. In a similar case, 106 individuals were affected and 60 of them were children and adolescents <18 years (Güloğlu et al. 2002). Of those hospitalized due to their severe condition, patients 0–1 and 2–7 years had the longest duration of hospitalization, suggesting an increased susceptibility among children than adults.

Sexton and Pronchik (1998) described the case of 13 children, ages 6–18, who were overcome by chlorine gas at two swimming pools. On admission to the Emergency Department, most children complained of eye and throat irritation, chest pain, anxiety, shortness of breath, wheezing, and chest tightness. Five patients were admitted to the hospital with hypoxia, but all were released after 2 days. Two weeks after the exposures, no patients complained of residual symptoms. In another swimming pool accident, 134 children inhaled chlorine vapors and acute respiratory symptoms occurred in 72% (Agabiti et al. 2001). The incidence of all symptoms tended to be higher among those who had a history of chronic respiratory disease and among those who were engaged in physical exercise during the accident. Lung function tests conducted in 82 children 15–30 days after exposure showed lower FVC, FEV_1, and $FEF_{25–75\%}$. A study of 10 children exposed to chlorine gas at a swimming pool reported that all children had respiratory distress and reduced lung function on admission to the hospital (Bonetto et al. 2006). Although lung function returned to normal values 15 days after the accident, biochemical markers of pulmonary inflammation were still elevated in exhaled breath condensate several months after the accident. Persistent respiratory alterations were also observed in a child who developed dyspnea, hypoxemia, and pneumonitis approximately 12 hours after exposure to chlorine gas (Vohra and Clark 2006). Pulmonary testing 4 months after the episode revealed the presence of mild obstructive reactivity of the airways. After being exposed to excess chlorine in an indoor pool, 18 children were followed for up to 28 days (Grasemann et al. 2007). Fractional exhaled NO (a measure of airway inflammation) was significantly increased on day 1 compared to days 8 and 28 in 13 of the children who had lower airway symptoms with cough or shortness of breath on day 1, but not in the total study group or in asymptomatic children. In the total group of children, FVC was decreased on day 1 compared to days 8 and 28, whereas a marker of pulmonary hyperinflation was elevated on day 1 compared to day 8 or 28.

There are no studies in animals that addressed possible differential susceptibilities to exposure to chlorine gas between young and older animals.

In children, as in adults, the gastrointestinal tract is the target for oral exposure to hypochlorite bleach. Children usually constitute a significant percentage of the reported cases of accidental ingestion of chlorine bleach. In general, ingestion of small amounts (less than a cup) of household bleach (5–5.5% sodium hypochlorite) does not result in serious effects, but fatalities have been reported. For example, a review of 129 cases of children who ingested Clorox® reported that no complications of consequences were found (Pike et al. 1963). Sixty-five cases were examined by esophagoscopy within 96 hours of the ingestion and only 2 showed evidence of esophageal injury. Another review of 393 children seen at a hospital who ingested bleach reported that there were no esophageal strictures or perforations and that about 50% of the patients received no treatment (Landau and Saunders 1964). Hook and Lowry (1974) reported that among 23 definite cases of children who ingested Clorox®, severe irritation of the esophageal mucosa was observed in only one case. Minor transient irritation was observed in some of the patients. A report from the German literature of 23 children who accidentally ingested 3–5% sodium hypochlorite indicates that there was only one case with signs of superficial burns in the esophagus, which had disappeared 2 weeks later when controlled by esophagoscopy (Mühlendahl et al. 1978). Severe gross lesions were seen in the mouth, tongue, glottis, epiglottis, esophagus, and stomach in a child who died after drinking an unknown amount of a 4.5% sodium hypochlorite solution in alkali (pH 12.0) (Jakobsson et al. 1991). The information available does not suggest that children are more or less sensitive to oral exposure to chlorine than adults.

There are no studies in animals that have examined whether young animals are more susceptible to the effects of ingestion of hypochlorite bleach than adult animals.

3.8 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).

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, 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 chlorine are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by chlorine are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Chlorine

There are no biomarkers that can be used to quantify exposure to chlorine gas or sodium hypochlorite in humans. However, a recent study documented a statistically significant exposure concentration-dependent increase in 3-chlorotyrosine (CY) and 3,5-dichlorotyrosine (dCY) in nasal tissue from F344 rats exposed to chlorine gas concentrations ranging from 0.5 to 2.5 ppm for 90 minutes (Sochaski et al. 2008). Preferential formation of CY and dCY was found in the respiratory and transitional epithelium versus the olfactory epithelium of the nasal cavity. The investigators point out that the method presented in the study is only applicable to animals, as the collection of tissue samples would be too invasive for use in humans; nevertheless, it represents a good starting point for future method development. It is not

known whether exposure to other chlorinated compounds could also produce CY and dCY, thus compromising the specificity of these biomarkers.

3.8.2 Biomarkers Used to Characterize Effects Caused by Chlorine

There are no specific biomarkers that can be used to characterize the effects of chlorine. Chlorine gas is a sensory and pulmonary irritant, and similar effects can be observed after exposure to many other irritants. Ingestion of sodium hypochlorite can irritate the upper gastrointestinal tract, but this effect is not specific to chlorine.

3.9 INTERACTIONS WITH OTHER CHEMICALS

The only relevant information is that exposure to chlorine gas may result in the development of cross-tolerance to other chemicals. A study showed rats pre-exposed to chlorine developed cross-tolerance to formaldehyde and vice versa, and the development of tolerance was a function of the duration of pre-treatment (Chang and Barrow 1984). Slight loss of cross-tolerance was observed following a recovery period of a few days. Interestingly, exposure of rats to 15 ppm formaldehyde did not induce tolerance to formaldehyde, but resulted in cross-tolerance to chlorine and, according to Chang and Barrow (1984), suggested the existence of different reactive sites at the trigeminal nerve endings.

As previously mentioned, hypochlorous acid is a very reactive chemical and has been shown to react with biomolecules found in food (Fuyakama et al. 1986). Hypochlorous acid reacts with proteins, amino acids, and unsaturated lipids to form chlorinated compounds, whereas the reaction with carbohydrates yields oxidation products. Scully et al. (1986) reported that chlorination of the stomach from rats resulted in the production of N-chloramines, tentatively identified as N-chloroalanine, N-chloroglycine, and N-chloro-phenylalanine. Chemicals such as chloroform, dichloroacetonitrile, and di- and trichloroacetic acids were shown to form *in vivo* in the stomach of rats following oral administration of sodium hypochlorite (Mink et al. 1983).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to chlorine than will most persons exposed to the same level of chlorine 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 result in reduced detoxification or excretion of chlorine, or compromised function of organs

affected by chlorine. Populations who are at greater risk due to their unusually high exposure to chlorine are discussed in Section 6.7, Populations with Potentially High Exposures.

Populations unusually susceptible to chlorine gas exposure include individuals with respiratory conditions such as asthma, hay fever, and chronic bronchitis, heavy smokers, and children. Rotman et al. (1983) described the case of an atopic individual who experienced severe distress during exposure to 1 ppm chlorine, a concentration that was tolerated by healthy subjects. D'Alessandro et al. (1996) also reported that subjects with airway hyperresponsiveness to methacholine exhibited a much more pronounced decrease in FEV_1 and $FEF_{25–75\%}$ than healthy subjects during exposure to 1 ppm chlorine. Shusterman et al. (1998) reported that subjects with seasonal allergic rhinitis experienced a significantly greater increase in nasal airway resistance (congestion) than non-rhinitic subjects following exposure to 0.5 ppm chlorine for 15 minutes. Following an accidental leak of chlorine, individuals who had a more prevent history of smoking and asthma exhibited more hypoxemia and were more likely to have tachypnea, crackles, and wheezes during examination than subjects without asthma and/or who smoked less (Hasan et al. 1983). In the former, signs and symptoms of chlorine intoxication resolved more slowly reduced and flow rates and lung volumes were still evident 2 weeks after acute exposure to chlorine. Similar observations regarding smokers have been made in studies of workers who have experienced occasional high exposures or "gassing" episodes (Chester et al. 1969; Gautrin et al. 1999; Henneberger et al. 1996).

In a swimming pool accident involving 126 adult and 134 children, among both children and adults, the incidences of all symptoms (eye, nose, and throat irritation) and respiratory problems (shortness of breath, wheezing, cough) were higher among those who had a history of chronic respiratory disease than among healthy people (Agabiti et al. 2001). In addition, in adults, incidences were higher among smokers and former smokers than among never smokers.

Some reports in which adults and children were accidentally exposed to high concentrations of chlorine have suggested that children might be more susceptible to the effects of chlorine than adults. For example, in a case involving 106 individuals, 60 of whom were children and adolescents <18 years old, of those hospitalized due to their severe condition, patients 0–1 and 2–7 years old had the longest duration of hospitalization, suggesting an increased susceptibility among children than adults (Güloğlu et al. 2002). In another case in which over 100 children may have been exposed to up 20 ppm chlorine, children aged 0–5 years had the highest prevalence of respiratory infections, rash, and vomiting (Agency for Toxic Substances and Disease Registry 1998).

No information was located regarding populations unusually susceptible to exposure to hypochlorite bleach.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to chlorine. 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 chlorine. 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 chlorine:

Ellenhorn MJ, Barceloux DG. 1988. Medical Toxicology: Diagnosis and Treatment of Human Poisoning. New York, NY: Elsevier, 878-879.

Ellenhorn MJ. 1997. Medical Toxicology: Diagnosis and Treatment of Human Poisoning. 2th ed. Philadelphia, PA: Williams and Wilkins, 1521.

Goldfrank LR, Flomenbaum NE, Lewin NA, et al. 2002. Goldfrank's Toxicologic Emergencies. 7th ed. New York, NY: McGraw-Hill, 1458-1459.

Viccellio P, Bania T, Brent J, et al. 1998. Chlorine gas. In: Emergency toxicology. 2nd ed. Philadelphia, PA: Lippincott-Raven Press, 444-445.

Additional information can be found in ATSDR's Medical Management Guideline for chlorine (ATSDR 2007).

3.11.1 Reducing Peak Absorption Following Exposure

There are no specific methods for reducing absorption of chlorine gas other than removing the patient from the source of the chlorine gas to fresh air and monitor for respiratory distress. It should be noted that rescuers should wear self-contained breathing apparatus and have protective clothing, if needed.

Sodium hypochlorite or hypochlorous acid is not absorbed in the gastrointestinal tract as such. Either substance will react with the acid in the stomach to form chlorine gas and/or with organic compounds present in the stomach fluid to form chlorinated compounds (Mink et al. 1983; Scully et al. 1989). Gastrointestinal decontamination procedures such as emesis, gastric lavage, and activated charcoal should be avoided following ingestion of chlorine bleach. However, dilution with water or milk is recommended, but the dilution amount should be small to avoid inducing vomiting. In case of exposure

of the skin to hypochlorite solutions, flushing with copious amounts of plain tepid water is recommended. In case of exposure of the eyes, irrigation with saline or Ringer's lactate is recommended.

3.11.2 Reducing Body Burden

There are no standard methods for reducing chlorine body burden. Studies in humans have shown that under low exposure conditions (<5 ppm), >95% of the inspired chlorine is absorbed in the upper airways and <5% is delivered to the lower airways (Nodelman and Ultman 1999a, 1999b). Chlorine that reacts with the mucosa of the upper respiratory airways eventually joins the pool of chloride ions in the body. Studies in animals also have shown that most of the chlorine ingested as hypochlorous acid is transformed and eliminated as chloride (Abdel-Rahman et al. 1983).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The toxic effects of chlorine gas are due to its oxidant properties and also to the added tissue damage caused by the hypochlorous and hydrochloric acids that result from the reaction of chlorine with water. There are no established methods to interfere with the oxidant properties of chlorine, but nebulized sodium bicarbonate has been used to neutralize the acid (Bosse 1994; Douidar 1997).

The treatment of exposure to chlorine gas is symptomatic, exposure to low concentrations may require only treatment for sensory irritation, but exposure to high concentrations may cause serious respiratory symptoms including pulmonary edema and respiratory failure and death. The information below has been extracted from the texts listed above and also from Baxter et al. (1989).

Before any treatment, the patient should be assessed for signs of corrosive injury to mucous membrane, eyes, and skin. The assessment should also include a check for lung sounds, peak flow, and vital signs. Patients heavily exposed who show breathing difficulties at rest should undergo baseline x-ray examination. The initial treatment consists of irrigation with water or saline and vasoconstrictive ophthalmic solutions for eye irritation, but eye damage may require referral to a health care facility. Nausea may be treated with Phenergan® and administration of clear liquids, whereas sore throat can be treated with throat lozenges or spray or a humidifier. Decongestants are recommended for rhinitis and antitussive agents for the treatment of cough. Skin burns should be treated as thermal burns. Patients exhibiting respiratory effects should receive 100% humidified oxygen, unless it is contraindicated by the medical history. As mentioned above, 5% nebulized bicarbonate has been used in patient with respiratory effects with favorable responses in at least some patients (Bosse 1994; Douidar 1997). Nebulized

bronchodilators may be used to treat bronchospasm. Therapy with corticosteroids has not been proved to produce improvement in chlorine gas poisoning (Baxter et al. 1989). Monitoring of respiratory function and arterial blood gases in important because pulmonary edema may occur up to 24 hours after exposure. If pulmonary edema occurs, emergent treatment and monitoring in an intensive care unit is often required. Caution should be exercised with the administration of intravenous fluids and because fluid overload is extremely dangerous in such patients. If fluid overload occurs, diuretics such as furosemide may be useful as indicated. Survivors of high chlorine exposure should be monitored periodically to determine possible persistent loss of pulmonary function.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of chlorine 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 chlorine.

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

3.12.1 Existing Information on Health Effects of Chlorine

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to chlorine gas and hypochlorite solutions chlorine are summarized in Figures 3-4 and 3-5, respectively. The purpose of these figures is to illustrate the existing information concerning the health effects of chlorine. 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* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific

Figure 3-4. Existing Information on Health Effects of Chlorine Gas

	Death	Systemic: Acute	Systemic: Intermediate	Systemic: Chronic	Systemic: Immunologic/Lymphoretic	Systemic: Neurologic	Systemic: Reproductive	Systemic: Developmental	Systemic: Genotoxic	Cancer
Inhalation	●	●	●	●		●	●	●		●
Oral										
Dermal		●								

Human

	Death	Systemic: Acute	Systemic: Intermediate	Systemic: Chronic	Systemic: Immunologic/Lymphoretic	Systemic: Neurologic	Systemic: Reproductive	Systemic: Developmental	Systemic: Genotoxic	Cancer
Inhalation	●	●	●	●	●	●	●		●	●
Oral										
Dermal				●						

Animal

● Existing Studies

Figure 3-5. Existing Information on Health Effects of Hypochlorite Solution

	Death	Systemic: Acute	Systemic: Intermediate	Systemic: Chronic	Systemic: Immunologic/Lymphoretic	Systemic: Neurologic	Systemic: Reproductive	Systemic: Developmental	Systemic: Genotoxic	Cancer
Inhalation										
Oral	●	●	●							
Dermal		●			●					

Human

	Death	Systemic: Acute	Systemic: Intermediate	Systemic: Chronic	Systemic: Immunologic/Lymphoretic	Systemic: Neurologic	Systemic: Reproductive	Systemic: Developmental	Systemic: Genotoxic	Cancer
Inhalation										
Oral	●	●	●	●	●	●	●	●	●	●
Dermal		●								●

Animal

● Existing Studies

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.

The existing information on the health effects of chlorine gas in humans comes from accounts of soldiers exposed during gas attacks in World War I, subjects exposed to chlorine at work, and members of the general population accidentally exposed due to leaks or explosions of storage tanks or due to the mishandling of bleach solutions or swimming pool chemicals. Regardless of the exposure scenario, the target for chlorine gas toxicity is the respiratory system. Effects of exposure to low concentrations may be limited to irritation of the eyes, upper respiratory tract, and skin, whereas exposure to high concentrations may cause serious pulmonary effects and death. There is limited information on neurological effects in humans exposed to chlorine gas. Oral exposure is not a relevant route of exposure to chlorine gas in humans or animals.

Acute-, intermediate-, and chronic inhalation studies of chlorine gas in animals are available. In these studies, the respiratory tract was also established as the target for chlorine toxicity. There are minimal data on neurological, lymphoreticular, reproductive, and genotoxic effects, as well as cancer, in animals.

The available information on health effects of hypochlorous acid or sodium hypochlorite in humans is derived almost exclusively from cases of accidental or intentional ingestion of chlorine bleach. These observations indicate that the principal target for oral exposure to hypochlorite is the upper gastrointestinal tract. Ingestion of small amounts of chlorine bleach may only cause esophageal irritation, but ingestion of strong solutions of bleach can cause severe damage to the upper gastrointestinal tract and even death. There are no data on long-term exposure of humans to hypochlorite bleach. Since no target for chlorine toxicity has been identified in oral studies of various durations with dose levels much higher than those that could be generally encountered by the general population, oral MRLs for hypochlorite were not derived. Additional oral studies are not considered necessary except for the few exceptions indicated in Section 3.12.2 below.

Information is available from acute-duration oral studies with hypochlorous acid or sodium hypochlorite in animals, which also indicates that the upper gastrointestinal tract is the main target of toxicity for concentrated bleach. Intermediate- and chronic-duration studies with hypochlorous acid or sodium hypochlorite in animals have examined systemic end points and also have provided limited information on immunological, neurological, reproductive, and developmental effects. There are cancer bioassays available with sodium hypochlorite in rats and mice.

There are data that indicate that dermal exposure to hypochlorite bleach can cause skin irritation in humans and in animals.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. Information regarding health effects of acute exposure to chlorine gas is available from studies with volunteers (Anglen 1981; D'Alessandro et al. 1996; Rotman et al. 1983; Schins et al. 2000; Shusterman et al. 1998, 2003b), from exposures of soldiers during World War I (i.e., Berghoff 1919; DOA 1933; Joy 1997; Meakins and Priestley; Sandall 1922), and from accidental exposures of workers and the general public following chlorine leaks in a variety of scenarios (i.e., Agabiti et al. 2001; Agency for Toxic Substances and Disease Registry 1998; Bhérer et al. 1994; Bonetto et al. 2006; CDC 1991, 2005; Chasis et al. 1947; Chester et al. 1977; Edwards et al. 1983; Hasan et al. 1983; Jones et al. 1986; Kowitz et al. 1967; Moulick et al. 1992; Salisbury et al. 1991; Schönhofer et al. 1996; Sexton and Pronchik 1998; Weill et al. 1969). These and many additional studies showed that the respiratory tract is the target for chlorine toxicity and that the effects range from sensory irritation at low exposures (<5 ppm) to severe pulmonary effects (40–60 ppm) and possibly death (>100 ppm). Information is also available regarding long-term effects of acute high exposures to chlorine; some studies did not find persistent effects (i.e., Chasis et al. 1947; Jones et al. 1986; Moulick et al. 1992; Weill et al. 1969), whereas others did (i.e., Bhérer et al. 1994; Kowitz et al. 1967; Salisbury et al. 1991). Further research on this issue is needed. The studies in volunteers provided sufficient information for derivation of an acute-duration inhalation MRL for chlorine gas. The studies in animals support the findings in humans and provided additional information regarding histopathological changes in the respiratory tract and duration and reversibility of the effects. Standard additional acute-duration inhalation studies of chlorine gas in animals do not seem necessary at this time.

Information regarding health effects of hypochlorous acid and sodium hypochlorite in humans is derived exclusively from cases of accidental or intentional ingestion of hypochlorite bleach. These reports indicate that the upper gastrointestinal tract is the main target of toxicity for the oral route of exposure (Landau and Saunders 1964; Pike et al. 1963; Ross and Spiller 1999). The animal data support the findings in humans, but the available studies are inadequate for constructing dose-response relationships. Earlier studies in animals tried to reproduce the lesions to the esophagus and/or stomach due to ingestion of bleach. In most of these studies, commercial bleach was administered through a tube directly into the esophagus and, in some cases, the distal end of the esophagus was artificially occluded to prolong and

monitor the contact time between the solution and the mucosa (Hook and Lowry 1974; Landau and Saunders 1964; Strange et al. 1951; Yarington 1970). Two more recent studies were of very limited scope (Cunningham 1980) or reported ambiguous results (Meier et al. 1985).

Dermal effects have been reported in a few cases of direct acute contact of the skin with high concentrations of chlorine gas in humans (Agency for Toxic Substances and Disease Registry 1998; Joyner and Durel 1962; NIOSH 1995), and eye irritation was reported in volunteers exposed to 1 ppm chlorine for up to 8 hours (Anglen 1981; Rotman et al. 1983). Information on dose-response for sensory irritation was used along with data on pulmonary effects to derive the acute-duration inhalation MRL for chlorine. Additional studies of sensory irritation with chlorine gas do not appear necessary at this time. Chlorine gas is not absorbed through the skin, so systemic effects due to contact of the skin with chlorine are not expected to occur. Dermal effects of hypochlorite bleach have been reported in humans and in animals (Habets et al. 1986; Hostynek et al. 1989, 1990; Nixon et al. 1975; Strange et al. 1951); therefore, additional dermal studies do not seem necessary at this time.

Intermediate-Duration Exposure. No studies of humans exposed specifically for intermediate duration to chlorine gas were located. However, it is likely that in many of the occupational studies available, some workers were exposed for intermediate durations. Only two intermediate-duration studies in animals are available (Barrow et al. 1979; Kutzman 1983). Both studies utilized rats and in both studies, the most sensitive target for chlorine exposure was the respiratory tract. Barrow et al. (1979) described inflammation of the nasal turbinates in rats exposed to ≥1 ppm chlorine, whereas loss of cilia and epithelium in the trachea was seen in rats exposed to ≥0.5 ppm in the Kutzman (1983) study. The Kutzman (1983) study was selected as the principal study for derivation of an intermediate-duration inhalation MRL for chlorine. Additional intermediate-duration inhalation studies in animals do not seem necessary at this time.

Two intermediate-duration studies in which volunteers were exposed to known amounts of total chlorine from chlorinated water provided no evidence of adverse effects (Lubbers et al. 1982; Wones et al. 1993). Few intermediate-duration studies in animals were located that examined a wide range of end points following exposure to hypochlorite. These studies showed that the main effect of exposure to solutions of hypochlorous acid or sodium hypochlorite, particularly at the higher concentrations levels, is a reduction of water intake that is due to taste aversion. The available intermediate-duration oral studies evaluated systemic toxicity (Abdel-Rahman et al. 1984; Cunningham 1980; Daniel et al. 1990, 1991; Furukawa et al. 1980) and also provided information, albeit limited, on immunological/lymphoreticular (Daniel et al.

1990, 1991; Exon et al. 1987), neurological (Daniel et al. 1990, 1991), reproductive (Carlton et al. 1986; Daniel et al. 1990, 1991), and developmental effects (Carlton et al. 1986). None of the available studies reported effects that could be attributed directly to chlorine or only reported effects that were considered of unknown toxicological significance. Additional intermediate-duration oral studies with hypochlorite do not seem necessary. It is also unclear what needed information would be provided in additional intermediate-duration dermal studies.

Chronic-Duration Exposure and Cancer. There are relatively few long-term studies in workers exposed to chlorine gas (Enarson et al. 1984; Ferris et al. 1967, 1979; Hyback 1999; Patil et al. 1970). Health evaluations of the workers in these studies, including pulmonary function monitoring, did not provide evidence of significant health problems. In the Enarson et al. (1984) and Patil et al. (1970) studies, it was estimated that the workers were exposed to a TWA mean of 0.15–0.18 ppm chlorine. Yet, due to limitations, these long-term studies were insufficient for quantitative risk assessment. In none of the studies available was the nasal cavity, a sensitive target of chlorine exposure in humans and animals, examined. Therefore, evaluations of workers currently exposed to chlorine should include examination of the nasal cavity. In addition, future studies should include more reliable methods to estimate exposure. Two chronic-duration inhalation studies in animals are available. One of them studied the effects of chlorine inhalation in monkeys (Klonne et al. 1987) and the other in rats and mice (Wolf et al. 1995). In both, the upper respiratory tract was most sensitive end point and the study in monkeys was selected as basis for derivation of a chronic-duration inhalation MRL for chlorine gas. These studies also evaluated hematology and clinical chemistry parameters and, for the most part, no significant alterations were found. Additional chronic-duration inhalation studies of chlorine gas in animals do not seem necessary at this time.

No chronic-duration human studies of exposure to hypochlorous acid or sodium hypochlorite were located. Three chronic-duration studies were available in rats and mice (Hasegawa et al. 1986; NTP 1992). All three studies evaluated a comprehensive number of end points including hematology and clinical chemistry and tissue and organ histopathology and did not find any significant toxicity attributed to exposure to hypochlorite solutions. Additional chronic-duration oral studies with hypochlorite solutions are not necessary at this time.

There are several studies of cancer in humans exposed to chlorine gas, and probably simultaneously to other chemicals that did not find any evidence that chlorine gas is carcinogenic (Barbone et al. 1992; Barregård et al. 1990; Bond et al. 1983, 1985, 1986; Heldaas et al. 1989). The chronic-duration

inhalation study in rats and mice exposed to chlorine for 2 years found no evidence of carcinogenicity at termination (Wolf et al. 1995). It is unclear what useful information additional studies with chlorine gas would provide. There are no studies of cancer in humans exposed to hypochlorite ions, and it is unlikely that such a cohort can ever be found. Studies of cancer in humans exposed to chlorinated water have been conducted, but the focus of these studies has not been the hypochlorite ion but the chlorinated byproducts derived from the reaction of chlorine with organic matter in the water (see Kantor [1994] for review). Long-term drinking water bioassays have been conducted in rats (Hasegawa et al. 1986; NTP 1992) and mice (Kurokawa et al. 1986; NTP 1992) and, with one exception, the results were negative. Equivocal evidence of increased leukemia was reported in female rats in the NTP (1992) study. A need to conduct additional drinking water studies in the F344 strain may be unwarranted given that: (1) mononuclear cell leukemia occurs with high incidence in F344 rats; (2) the increase in leukemia was slight and not clearly dose-related; (3) there was no decrease in tumor latency; and (4) the incidence in concurrent controls was less than in historical controls. Although an option would be to conduct a study in a different strain of rat, the need would still have to be balanced with the fact that epidemiologic data do not suggest such an effect in humans.

Genotoxicity. No studies were located regarding genotoxic effects of chlorine gas in humans. The only information available in animals is that from a study in which blood and bone marrow from rats exposed to chlorine gas for 62 days showed no evidence on increased incidence of sister chromatid exchanges, chromosomal aberrations, or of cellular proliferation (Kutzman 1983). It does not appear that there is a need for additional genotoxicity studies for chlorine gas. Studies examining the *in vivo* genotoxicity of hypochlorite ions in mammals gave negative results (Hayashi et al. 1988; Meier et al. 1985) and studies *in vitro* in animal cells and bacteria gave mixed results (Hagiwara et al. 2006; Ishidate et al. 1984; Le Curieux et al. 1993; Miyachi and Tsutsui 2005; Sasaki et al. 1980). It is unlikely that additional studies with hypochlorite will settle the issue.

Reproductive Toxicity. The only information available regarding effect of chlorine gas in humans is that evaluation of the outcome of 15 pregnancies among female workers at a chlorine plant in 1932–1933 did not provide any evidence of reproductive toxicity (Skljanskaja et al. 1935). Pre-exposure of male or female rats to up to 5 ppm chlorine for 62 days followed by mating with untreated rats resulted in no significant effects on fertility, number of corpora lutea, viable embryos, early or late deaths, or pre-implantation loses. In addition, in males exposed for 62 days there were no histological alterations in the testes, and sperm morphology was unremarkable (Kutzman 1983). Chronic-duration inhalation studies with monkeys, rats, and mice exposed to up to 2.5 ppm chlorine did not observe gross or microscopic

lesions in the reproductive organs (Klonne et al. 1987; Wolf et al. 1995). It is plausible that accidental exposure of pregnant women to concentrations of chlorine high enough to produce severe hypoxia may affect pregnancy outcomes (i.e., stillbirth, abortions). Therefore, identification and evaluation of women who were pregnant during past chlorine accidents may provide valuable information. This can also be tested in animal models.

There is no information regarding reproductive effects in humans exposed to hypochlorite solutions and there are limited data in animals. An acute-duration study reported sperm abnormalities in mice treated with sodium hypochlorite, but not in mice treated with hypochlorous acid (Meier et al. 1985). This and other internal inconsistencies make this finding of unknown toxicological significance. A study in rats exposed to hypochlorous acid by gavage before and during breeding reported no significant effects on fertility or on histopathology of the reproductive organs of males or females (Carlton et al. 1986). Sperm was examined in this study and no significant alterations were reported. Long-term drinking water studies in rats and mice did not report any gross or microscopic alterations in the reproductive organs of males and females (Daniel et al. 1991, 1991; Furukawa et al. 1980; Hasegawa et al. 1986; NTP 1992). Although 2-generation reproductive studies with sodium hypochlorite (in water devoid of organic matter to eliminate the formation of chlorination byproducts) have not been conducted, the available information does not suggest that reproductive parameters are sensitive targets for hypochlorite. Thus, additional oral studies do not appear necessary at this time. There are no studies that evaluated reproductive parameters in humans or animals following dermal exposure to hypochlorite. However, dermal exposure to hypochlorite bleach is expected to affect only the site of exposure.

Developmental Toxicity. The only information available in humans exposed to chlorine gas is that evaluation of the outcome of 15 pregnancies among female workers at a chlorine plant in 1932–1933 did not provide any evidence of teratogenic effects (Skljanskaja et al. 1935). The same investigators reported that rabbits exposed to low chlorine concentrations (0.6–1.6 ppm) during pregnancy gave birth to healthy offspring (Skljanskaja and Rappoport 1935). There are no modern developmental studies of chlorine gas in animals. As mentioned in the preceding paragraph, exposure to high concentrations of chlorine gas during pregnancy could affect fetal or neonatal development, although so far, no study has examined that possibility. Therefore, identification and evaluation of women that were pregnant during past accidents in which chlorine gas was released may provide valuable information. Again, this can also be tested in animal models.

There is no information regarding developmental effects in humans exposed to hypochlorite. Only one reliable study in animals was available. In that study, exposure of pregnant rats to hypochlorous acid by gavage had no effect on neonate viability, weight gain, incidence of gross external abnormalities, or developmental landmarks (Carlton et al. 1986). Additional studies using a relevant means of administering chlorine, such as drinking water rather than gavage, may be necessary to confirm or refute the results of Carlton et al. (1986). There are no studies that evaluated developmental parameters in humans or animals following dermal exposure to hypochlorite bleach. However, as mentioned above, dermal exposure to bleach is expected to affect only the site of exposure.

Immunotoxicity. No studies were located that evaluated immunocompetence or effects on lymphoreticular organs in humans following exposure to chlorine gas. Studies in workers did not provide any evidence that exposure to chlorine gas may affect immunocompetence. A 6-week study reported that the spleen and thymus from rats exposed to 9 ppm chlorine showed decreased content of lymphoid elements, but according to the investigators, this may have been a function of the poor physical condition and decreased nutritional state of the rats in that dosing group (Barrow et al. 1979). Chronic-duration inhalation studies in moneys, rats, and mice found no gross or microscopic lesions in lymphoreticular organs and tissues (Klonne et al. 1987; Wolf et al. 1995). The immune system does not seem to be a sensitive target for chlorine gas toxicity; additional studies of the immune system in animals exposed by inhalation to chlorine gas are not necessary at this time.

There is no information regarding effects on the immune system in humans following oral exposure to chlorine. One study reported that exposure of rats to chlorine in the drinking water for 8 weeks resulted in alterations in some immune parameters (Exon et al. 1987). The toxicological significance of these findings is difficult to ascertain because there is no known mechanism by which oral administration of chlorine could induce immunological effects and no additional studies that could corroborate these findings. It is possible that oxidative reactions play a role in the effects reported by Exon et al. (1987). Other intermediate-duration studies and chronic-duration studies in rats and mice dosed with much higher doses of chlorine in the drinking water found no gross or microscopic alterations in lymphoreticular organs, but did not examine immunocompetence (Daniel et al. 1990, 1991; Furukawa et al. 1980; Hasegawa et al. 1986; NTP 1992). It would be useful to try to replicate the findings from Exon et al. (1987) adding to the protocol challenges with microorganisms to determine whether the reported alterations translate into decreased immunity.

Sodium hypochlorite is generally not considered a skin sensitizer, but several cases of allergic contact dermatitis have been reported (Eun et al. 1984; Habets et al. 1986; Osmundsen 1978; Van Joost et al. 1987). Additional dermal studies are not necessary

Neurotoxicity. A series of studies by Kilburn (1995, 2000, 2003b) suggested that brief exposures to high concentrations of chlorine gas can result in long-term neurological alterations in humans. No other high-exposure studies in humans have reported similar effects, but no neurobehavioral tests have been conducted in these studies. Therefore, it would be useful to conduct neurobehavioral evaluations in subjects known to have been exposed to high concentrations of chlorine to confirm or refute Kilburn's findings. The study should include comparison populations matched for the prior occurrence of a non-chemically related traumatic event. In addition, there are several validated animal models that have been used to test for neurobehavioral effects of chemicals (i.e., lead) that could be used to test for possible chlorine effects. The available studies in animals have provided no evidence of gross or microscopic alterations in tissues of the central or peripheral nervous system following exposure to chlorine gas.

There is no information regarding neurological effects in humans following exposure to hypochlorite bleach. The only information relevant information in animals is that from 90-day and 2-year studies that found no gross or microscopic alterations in the brain from rats and mice exposed to chlorine in the drinking water (Daniel et al. 1990, 1991; Furukawa et al. 1980; Hasegawa et al. 1986; NTP 1992). No further neurological end points were evaluated in these studies. Since the nervous system does not seem to be a sensitive target for oral chlorine, there is no compelling reason to conduct additional studies.

Epidemiological and Human Dosimetry Studies. There is a considerable number of studies of humans exposed to chlorine gas (see Section 3.2.1.2 for representative references). The effects of acute exposure to high concentrations of chlorine gas are known and concentration-response relationships have been established (Ellenhorn and Barceloux 1988). However, less is known about long-term effects of high acute exposures to chlorine gas and low-level, long-term exposure. As previously mentioned, some studies have described persistent pulmonary alterations following acute exposure to chlorine gas (i.e., Bhérer et al. 1994; Kowitz et al. 1967; Salisbury et al. 1991), whereas others have not (i.e., Chasis et al. 1947; Jones et al. 1986; Moulick et al. 1992; Weill et al. 1969). Better exposure data and baseline health information would seem necessary to establish reliable correlations between exposure and effects. Also, evaluation of the nasal cavity of low-level, long-term exposure to chlorine gas seems warranted in light of the findings of Klonne et al. (1987) in monkeys exposed to chlorine for 1 year.

Exposure to hypochlorous acid or sodium hypochlorite can occur by accidental or intentional ingestion of chlorine bleach. This type of exposure is generally of acute duration and, in most cases, the effects are restricted to esophageal irritation without long-term consequences (Hook and Lowry 1974; Landau and Saunders 1964; Pike et al. 1963). As discussed in Chapters 4 and 6, the level of dissolved chlorine in drinking water is extremely low and most of the free chlorine is as hypochlorous acid at the normal pH of drinking water. The highest level of chlorine allowed in drinking water is 4 ppm (EPA 2006a), which is considerably lower than the maximal concentration of chlorine used in long-term studies (275 ppm available chlorine) in rats and mice (NTP 1992), which caused no significant toxicity. Therefore, it seems unlikely that free chlorine in drinking water will represent a health concern for humans. It should be noted, however, that chlorinated water contains a variety of chlorinated byproducts whose biological effects continue to be studied.

Biomarkers of Exposure and Effect.

Exposure. There are no specific biomarkers of exposure for chlorine in humans. Chlorine gas that enters the airways or chlorine ingested as sodium hypochlorite eventually joins the chloride pool in the body.

A recent study showed that exposure of rats to chlorine gas (0.5–2.5 ppm) for 90 minutes resulted in dose-related formation of 3-chlorotyrosine and 3,5-dichlorotyrosine in nasal tissue (Sochaski et al. 2008). Since the method could not be used in humans due to the invasive nature of the tissue collection, additional research needs to be conducted to make the method less invasive. In addition, it would be valuable to determine whether exposure to other chlorinated compounds also result in the formation of these potential biomarkers.

Effect. There are no biomarkers of effect specific for chlorine. The sensory irritation and respiratory alterations caused by exposure to chlorine gas or the esophageal irritation caused by ingestion of hypochlorite bleach can also be caused by other chemicals.

Absorption, Distribution, Metabolism, and Excretion. The only information regarding pharmacokinetics of chlorine gas is that from experiments in volunteers conducted by Nodelman and Ultman (1999a, 1999b) that showed that almost all (>95%) of a bolus dose of chlorine gas inhaled through the mouth or the nose is cleared by the upper respiratory tract and none reaches the lungs. This was observed over a 0.5–3 ppm exposure range. The methodology used to generate the bolus and to monitor the concentrations of chlorine in the airways could probably be adapted to studies in animals,

particularly monkeys, to test a wider range of concentrations and to correlate internal concentrations of chlorine with lesions in the respiratory tract.

There is only one study of the pharmacokinetics of hypochlorite, the study by Abdel-Rahman et al. (1983) that evaluated absorption, metabolism, distribution, and excretion of chlorine in rats following gavage doses or radiolabeled (^{36}Cl) hypochlorous acid. Additional studies may be useful to confirm or refute the findings of Abdel-Rahman et al. (1983). On the other hand, as Scully et al. (1989) pointed out, because hypochlorite is a potent oxidant, pharmacokinetic studies of radiolabeled hypochlorous acid (^{36}Cl) in animals do not reveal what happens to the parent compound, but rather to the product of the reactions of these compounds *in vivo*. Therefore, the usefulness of additional studies is questionable. As previously mentioned, a computational fluid dynamics-physiologically based pharmacokinetic model is being developed for chlorine (Jarabek et al. 2007).

Comparative Toxicokinetics. The nature and distribution of lesions in the respiratory tract of monkeys were compared with those in rats and mice following chronic exposure to comparable concentrations (Ibanes et al. 1996). The investigators noted that monkeys and rodents exhibited both differences and similarities that were most likely related to the differences in airflow characteristics. Intuitively, it would seem that monkeys are a better model for human risk assessment because rodents are obligate nose breathers, whereas humans and monkeys are not. However, Ibanes et al. (1996) concluded that with appropriate exposure and response adjustments, both rodents and Rhesus monkeys appear to be valid models for human risk assessment.

An animal model for human exposure to hypochlorite has not been identified. Studies in rats and mice exposed to sodium hypochlorite by the oral route for 90 days or 2 years showed practically no toxicity of at the concentrations of chlorine tested (Daniel et al. 1990, 1991; NTP 1992). The gastrointestinal effects observed in humans after ingestion of high amounts of hypochlorite bleach (i.e., Ross and Spiller 1999) are similar to those described in earlier studies in dogs and rabbits exposed also to high amounts of hypochlorite bleach (Landau and Saunders 1964; Strange et al. 1951; Yarington 1970). Additional comparative studies do not seem necessary at this time.

Methods for Reducing Toxic Effects. The treatment of chlorine exposure is mostly supportive of respiratory and cardiovascular functions. The efficacy of some specific agents such as corticosteroids or nebulized sodium bicarbonate for the treatment of respiratory alterations due to exposure to chlorine gas has not been properly documented and further studies in animal models would be valuable.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

Children exposed to high concentrations of chlorine gas or hypochlorite solutions have experienced effects similar to those observed in adults, although some reports have suggested that children may more susceptible to chlorine gas toxicity than adults (Agency for Toxic Substances and Disease Registry 1998; Güloğlu et al. 2002). Children may be at increased risk for exposure to chlorine gas because they have a greater lung surface area:body weight ratio and an increased minute volume:weight ratio. Children may also be more vulnerable than adults because of the smaller diameter of their airways. Prolonged low-level exposures to chlorine gas are not relevant to children since this type of exposure occurs only in occupational settings. There are no studies that have examined whether young animals are more or less susceptible than adults to chlorine gas or hypochlorite toxicity. Additional information on this issue would be useful.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

The following ongoing studies pertaining to chlorine were identified in the Federal Research in Progress database (FEDRIP 2009):

Dr. M.D. Gunn and coworkers at Duke University, plan to conduct a series of murine studies to determine the extent to which specific pulmonary inflammatory cell types contribute to chlorine-induced lung injury and the consequent epithelial damage, bronchial reactivity, morbidity, and mortality. He will then examine the ability of the chemokine receptor antagonist to reduce these toxicities. The research is sponsored by the National Institute of Environmental Health Sciences (NIEHS).

Dr. S-E. Jordt and coworkers at the University of Alabama, Birmingham, intend to develop pharmacological approaches for the treatment of asthma patients after exposure to chlorine. Based on preliminary results indicating that the TRPA1, an ion channel of the transient receptor potential (TRP) gene family, is strongly activated by chlorine in primary and heterologous cells, the investigators

hypothesized that post-exposure administration of TRP channel antagonist, in combination with adrenergic agonists and antioxidants, will counteract life-threatening hypersensitivity responses in asthma patients to chlorine and other pulmonary chemical threats. Specifically the investigators intend to (1) compare responses of wild-type and TRP-channel deficient mice to chlorine in the background of the ovalbumin asthma model, (2) establish pharmacological measures to counteract chlorine hypersensitivity responses and chlorine-induced tissue damage in asthma, and (3) analyze the mechanism of TRPA1 activation and sensitization by chlorine, and its reversal. The research is sponsored by the NIEHS.

Dr. S. Matalon and associates at the University of Alabama, Birmingham, plan to test the hypothesis that the toxic effects of C12 will be heightened in animals infected with respiratory syncytial virus (RSV) or challenged with ova albumin. For that purpose, they will perform a number of biochemical, biophysical, physiological, and morphometric measurements in RSV infected and ova albumin challenged mice as well as normal rats prior to and following chlorine exposure to document the onset and progression of injury to lung epithelia and pulmonary and systemic vasculature. They will then treat the mice with antioxidants, TRP antagonists, and nitrite administered at various intervals post chlorine exposure either intratracheally or via aerosolization, or intraperitoneally (antioxidants and nitrite) and quantify recovery by specific functional measurements. The research is sponsored by the NIEHS.

Dr. S. Matalon and coworkers at the University of Alabama, Birmingham, also plan to determine whether systemic administration of reactive species scavengers, as well as agents that augment surfactant levels, ion transport and paracellular resistance, and a recently described peptide based on the lectin region of TNFa (tip peptide), shortly after exposure to chlorine will decrease lung injury, morbidity and mortality. This hypothesis will be tested by exposing either confluent monolayers of rat alveolar type II (ATII) epithelial cells or rats to chlorine (50–200 ppm for 30 minutes) and measuring a number physiological and biochemical indices of lung function 0.5, 6, 12, and 24 hours post exposure. The measurements will be repeated following intravenous injections of NAC, ascorbate, and deferoxamine as well as albuterol and the tip peptide. They will also assess the efficacy of intratracheally instilled ascorbate, NAC, deferoxamine, Infasurf (a surfactant replacement mixture), albuterol and the tip peptide, as well as aerosolized albuterol, in prolonging survival of rats with respiratory failure following chlorine exposure. The research is sponsored by the NIEHS.

Dr. P.R. Patel and coworkers at the University of Alabama, Birmingham, hypothesize that chlorine gas exposure induces both pulmonary and systemic vascular injury, which will be inhibited by nitrite-dependent NO formation. The investigators propose to test this via the following specific aims: (1) test

the hypothesis that inactivation of eNOS leads to compromised pulmonary and systemic vascular function, (2) establish protective effects of nitrite administration to rats following chlorine gas exposure on cardiopulmonary function, and (3) determine the mechanism by which nitrite therapy protects against C12 gas induced cardiopulmonary toxicity.